



## Conformation of an immunoreactive undecapeptide fragment (10–20) of Asp f 1 by NMR and molecular modeling

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### Summary

Allergic bronchopulmonary aspergillosis (ABPA), caused by *Aspergillus fumigatus*, is a complication of allergic asthma. Asp f 1 secreted by *A. fumigatus* is reported to be a major allergen/antigen involved in pathogenesis of aspergillosis. A 11-mer immunodominant epitope (Leu-Asn-Pro-Lys-Thr<sup>5</sup>-Asn-Lys-Trp-Glu-Asp<sup>10</sup>-Lys) of Asp f 1 has shown immunoreactivity with specific IgG and IgE antibodies in the sera of patients with ABPA in ELISA inhibition assay. Various studies have suggested that the peptide has a potential use in the development of ELISA based diagnostic kit for early diagnosis of infections caused by *A. fumigatus*. In view of these interesting properties of the undecapeptide we have embarked on an investigation of its conformation to understand the relationship between structure and immunoreactivity. NMR and molecular modeling studies of the peptide suggest a structure with a  $\beta$ -turn spanning residues Asn<sup>6</sup> – Glu<sup>9</sup> in water at pH 4.0, a  $\beta$ -pleated sheet in DMSO and a  $\alpha$ -helix in 40% HFA.

**Abbreviations:** ABPA, allergic bronchopulmonary aspergillosis; Boc, butyloxycarbonyl; CSI, chemical shift index; DDS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; DIC, 1,3-diisopropyl carbodiimide; DMAP, dimethylaminopyridine; DMF, dimethylformamide; DQF-COSY, double quantum filtered correlated spectroscopy; ELISA, enzyme-linked immunosorbent assay; FAB-MS, fast atom bombardment-mass spectrometry; Fmoc, fluorenylmethyloxycarbonyl; HFA, hexafluoroacetone; HoBt, hydroxy benzotriazole; HSQC, hetero nuclear single quantum coherence; IRMA, iterative relaxation matrix analysis; MD, molecular dynamics; DMSO, dimethyl sulfoxide-d<sub>6</sub>; NOESY, nuclear Overhauser effect spectroscopy; nOe, nuclear Overhauser effect; OBU, oxybutyl; RMSD, root mean squared deviation; ROESY, rotating frame nuclear Overhauser effect spectroscopy; TFE, trifluoroethanol; TOCSY, total correlated spectroscopy.

### Introduction

*Aspergillus fumigatus*, a ubiquitous fungus present in nature, causes a variety of respiratory disorders including ABPA. Invasive aspergillosis is generally observed in immunocompromised patients and is usually fatal in these cases [1]. ABPA is a complication of allergic asthma. It is a progressively disabling lung disease characterized by elevated total and anti-*Aspergillus* IgE, pulmonary and peripheral blood eosinophilia, central bronchiectasis, and an obstructive airway dis-

ease [2, 3]. Early diagnosis of aspergillosis can lead to more effective therapy and an increase in survival rate.

Asp f 1 is an 18 kDa protein secreted by *A. fumigatus* that is reported to be a major allergen/antigen involved in pathogenesis of Aspergillosis [4]. Several epitopes of Asp f 1 have been identified and their immunological properties studied [5]. These epitopes have the potential for development as synthetic peptide based diagnostics, which hitherto has been dependent on the crude antigen.

Recently, an eleven amino acid immunodomin-

ant epitope (Leu-Asn-Pro-Lys-Thr-Asn-Lys-Trp-Glu-Asp-Lys) present in the N-terminal region of Asp f 1 (10–20) has been identified based on algorithmic predictions [6]. The synthetic peptide showed immunoreactivity with specific IgG and IgE antibodies in the sera of patients of ABPA in ELISA inhibition assay.

The ELISA absorbance values obtained with synthetic peptide are comparable to those obtained with mixture of potent antigens, indicating that this peptide can be used for diagnosis of *A. fumigatus* specific antibodies in sera of APBA patients. This suggests a potential for the peptide to be used in the development of ELISA based diagnostic kit, for early diagnosis of *A. fumigatus* [7].

Several epitopes corresponding to the N- or C-terminal regions of Asp f 1 and other antigenic/allergenic proteins have been reported to bind IgG and IgE immunoglobulin [5]. However, no sequence homology has been observed among the epitopes. The antibody binding affinity and specificity of these epitopes have been attributed to their conformational properties [8].

In view of the interesting immunological properties of the undecapeptide [7] we have embarked on a study of its conformation in diverse media that could be used in structure activity relationship studies.

## Methods

### *Synthesis of peptide*

The peptide was synthesized by solid phase synthesis using Fmoc chemistry on Wang resin [9]. Fmoc-Lys(Boc)-OH was immobilised on Wang resin (Sigma) by treatment with DIC and DMAP. The loading was found to be 0.51 meq. g<sup>-1</sup> [10]. Fmoc group from the resin (500 mg) was removed by 50% piperidine in DMF and Fmoc-Asp(OBu)-OH was coupled by DIC/HOBt method. The coupling was monitored by Kaiser's test. Subsequently, remaining amino acids, appropriately protected, were coupled on the growing sequence. After all couplings the Fmoc group was removed and the resin dried. The peptide was cleaved from the resin by treatment with 95% trifluoroacetic acid and 5% thioanisol in presence of suitable scavengers for 1.5 hr. The reaction mixture was filtered and the solvent removed under reduced pressure and the peptide precipitated by addition of anhydrous ether. The hygroscopic precipitate was precipitated twice from absolute methanol/ether. The crude peptide was purified by reversed phase HPLC on a C<sub>18</sub> column using gradient elution (0–90% B in 90 min; A = 0.1%

trifluoroacetic acid in water, B = 0.1% acetonitrile). The pure fractions were pooled and lyophilized to get the undecapeptide as a white powder (370 mg). The peptide was characterized by FAB-MS (M + 1 1373).

DMSO (99.9%) was purchased from Sigma Chemical Co., (USA), D<sub>2</sub>O and DSS from Merck (Germany), and HFA from Aldrich Chemical Co., (USA).

The peptide (3 mg) was dissolved in 0.6 ml DMSO (4 mM solution) in a nitrogen atmosphere. For studies in water, the same amount of peptide was dissolved in a 0.6 ml solution of 95% H<sub>2</sub>O:5% D<sub>2</sub>O mixture. It is now well established that besides the solvents mentioned above, TFE and HFA are invaluable media for probing biomolecular structure, function, dynamics and protein folding. There is strong evidence that structures seen in such solvents are similar to those adopted by the peptide in their active conformation. The study of the conformation and dynamics of membrane bound peptides is a challenging one. A number of model systems have been suggested to mimic features of the membrane. It is now emerging that TFE and HFA are suitable model systems for the membrane [11, 12]. To study the effect of HFA, a mixture of 55% H<sub>2</sub>O:40% HFA:5% D<sub>2</sub>O was used. About 10 μL of a 0.1% solution of DSS in appropriate solvent was used as internal standard for the three solvents. The pH of aqueous samples was adjusted to 4.0 using dilute NaOH. The solutions were filtered and transferred to 5 mm NMR tubes.

### *NMR*

All NMR experiments were performed on a Varian Unity Plus 600 MHz FT NMR spectrometer using a 5 mm ID probe with 90° proton pulse length of 7.5 μs at a transmission power of 60 db. Data were collected using VNMR (v. 6.1b) on a Sun Sparc workstation. The length of the 90° pulse was checked at the beginning of the session and the temperature was controlled throughout the experiments to ± 0.1 K. The data was processed using FELIX software (v. 97, MSI, USA) running on a Silicon Graphics O2 workstation. All 2D spectra were acquired without spinning, whereas samples were spun (at 20 rpm) for all 1D experiments. 1D-NMR spectra of serially diluted samples were recorded to confirm that the peptide does not aggregate under the conditions of study.

All experiments were recorded with the States-Haberkorn [13] method of quadrature detection. A sweep width of 9500 Hz was used in both dimensions. Typically, 2K data points in D2 and 512 experiments in

D1 were acquired. All 2D experiments were preceded with 32 dummy scans, but none were applied between individual D1 increments. The pre-acquisition  $\alpha$  was individually adjusted. A relaxation delay of 1.5 s was used for all experiments.

The TOCSY experiment [14, 15] was carried out with the basic pulse sequence proposed by Bax. A 2 ms trim pulse preceded the MLEV-17 spin lock of 80 ms. ROESY [16] with a mixing time of 300 ms was also recorded. The mixing was achieved by continuous wave. Standard pulse sequences for DQF-COSY [17] and NOESY [18] were used. Several NOESY spectra with mixing times of 50, 100, 150, 250 and 400 ms were recorded.  $^1\text{H}$ - $^{13}\text{C}$  Gradient-HSQC experiments [19] with sensitivity enhancement was recorded to obtain  $^{13}\text{C}$  chemical shifts.

For the samples in water and HFA, solvent suppression was achieved using the WATERGATE (Water suppression by Gradient Tailored Excitation) technique for TOCSY [20] and NOESY [21] experiments. The number of transients per t1 increment for DQF-COSY was 64, 96 for HSQC and 32 for all other experiments.

A  $\pi/2$  phase-shifted sine squared window function was applied to the data along both dimensions. In some spectra,  $\pi/4$  phase-shifted sine squared window function was used to obtain better resolution when the peaks overlapped. The data was zero filled to a matrix of size  $4\text{K} \times 4\text{K}$  points for DQF-COSY and  $2\text{K} \times 2\text{K}$  points for all other experiments, prior to Fourier transformation. Chemical shifts were referenced to DSS.

Temperature coefficients for amide protons were determined from TOCSY experiments carried out at 298 K, 308 K and 318 K for DMSO and HFA, and at 283 K and 298 K for water. All other experiments for DMSO and HFA samples were recorded at 298 K and for water at 283 K.  $^3J_{\text{NH}\alpha}$  coupling constants were extracted from the 1D spectra.

Identification of spin systems was done using DQF-COSY and TOCSY and sequential assignments were made according to the methods described by Wüthrich *et al.* [22].

### *Theoretical methods*

#### *Secondary structure prediction*

Methods available in the *BIOPOLYMER* module in *SYBYL* (v. 6.4 Tripos, USA) were employed. We have used the methods developed by Qian/Sejnowski (based on Neural Networks) [23],

Garnier/Osguthorpe/Robson (employing Information Theory) [24] and Maxfield/Scheraga (using Bayes Statistics) [25], for predicting the most likely secondary structure for the peptide.

#### *Chemical Shift Index (CSI)*

The CSI of a proton is defined as the difference between the observed chemical shift and the chemical shift for that proton when the peptide is in a random coil structure. A negative deviation of the  $\alpha\text{H}$  chemical shift from the random coil value for a length of at least 3 or 4 residues, is a pointer of helical or turn-like structures, while a positive deviation, indicates the presence of a  $\beta$ -sheet structure [26]. For  $\alpha^{13}\text{C}$ , the relationship between the deviation (positive or negative) from the random coil values, and the secondary structure, is opposite to that described above for the proton.

#### *Molecular Dynamics (MD) simulations*

The NMR parameters of chemical shift, temperature coefficients of exchangeable protons and nOe's provide information about the secondary structure of a molecule. However, this data is insufficient to fully define all the degrees of freedom of the molecule. Techniques like distance geometry and molecular dynamics simulation are often used in conjunction with NMR data to obtain the 3D structure of molecules. We have used MD simulations with distance and dihedral restraints, to generate the solution structures of our peptide. Computations were done on a Silicon Graphics O2 workstation, with molecular modeling software from MSI, USA (*INSIGHT II* and *DISCOVER*, version 98). An extended structure of the peptide was built with the *BIOPOLYMER* module as the starting conformation for the simulations. The energy of the system was calculated by the CFF91 forcefield [27]. During the simulations, the *omega* torsions were forced to  $180^\circ$  with a force constant of  $100 \text{ kcal mole}^{-1} \text{ rad}^{-2}$ . The bond stretching in the energy equation was represented by a harmonic function. A dielectric constant of 1.0 was used for evaluation of the electrostatics. The Newton's equations of motion were integrated with the Verlet algorithm [28], with a step size of 1 fs. During equilibration, temperature was controlled by direct scaling of the atom velocities, while during the data collection period, a weak coupling to a temperature bath with a time constant of 0.1 ps was used. Distance and dihedral restraints were applied for all simulations as discussed further. Three simulations - (S1, S2, S3), each utilizing NMR data

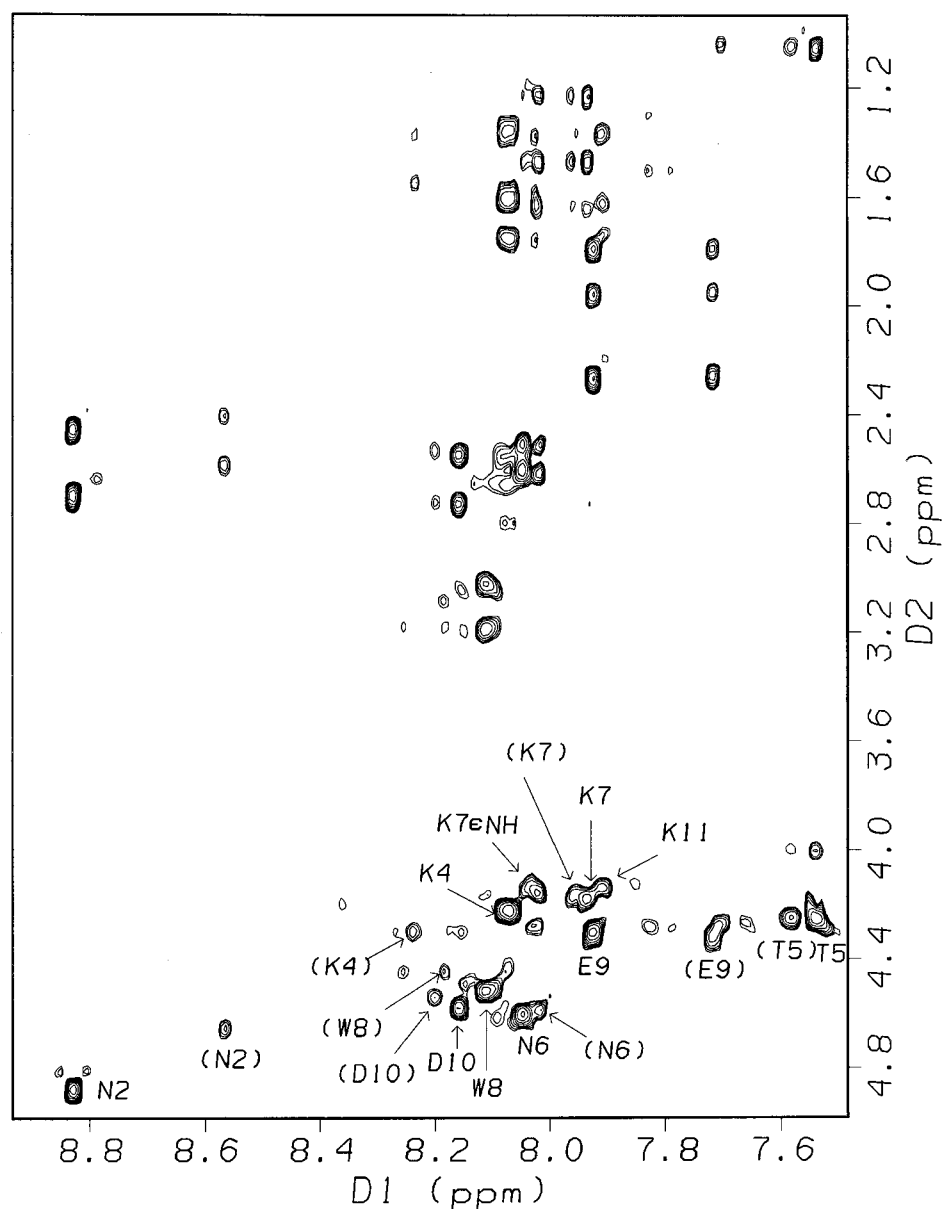


Figure 1. 600 MHz TOCSY spectrum of Asp f 1 (10–20) in Me<sub>2</sub>SO at 298 K; D1: 7.50–8.90 ppm., D2: 1.00–5.00 ppm.

obtained for the peptide in water, DMSO and HFA respectively, were performed with the general methodology as stated below. In all three simulations, the starting extended structure was thoroughly minimized by a combination of steepest descents and conjugate gradients, to a gradient of  $0.01 \text{ kcal mole}^{-1} \text{ \AA}$ . In simulation S1, this was followed by an MD run at 300 K, beginning with 3 ps of equilibration followed by 25 ps of productive dynamics. In case of simulations S2 and S3, the dynamics was conducted at 600 K and

1000 K, respectively. The temperature was reached by a slow ‘heating’ in 100 K steps with a 2.5 ps dynamics run. As in the previous simulation, the data collection period was restricted to 25 ps. These 25 structures were then ‘cooled’ to 300 K, in steps of 100 K, by a 2.5 ps dynamics run. The 300 K structures, in all three simulations, (each 25 in number), were then energy minimized with restraints first by steepest descents, followed by conjugate gradients, and ended with the Newton-Raphson (VA09A) method, to yield structures

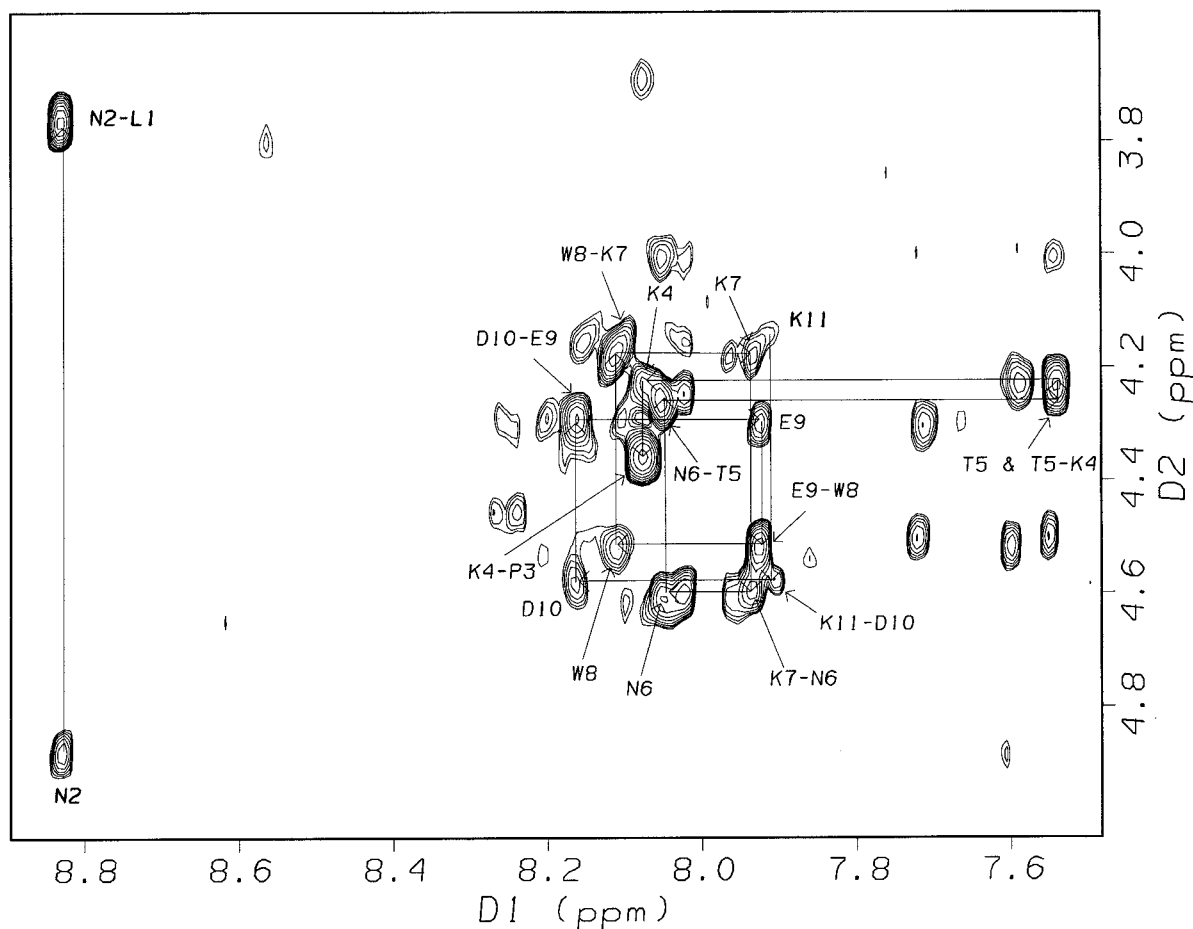


Figure 2. 600 MHz NOESY spectrum of Asp f 1 (10–20) in Me<sub>2</sub>SO at 298 K, recorded with mixing time 400 ms.; D1: 7.50–8.90 ppm., D2: 3.60–5.00 ppm.

having a gradient of 0.001 kcal mole<sup>-1</sup> Å or lower. The stability of the solution conformations was proved by final minimization without NMR restraints.

### Restraints

#### Distance restraints

Approximate distance restraints were calculated from the linear portion of the nOe build up curve, using the relationship  $\eta_{ij}/\eta_{kl} = (r_{kl}/r_{ij})^6$ , where  $\eta_{ij}$  and  $\eta_{kl}$  are the nOe intensities for the atom pairs i,j and k,l separated by distances  $r_{ij}$  and  $r_{kl}$  respectively. The intensity of the nOe between side chain amide protons of Asn<sup>2</sup> for water,  $\beta$  protons of Glu<sup>9</sup> for DMSO, and  $\beta$  protons of Lys<sup>4</sup> for HFA, where the distance 1.74 Å, was used as the reference. The distances calculated for methyl and methylene groups, were corrected according to the rules formulated by Wüthrich *et al* [22]. The

distances were cast as restraints, using force constants that ranged from 1 to 25 kcal mol<sup>-1</sup> rad<sup>-2</sup> and a  $\pm 0.3$  Å allowance put on the calculated distances, to represent the upper and lower bounds. The total number of distance restraints in the three simulations, was 48, 70 and 78 respectively.

#### Dihedral restraints

The  $^3J_{NH\alpha}$  coupling is a function of the dihedral angle  $\phi$  as given by the modified Karplus Equation [29]

$$^3J_{NH\alpha} = 6.51 \cdot \cos^2(\phi - 60) - 1.76 \cdot \cos(\phi - 60) + 1.60$$

The coupling constants were transformed to  $\phi$  values, and introduced as dihedral restraints, allowing a range of  $\pm 10^\circ$  on the calculated values. Force constants in the range of 1 to 30 kcal mol<sup>-1</sup> rad<sup>-2</sup>, were applied. As many coupling constants that could be accurately estimated, were used as restraints.

Table 1. Chemical shifts ( $\delta$  ppm),  $^3J_{\text{NH}\alpha}$  coupling constants (Hz) and temperature coefficients of NH chemical shifts (p.p.b./K) of Asp f 1 (10–20) in water<sup>a,b,c</sup>

| Residue           | NH             | $\alpha$ H     | $\beta$ H                  | Others                                                                                                                                          | $^3J_{\text{NH}\alpha}$ | Temp. Coeff. |
|-------------------|----------------|----------------|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------|
| LEU <sup>1</sup>  | –              | 3.99           | 1.69                       | $\gamma$ H 1.61; $\delta$ CH <sub>3</sub> 0.93, 0.92                                                                                            | –                       | –            |
| ASN <sup>2</sup>  | 9.02           | 5.03           | 2.91, 2.71                 | $\gamma$ NH <sub>2</sub> 7.75, 7.08                                                                                                             | –                       | 7.2          |
| PRO <sup>3</sup>  | –              | 4.42           | 2.29, 1.94                 | $\gamma$ CH <sub>2</sub> 2.01; $\delta$ CH <sub>2</sub> 3.83, 3.75                                                                              | –                       | –            |
| LYS <sup>4</sup>  | 8.46<br>(8.64) | 4.32           | 1.84, 1.76                 | $\gamma$ CH <sub>2</sub> 1.46, 1.41; $\delta$ CH <sub>2</sub> 1.68;<br>$\gamma$ CH <sub>2</sub> 2.98; $\epsilon$ NH <sub>2</sub> 7.57           | –                       | 7.2          |
| THR <sup>5</sup>  | 8.12<br>(7.88) | 4.31<br>(4.16) | 4.20                       | $\gamma$ CH <sub>3</sub> 1.17                                                                                                                   | 7.6                     | 7.1          |
| ASN <sup>6</sup>  | 8.46<br>(8.51) | 4.65           | 2.61                       | $\gamma$ NH <sub>2</sub> 7.61, 6.94                                                                                                             | –                       | 9.2          |
| LYS <sup>7</sup>  | 8.33<br>(8.36) | 4.21           | 1.63                       | $\gamma$ CH <sub>2</sub> 1.24; $\delta$ CH <sub>2</sub> 1.58;<br>$\epsilon$ CH <sub>2</sub> 2.89; $\epsilon$ NH <sub>2</sub> 7.54               | 5.0                     | 8.2          |
| TRP <sup>8</sup>  | 8.15<br>(8.06) | 4.63<br>(4.69) | 3.28                       | Ar. 7.64, 7.25; NH 10.21                                                                                                                        | 7.0                     | 5.2          |
| GLU <sup>9</sup>  | 8.04<br>(7.96) | 4.23<br>(4.30) | 1.96, 1.79<br>(2.01, 1.80) | $\gamma$ CH <sub>2</sub> 2.15                                                                                                                   | 7.1                     | 1.9          |
| ASP <sup>10</sup> | 8.24<br>(8.19) | 4.55<br>(4.48) | 2.76, 2.60                 | –                                                                                                                                               | 6.0                     | 2.9          |
| LYS <sup>11</sup> | 7.87<br>(7.77) | 4.16<br>(4.12) | 1.81, 1.70<br>(1.76, 1.71) | $\gamma$ CH <sub>2</sub> 1.34 (1.31); $\delta$ CH <sub>2</sub> 1.64<br>(1.59); $\epsilon$ CH <sub>2</sub> 2.94; $\epsilon$ NH <sub>2</sub> 7.56 | 7.4                     | 6.2          |

<sup>a</sup> Chemical shifts were obtained at 283 K.

<sup>b</sup> Values in parentheses are for the minor conformation.

<sup>c</sup> Pairs of geminal protons x (x =  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) were not assigned stereospecifically. When two distinct lines were observed, the larger chemical shift was arbitrarily assigned to x and the smaller to x'.

#### Iterative Relaxation Matrix Analysis (IRMA)

IRMA is a computational method based on NMR relaxation theory [30]. For a given molecular structure, the nOe intensities can be accurately calculated by IRMA, since it takes into consideration, the entire spin-relaxation network and molecular flexibility. The calculated and experimental nOe's are compared, and the structure is changed gradually, until the two match as close as possible.

We have used IRMA on each set of 25 structures generated by the three simulations S1, S2 and S3, to evaluate the precision and accuracy of these NMR structures. This is measured by the R-factor defined as follows [31]

$$R = \frac{\sum_{ijm} W_{ij}(\tau_m) |A_{ij}^{\text{calc}}(\tau_m) - A_{ij}^{\text{exp}}(\tau_m)|}{\sum_{ijm} W_{ij}(\tau_m) A_{ij}^{\text{exp}}(\tau_m)}$$

where  $A_{ij}^{\text{calc}}(\tau_m)$  and  $A_{ij}^{\text{exp}}(\tau_m)$  denote respectively, the theoretical and experimental nOe intensities, for the pair (i, j), for a given mixing time ( $\tau_m$ ). Weight factors  $W_{ij}(\tau_m)$ , which account for measurement errors, particularly noise levels, were set to 1.0.

The rotational correlation time ( $\tau_c$ ) for the molecule was estimated as 1 ns for water and HFA, and 2 ns for DMSO, from the nOe buildup of the reference peaks (same as those used for calculating distance restraints), for respective samples [32].

#### Results and Discussion

The unique spin systems of Thr ( $A_3MX$ ) and Glu [ $AM(PT)X$ ] were distinctly identified from the DQF-COSY and TOCSY spectra. Trp was assigned based on the cross peak between the indole NH and 2H (aromatic) in the TOCSY spectrum and a cross peak between 2H and  $\alpha$ ,  $\beta$  protons in the NOESY spectrum. The three AMX spin systems were assigned to two Asn and one Asp residues. The Asp residue was then identified based on the fact that its  $\delta$  protons resonate downfield compared to those of Asn residues. The Pro residue was assigned from the typical chemical shift values of its  $\delta$  protons. The three Lys residues were identified from the typical spin systems [ $A_2(F_2T_2)MPX$ ] seen in the TOCSY spectrum.

Table 2. Chemical shifts ( $\delta$  ppm),  $^3J_{\text{NH}\alpha}$  coupling constants (Hz) and temperature coefficients of NH chemical shifts (p.p.b./K) of Aspf1 (10–20) in DMSO<sup>a,b,c</sup>

| Residue           | NH             | $\alpha$ H     | $\beta$ H            | Others                                                                                                                            | $^3J_{\text{NH}\alpha}$ | Temp. Coeff. |
|-------------------|----------------|----------------|----------------------|-----------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------|
| LEU <sup>1</sup>  | –              | 3.76           | 1.53                 | $\gamma$ H 1.66; $\delta$ CH <sub>3</sub> 0.91, 0.89                                                                              | –                       | –            |
| ASN <sup>2</sup>  | 8.83<br>(8.56) | 4.88<br>(4.66) | 2.69<br>(2.59, 2.41) | $\gamma$ NH <sub>2</sub> 7.61, 7.06                                                                                               | 5.9                     | 3.8          |
| PRO <sup>3</sup>  | –              | 4.36           | 2.04, 1.92           | $\gamma$ CH <sub>2</sub> 1.91; $\delta$ CH <sub>2</sub> 3.69                                                                      | –                       | –            |
| LYS <sup>4</sup>  | 8.07<br>(8.24) | 4.22<br>(4.29) | 1.75, 1.61<br>(1.73) | $\gamma$ CH <sub>2</sub> 1.36; $\delta$ CH <sub>2</sub> 1.59 (1.54);<br>$\epsilon$ CH <sub>2</sub> 2.79                           | 8.4                     | 3.4          |
| THR <sup>5</sup>  | 7.54<br>(7.58) | 4.25           | 4.00                 | $\gamma$ CH <sub>3</sub> 1.05; OH 10.62                                                                                           | –                       | 3.4          |
| ASN <sup>6</sup>  | 8.05<br>(8.01) | 4.61           | 2.60, 2.52           | $\gamma$ NH <sub>2</sub> 7.52, 7.05                                                                                               | 9.8                     | 4.2          |
| LYS <sup>7</sup>  | 7.93<br>(7.96) | 4.18           | 1.64, 1.47           | $\gamma$ CH <sub>2</sub> 1.23; $\delta$ CH <sub>2</sub> 1.48;<br>$\epsilon$ CH <sub>2</sub> 2.72; $\epsilon$ NH <sub>2</sub> 8.02 | –                       | 3.8          |
| TRP <sup>8</sup>  | 8.11<br>(8.18) | 4.52<br>(4.44) | 3.18, 3.03           | Ar. 7.59, 7.17; NH 10.79                                                                                                          | 8.1                     | 3.9          |
| GLU <sup>9</sup>  | 7.92<br>(7.71) | 4.29           | 1.96, 1.79           | $\gamma$ CH <sub>2</sub> 2.27                                                                                                     | 7.5                     | 4.2          |
| ASP <sup>10</sup> | 8.16<br>(8.20) | 4.58<br>(4.54) | 2.73, 2.55           | –                                                                                                                                 | 7.9                     | 4.3          |
| LYS <sup>11</sup> | 7.91           | 4.14           | 1.74, 1.62           | $\gamma$ CH <sub>2</sub> 1.36, $\delta$ CH <sub>2</sub> 1.62;<br>$\epsilon$ CH <sub>2</sub> 2.78                                  | 7.5                     | 5.3          |

<sup>a</sup> Chemical shifts were obtained at 298 K.

<sup>b</sup> Values in parentheses are for the minor conformation.

<sup>c</sup> Pairs of geminal protons x (x=  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) were not assigned stereospecifically. When two distinct lines were observed, the larger chemical shift was arbitrarily assigned to x and the smaller to x'.

The sequence specific assignments were achieved with the NOESY spectrum wherein, nOe's between NH of a residue and  $\alpha$ H of the residue preceding it, are observed. By identifying the NH- $\alpha$ H cross peak and tracing the connection to the NH<sub>*i*</sub>- $\alpha$ <sub>*i*-1</sub>H cross peak, the individual amino acids could be assigned. A break in assignment occurred due to absence of an NH proton for Pro. This break was bridged by presence of an nOe between the Pro<sup>3</sup>  $\delta$  and Asn<sup>2</sup>  $\alpha$  protons in the NOESY spectrum. The <sup>13</sup>C assignments were made from the HSQC spectrum where the protonated <sup>13</sup>C NMR resonances were correlated to the assigned proton NMR signals. The HSQC spectrum also helped in resolving ambiguities in the assignments, if any, due to overlapping peaks in DQF-COSY, TOCSY and NOESY spectra. As an example, a portion of the TOCSY spectrum of the peptide in DMSO, showing the spin systems, and a NOESY spectrum outlining the sequential assignments, are presented in Figures 1 and 2 respectively.

The <sup>1</sup>H chemical shift of the peptide along with temperature coefficients of amide protons, and  $^3J_{\text{NH}\alpha}$  coupling constants, in the three solvents viz. water, DMSO and HFA are presented in Tables 1, 2 and 3 respectively. The <sup>13</sup>C chemical shifts are listed in Table 4.

Average values of torsion angles of structures obtained by the three MD simulations, are given in Tables 5, 6 and 7, respectively. Analysis of the *ensemble* of structures obtained from MD simulations is given in Table 8.

The TOCSY and NOESY spectra of the peptide in all three solvents displayed additional set of weak peaks suggesting the presence of a minor conformation in equilibrium with the major component. The ratio of the minor component was estimated to be about 10%, based on intensities of the cross peaks of the minor and major conformations in the TOCSY spectra. Presence of strong nOe's between Asn<sup>2</sup>  $\alpha$ H-Pro<sup>3</sup>  $\delta$ H, and absence of cross peaks between Asn<sup>2</sup>  $\alpha$ H-Pro<sup>3</sup>  $\delta$ H, suggest a *trans* conformation for the

Table 3. Chemical shifts ( $\delta$  ppm),  $^3J_{\text{NH}\alpha}$  coupling constants (Hz) and temperature coefficients of NH chemical shifts (p.p.b./K) of Asp f 1 (10–20) in HFA<sup>a,b,c</sup>

| Residue           | NH             | $\alpha$ H     | $\beta$ H                  | Others                                                                                                                            | $^3J_{\text{NH}\alpha}$ | Temp. Coeff. |
|-------------------|----------------|----------------|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------|
| LEU <sup>1</sup>  | –              | 3.99           | 1.69                       | $\gamma$ H 1.59; $\delta$ CH <sub>3</sub> 0.92, 0.91                                                                              | –                       | –            |
| ASN <sup>2</sup>  | 8.65<br>(8.22) | 5.08<br>(4.71) | 3.00, 2.78                 | $\gamma$ NH <sub>2</sub> 7.57, 6.67                                                                                               | –                       | 3.7          |
| PRO <sup>3</sup>  | –              | 4.41           | 2.32, 1.98                 | $\gamma$ CH <sub>2</sub> 2.03; $\delta$ CH <sub>2</sub> 3.79                                                                      | –                       | –            |
| LYS <sup>4</sup>  | 8.11<br>(8.04) | 4.30<br>(4.40) | 1.89, 1.81<br>(1.93, 1.80) | $\gamma$ CH <sub>2</sub> 1.49, 1.41 (1.48);<br>$\delta$ CH <sub>2</sub> 1.70 (1.73); $\epsilon$ CH <sub>2</sub> 2.99              | –                       | 2.8          |
| THR <sup>5</sup>  | 7.77<br>(7.64) | 4.31           | 4.28                       | $\gamma$ CH <sub>3</sub> 1.19                                                                                                     | 7.0                     | 1.9          |
| ASN <sup>6</sup>  | 8.11<br>(7.84) | 4.64<br>(4.46) | 2.65                       | $\gamma$ NH <sub>2</sub> 7.34, 6.68                                                                                               | –                       | 2.3          |
| LYS <sup>7</sup>  | 7.99           | 4.26           | 1.74                       | $\gamma$ CH <sub>2</sub> 1.29; $\delta$ CH <sub>2</sub> 1.63;<br>$\epsilon$ CH <sub>2</sub> 2.93; $\epsilon$ NH <sub>2</sub> 7.92 | –                       | 3.1          |
| TRP <sup>8</sup>  | 7.88<br>(7.81) | 4.65           | 3.33                       | Ar. 7.65, 7.25; NH 9.88                                                                                                           | –                       | 2.3          |
| GLU <sup>9</sup>  | 7.99<br>(7.79) | 4.25           | 2.04, 1.88                 | $\gamma$ CH <sub>2</sub> 2.23, 2.13                                                                                               | –                       | 2.5          |
| ASP <sup>10</sup> | 8.07<br>(7.77) | 4.64<br>(4.71) | 2.79, 2.68                 | –                                                                                                                                 | –                       | 1.6          |
| LYS <sup>11</sup> | 7.67           | 4.22           | 1.88, 1.75                 | $\gamma$ CH <sub>2</sub> 1.41, $\delta$ CH <sub>2</sub> 1.66;<br>$\epsilon$ CH <sub>2</sub> 2.98                                  | –                       | –            |

<sup>a</sup> Chemical shifts were obtained at 298 K.

<sup>b</sup> Values in parentheses are for the minor conformation.

<sup>c</sup> Pairs of geminal protons x (x =  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) were not assigned stereospecifically. When two distinct lines were observed, the larger chemical shift was arbitrarily assigned to x and the smaller to x'.

proline residue in the major component in all three solvents. However, unequivocal deduction of the complete structure of the minor conformation was not possible due to the limited information presented in the spectra.

#### Conformation in water

A low temperature coefficient for Glu<sup>9</sup> amide proton, along with the following nOe's – Asn<sup>6</sup>  $\alpha$ H Glu<sup>9</sup> NH, Lys<sup>7</sup>  $\alpha$ H Glu<sup>9</sup> NH, Trp<sup>8</sup> NH Glu<sup>9</sup> NH (moderate intensity); and  $^3J_{\text{NH}\alpha}$  coupling constants of 5 and 7 Hz for Lys<sup>7</sup> and Trp<sup>8</sup> respectively, suggest a Type I  $\beta$ -turn across the residues Asn<sup>6</sup> to Glu<sup>9</sup> [22]. The low temperature coefficient of 2.9 for the Asp<sup>10</sup> amide proton points towards a possibility of this proton being involved in intramolecular hydrogen bonding. The structural feature contributing to the low temperature coefficient was revealed by MD simulations as discussed below (*vide infra*).

Lack of significant nOe's and relatively high amide temperature coefficients for almost all other residues

(Table 1), indicate an extended or random coil like structure for rest of the peptide.

The MD simulations returned a Type I  $\beta$ -turn for the residues Asn<sup>6</sup> – Glu<sup>9</sup> (Figure 3). A  $\gamma$ -bend at Glu<sup>9</sup> was also observed in all the structures. This nicely explains the low temperature coefficient found for Asp<sup>10</sup> amide proton (Table 1). The average backbone torsions for the 25 structures of all the residues (Table 5), excluding those involved in the  $\beta$ -turn and the  $\gamma$ -bend, are more or less extended. The side chains of most residues are folded in  $g^+$  or  $g^-$  conformations. Some extended structures are seen for Leu<sup>1</sup>, Lys<sup>4</sup> and Asp<sup>10</sup> residues, together with folded conformations (Table 5).

A constraint free minimization of the 25 MD structures retained the  $\beta$ -turn in all structures while 20 structures had the  $\gamma$ -bend at Glu<sup>9</sup>. The two sets of structures (with and without restraints) are very homogeneous, differing in only 0.35 RMSD for the backbone atoms. This is an excellent test of the stability and validity of the structures, generated using restrained

Table 4.  $^{13}\text{C}$  Chemical shifts ( $\delta$  ppm) of Asp f 1 (10–20) in water, DMSO and HFA<sup>a</sup>

| Solvent | Residue           | $\alpha\text{C}$ | $\beta\text{C}$ | $\gamma\text{C}$ | $\delta\text{C}$ | $\varepsilon\text{C}$ |
|---------|-------------------|------------------|-----------------|------------------|------------------|-----------------------|
| Water   | LEU <sup>1</sup>  | 54.0             | 42.2            | 26.1             | 23.3, 24.0       | –                     |
| DMSO    |                   | 52.2             | 41.8            | 25.0             | 24.3, 23.3       | –                     |
| HFA     |                   | 54.3             | 42.1            | 26.1             | 23.0, 23.5       | –                     |
| Water   | ASN <sup>2</sup>  | 50.9             | 37.9            | –                | –                | –                     |
| DMSO    |                   | 49.2             | 38.2            | –                | –                | –                     |
| HFA     |                   | 50.4             | 38.6            | –                | –                | –                     |
| Water   | PRO <sup>3</sup>  | 62.8             | 31.8            | 26.8             | 50.3             | –                     |
| DMSO    |                   | 61.1             | 30.8            | 25.8             | 48.5             | –                     |
| HFA     |                   | 63.5             | 31.5            | 26.4             | 50.2             | –                     |
| Water   | LYS <sup>4</sup>  | 56.1             | 32.4            | 24.5             | 28.7             | 41.7                  |
| DMSO    |                   | 54.2             | 32.1            | 23.8             | 28.1             | 40.3                  |
| HFA     |                   | 56.6             | 32.0            | 24.5             | 28.5             | 41.7                  |
| Water   | THR <sup>5</sup>  | 61.3             | 69.4            | 21.1             | –                | –                     |
| DMSO    |                   | 59.3             | 68.3            | 20.8             | –                | –                     |
| HFA     |                   | 61.5             | 69.3            | 20.7             | –                | –                     |
| Water   | ASN <sup>6</sup>  | 52.6             | 38.4            | –                | –                | –                     |
| DMSO    |                   | 51.2             | 38.5            | –                | –                | –                     |
| HFA     |                   | 52.9             | 38.2            | –                | –                | –                     |
| Water   | LYS <sup>7</sup>  | 56.3             | 32.3            | 24.0             | 28.6             | 41.5                  |
| DMSO    |                   | 54.4             | 32.4            | 23.5             | 28.1             | 40.2                  |
| HFA     |                   | 56.2             | 32.2            | 23.8             | 28.5             | 41.6                  |
| Water   | TRP <sup>8</sup>  | 57.1             | 28.8            | –                | –                | –                     |
| DMSO    |                   | 55.2             | 28.8            | –                | –                | –                     |
| HFA     |                   | 57.2             | 28.7            | –                | –                | –                     |
| Water   | GLU <sup>9</sup>  | 55.7             | 29.9            | 34.6             | –                | –                     |
| DMSO    |                   | 53.5             | 29.0            | 31.5             | –                | –                     |
| HFA     |                   | 56.6             | 29.7            | 34.7             | –                | –                     |
| Water   | ASP <sup>10</sup> | 53.8             | 40.1            | –                | –                | –                     |
| DMSO    |                   | 51.2             | 37.7            | –                | –                | –                     |
| HFA     |                   | 53.6             | 40.0            | –                | –                | –                     |
| Water   | LYS <sup>11</sup> | 57.0             | 33.3            | 24.1             | 28.7             | 41.6                  |
| DMSO    |                   | 53.5             | 32.1            | 23.8             | 28.1             | 40.2                  |
| HFA     |                   | 56.9             | 33.2            | 23.9             | 28.4             | 41.5                  |

<sup>a</sup> Chemical shifts were obtained at 283 K in water and 298 K in DMSO and HFA.

MD. IRMA refinement of the structures generated by restrained MD simulation gave an average R-factor of 0.40 (Table 8). This is considered as reasonably good value in view of the sparse NMR data generally observed for small peptides [32]. For small peptides R-factors in the range of 0.30 to 0.50 have been reported [31, 33].

#### Conformation in DMSO

The NOESY spectrum in DMSO displays a significant number of nOe's suggesting a more or less rigid

structure for the peptide. Sequential nOe's like  $d_{\text{NN}}$  ( $i, i + 1$ ) and  $d_{\alpha\text{N}}$  ( $i, i + 1$ ) are observed for almost all the residues. Further, the  $d_{\alpha\text{N}}$  ( $i, i + 1$ ) peaks are more intense than the corresponding  $d_{\alpha\text{N}}$  ( $i, i$ ) peaks. Most of the inter-residue nOe's are between neighboring amino acids. All NH protons have a temperature coefficient that suggests absence of any intramolecular hydrogen bonding. Nearly all residues have a  $^3\text{J}_{\text{NH}\alpha}$  coupling constant greater than 7 Hz. These observations are typical signatures of a  $\beta$ -pleated sheet. The  $\alpha^{13}\text{C}$  CSI for all residues are in line with a  $\beta$ -sheet structure for the peptide.

Table 5. The torsion angles ( $\phi$ ,  $\Psi$ ,  $\chi$ ) averaged over the trajectory of 25 structures obtained in simulation S1<sup>a,b</sup>

| Residue           | $\phi$          | $\Psi$                     | $\chi_1$       | $\chi_2$         | $\chi_3$        | $\chi_4$ | $\chi_5$                  |
|-------------------|-----------------|----------------------------|----------------|------------------|-----------------|----------|---------------------------|
| LEU <sup>1</sup>  | –               | –176(0), 106(0),<br>164(0) | –173(0), 73(1) | –179(1), 174 (0) | –177(0), –57(1) | –        | –                         |
| ASN <sup>2</sup>  | –173(2), –69(0) | 169(1)                     | 50(5)          | 94(3)            | –178(1)         | –        | –                         |
| PRO <sup>3</sup>  | –76(1)          | –179(1)                    | –34(0)         | 41(0)            | –               | –        | –                         |
| LYS <sup>4</sup>  | –171(1)         | 105(2)                     | –177(1)        | –61(3)           | –171(0)         | –172(2)  | –173(0), –54(1),<br>63(1) |
| THR <sup>5</sup>  | –158(0)         | 117(5)                     | –54(0)         | –61(2)           | –               | –        | –                         |
| ASN <sup>6</sup>  | –100(8)         | 167(3)                     | –64(1)         | –82(1)           | –180(5)         | –        | –                         |
| LYS <sup>7</sup>  | –60(0)          | –24(2)                     | –150(6)        | –62(9)           | 90(2)           | –136(14) | –62(0), 57(0)             |
| TRP <sup>8</sup>  | –91(1)          | –12(2)                     | 55(1)          | 77(1)            | –               | –        | –                         |
| GLU <sup>9</sup>  | –82(1)          | 63(2)                      | –69(1)         | 119(3)           | 23(3)           | –        | –                         |
| ASP <sup>10</sup> | –176(1)         | –99(8)                     | 67(2), 161(0)  | 51(1), 143(0)    | –               | –        | –                         |
| LYS <sup>11</sup> | –173(0)         | –                          | –32(2)         | 67(1)            | 38(1)           | –69(1)   | –171(4), 67(3)            |

<sup>a</sup> Torsions are in degrees.<sup>b</sup> Numbers in parentheses are standard deviation values.Table 6. The torsion angles ( $\phi$ ,  $\Psi$ ,  $\chi$ ) averaged over the trajectory of 25 structures obtained in simulation S2<sup>a,b</sup>

| Residue           | $\phi$   | $\Psi$  | $\chi_1$       | $\chi_2$                 | $\chi_3$                 | $\chi_4$       | $\chi_5$                  |
|-------------------|----------|---------|----------------|--------------------------|--------------------------|----------------|---------------------------|
| LEU <sup>1</sup>  | –        | 134(0)  | –59(0), 49(2)  | 60(1), 176(0)            | –62(6), 52(0)            | –              | –                         |
| ASN <sup>2</sup>  | –113(1)  | 127(2)  | 179(1)         | 84(1)                    | 177(0)                   | –              | –                         |
| PRO <sup>3</sup>  | –85(0)   | 107(1)  | 37(0)          | –8(0)                    | –                        | –              | –                         |
| LYS <sup>4</sup>  | –123(11) | 106(1)  | –68(0), 178(1) | –59(1), 178(2)           | 62(2), 179(0)            | –62(1), 178(0) | –63(3), 66(7)             |
| THR <sup>5</sup>  | –106(0)  | 109(1)  | 55(1)          | 58(1)                    | –                        | –              | –                         |
| ASN <sup>6</sup>  | –109(1)  | 132(1)  | 53(2)          | –85(1)                   | –172(1)                  | –              | –                         |
| LYS <sup>7</sup>  | –127(3)  | 121(10) | –44(1), 57(2)  | –176(2)                  | –177(2)                  | –64(3), 63(2)  | –178(1), –59(2),<br>62(3) |
| TRP <sup>8</sup>  | –120(13) | 133(3)  | –34(2), 29(3)  | –87(3), 94(2)            | –                        | –              | –                         |
| GLU <sup>9</sup>  | –113(9)  | 123(12) | –62(5)         | –179(1), –48(3)<br>75(1) | –173(1), –67(2)          | –              | –                         |
| ASP <sup>10</sup> | –132(0)  | 132(2)  | –170(1)        | –163(1)                  | –179(2)                  | –              | –                         |
| LYS <sup>11</sup> | –112(9)  | –       | –117(3)        | –71(2), 60(3),<br>175(9) | –66(2), 62(3),<br>175(6) | 62(4), 177(3)  | –176(5), –65(3),<br>62(3) |

<sup>a,b</sup> See footnotes under Table 5.

Most of the restraints were satisfied in all the simulated structures with very few violations. The average  $\phi$ ,  $\psi$  values for the 25 structures (Table 6) fall in the range seen for  $\beta$ -pleated structures (Figure 3). The side chain conformation picture is slightly different from that seen in water. Many of the side chain torsions tend to adopt two different values.

Constraint free minimization of the structures obtained by MD simulation, maintained the  $\beta$ -pleated structure. The deviation in the two sets of structures, (with and without restraints), is 1.1 RMSD for the backbone atoms.

IRMA refinement of the structures generated by restrained MD simulation gave an average R-factor of 0.43 (Table 8).

#### Conformation in HFA

A number of both short and long range inter-residue nOe's have been observed, these being  $i - i + 1$  ( $\alpha$ H-NH, NH-NH,  $\beta$ H-NH), medium range peaks like  $i - i + 2$  (NH-NH),  $i - i + 3$  ( $\alpha$ H-NH) and long range  $i - i + 4$  ( $\alpha$ H-NH) peaks. The pattern of sequential, medium and long range nOe's, suggest the presence of a helical structure for a significant population of the

Table 7. The torsion angles ( $\phi$ ,  $\Psi$ ,  $\chi$ ) averaged over the trajectory of 25 structures obtained in simulation S3<sup>a,b</sup>

| Residue           | $\phi$ | $\Psi$ | $\chi_1$        | $\chi_2$        | $\chi_3$       | $\chi_4$        | $\chi_5$                  |
|-------------------|--------|--------|-----------------|-----------------|----------------|-----------------|---------------------------|
| LEU <sup>1</sup>  | –      | –47(1) | –160(2), 173(3) | –66(2), 63(4)   | –169(5), 64(3) | –               | –                         |
| ASN <sup>2</sup>  | –56(1) | –35(1) | –46(3), 34(2)   | –153(3), 161(7) | –2(0), 171(2)  | –               | –                         |
| PRO <sup>3</sup>  | –64(2) | –40(3) | –41(1)          | 39(1)           | –              | –               | –                         |
| LYS <sup>4</sup>  | –78(1) | –27(4) | 77(2), 102(4)   | 79(7)           | –150(1), 40(3) | –131(3), 148(6) | –63(2), 64(3),<br>178(1)  |
| THR <sup>5</sup>  | –69(4) | –48(2) | 64(1)           | 76(4)           | –              | –               | –                         |
| ASN <sup>6</sup>  | –71(2) | –51(5) | –71(3), 164(4)  | –85(3), 63(4)   | –160(1), 1(0)  | –               | –                         |
| LYS <sup>7</sup>  | –78(6) | –31(3) | –69(3)          | –51(12)         | –58(2), 116(5) | –83(3), 112(3)  | –156(3), 52(4),<br>175(6) |
| TRP <sup>8</sup>  | –74(2) | –43(4) | –173(4), –75(3) | –137(3), –74(4) | –              | –               | –                         |
| GLU <sup>9</sup>  | –74(4) | –42(5) | –75(4)          | 66(8)           | –63(3), 110(4) | –               | –                         |
| ASP <sup>10</sup> | –71(1) | –28(2) | 79(7)           | –151(3), –5(2)  | –              | –               | –                         |
| LYS <sup>11</sup> | –59(7) | –      | 53(9)           | 62(2)           | –79(7)         | 4(2), 65(4)     | –66(2), 54(1),<br>168(4)  |

<sup>a,b</sup> See footnotes under Table 5.

Table 8. Summary of experimental restraints and statistical analysis of family of structures generated by simulated annealing

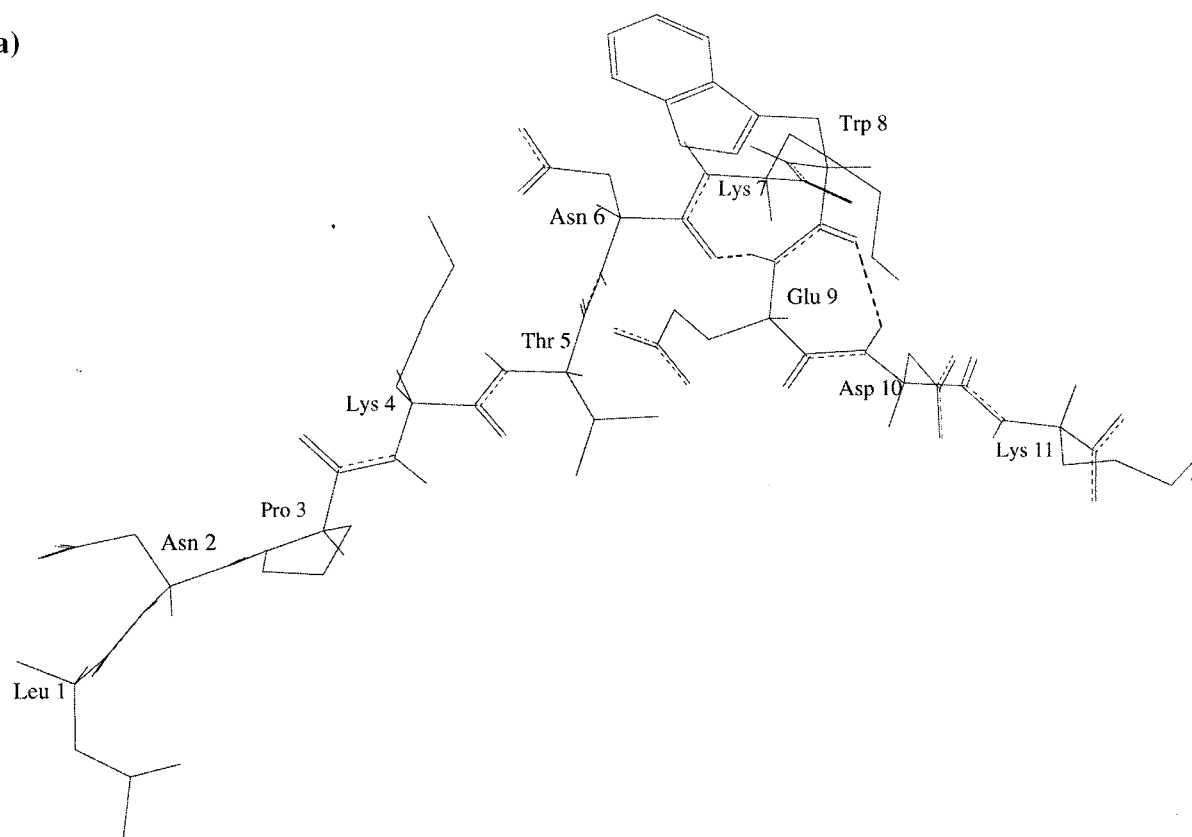
| Parameters                                          | Values in different solvents |                  |                  |
|-----------------------------------------------------|------------------------------|------------------|------------------|
|                                                     | Water (S1)                   | DMSO (S2)        | HFA (S3)         |
| <b>Distance restraints</b>                          |                              |                  |                  |
| All                                                 | 48                           | 70               | 78               |
| Intra-residue                                       | 27                           | 52               | 42               |
| Inter-residue                                       | 21                           | 18               | 36               |
| Sequential                                          | 16                           | 18               | 19               |
| Medium range                                        | 5                            | –                | 17               |
| Long range                                          | –                            | –                | –                |
| <b>Fractional Avg. violation/constraint</b>         | 0.021 $\pm$ 0.01             | 0.042 $\pm$ 0.02 | 0.071 $\pm$ 0.04 |
| <b>Avg. no. of violations/structure<sup>a</sup></b> | 0.04                         | 0.12 $\pm$ 0.02  | 0.16 $\pm$ 0.03  |
| <b>RMSD 's with avg. structure:</b>                 |                              |                  |                  |
| <b>Backbone atoms</b>                               |                              |                  |                  |
| Maximum                                             | 0.852                        | 0.964            | 0.467            |
| Minimum                                             | 0.413                        | 0.452            | 0.314            |
| Average                                             | 0.49 $\pm$ 0.27              | 0.67 $\pm$ 0.33  | 0.35 $\pm$ 0.14  |
| <b>Average pair-wise RMSD</b>                       | 0.58 $\pm$ 0.33              | 0.88 $\pm$ 0.44  | 0.47 $\pm$ 0.23  |
| <b>IRMA: R-factor</b>                               | 0.40                         | 0.43             | 0.48             |

<sup>a</sup> A  $\pm$  0.2 Å difference from the imposed distance restraint was considered a violation.

peptide in HFA. The low temperature coefficients for almost all the amide protons (Table 3) further support this structure.  $^3J_{\text{NH}\alpha}$  coupling constant in the range of 4.0 to 5.0 would further strengthen a helical structure for the peptide, however, these could not be extracted for most of the residues, either from the 1D or the DQF-COSY spectra, due to strong overlap of many peaks.

The Garnier/Osguthorpe/Robson secondary structure prediction method indicates the probability of a helical structure for the Lys<sup>7</sup> – Lys<sup>11</sup> segment of the peptide. Other evidences for a helical structure are the CSI values for  $\alpha\text{H}$  and  $\alpha^{13}\text{C}$ . The  $\alpha\text{H}$  chemical shifts in HFA show a negative deviation, and the  $\alpha^{13}\text{C}$  chemical shifts have a positive deviation from the random coil value.

a)



b)

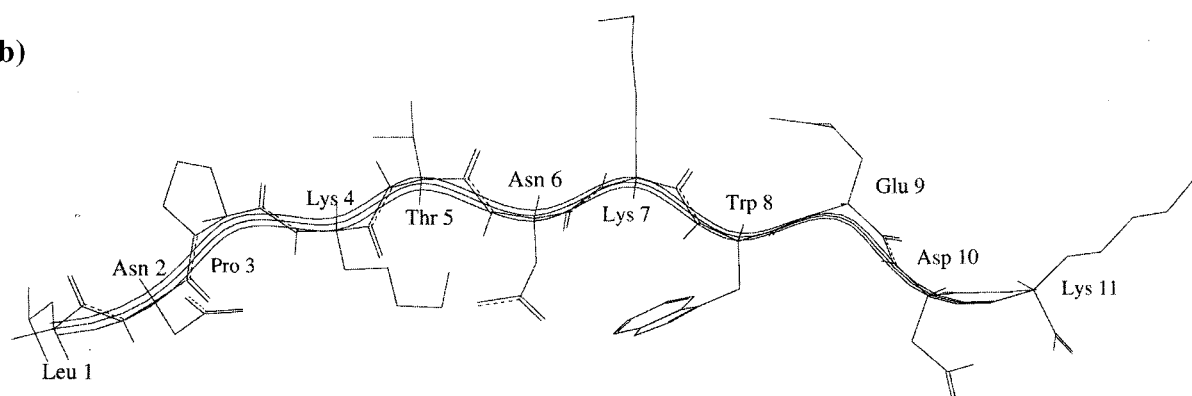


Figure 3. Structure of Asp f1 (10–20) in (a) water, (b) DMSO and (c) HFA as obtained by MD simulations incorporating NMR data.

MD simulations produced an *ensemble of structures* with average pair-wise RMSD of 0.47. The average phi and psi values of the resultant structures (Table 7) show a  $\alpha$ -helix stretched over all the residues (Figure 3). The quality of the simulated structures is judged by a negligible violation on the imposed distance restraints. Constraint free minimization of these structures, maintained the overall  $\alpha$ -helical structure,

indicating that the  $\alpha$ -helix is indeed a stable structure. The average RMSD between the two sets of structures was found to be 0.95. The average IRMA R-factor for the simulated structures is 0.48 (Table 8).

c)

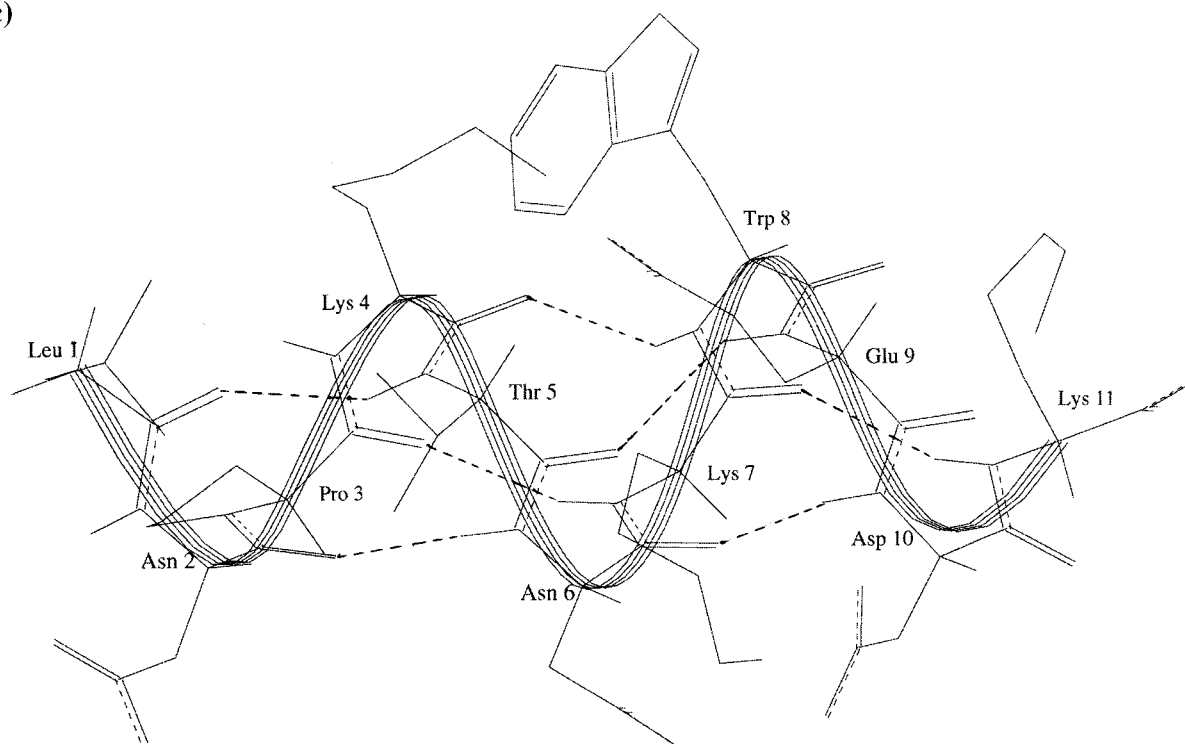


Figure 3. continued.

## Conclusion

The conformation of an eleven amino acid immunodominant epitope of Asp f 1 protein secreted by *A. fumigatus* has been investigated by 2D-NMR and molecular modeling methods. The peptide has been found in various structures depending on the medium in which it has been studied. Thus in DMSO, a  $\beta$ -pleated structure has been seen, while in water at pH 4.0, the major structural feature is a Type I  $\beta$ -turn at Asn<sup>6</sup>-Lys<sup>7</sup>-Trp<sup>8</sup>-Glu<sup>9</sup>. HFA is seen to induce an  $\alpha$ -helical structure across the entire length of the peptide. If we regard structure in HFA as being closely related to biologically active conformations, then it may be said that for this peptide the  $\alpha$ -helix structure may be relevant to its antibody binding. Other such peptides are being synthesized and studied to verify this hypothesis.

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