



ENHANCED IMMUNOREACTIVITY AND PREFERENTIAL HETERODIMER FORMATION OF REASSOCIATED *Fel d I* RECOMBINANT CHAINS

KATHLEEN M. KEATING, DEBRA B. SEGAL, SANDRA J. CRAIG,
ANNELIESE K. NAULT, VICTOR SEMENSI, ANDREW S. WASSERMAN,
CATHERINE M. COUNSELL and JULIAN F. BOND*

ImmuLogic Pharmaceutical Corporation, 610 Lincoln Street, Waltham, MA 02154, U.S.A.

(First received 28 April 1994; accepted in revised form 21 September 1994)

Abstract—In this study we have addressed the question of whether reassociating the two recombinant protein chains that comprise the major cat dander allergen, *Fel d I*, would change the overall IgE and allergic patient T cell immunoreactivity compared to the native molecule. To accomplish this, the chains were combined under reducing and denaturing conditions, then allowed to reassociate by dilution and extensive dialysis against a physiological buffer. An initial examination of the reaction products using quantitative capture ELISA demonstrated comparable reactivity to *Fel d I*. Further analysis, using a pool of cat allergic patient plasma, showed that the products of the reassociation reaction (r*Fel d I*) also possessed an enhanced IgE binding capacity. Depletion ELISA results gave only a 5% difference in reactivity between r*Fel d I* and the native protein versus a 20% difference with the mixture of the two chains. Comparative secondary T cell stimulation assays were subsequently performed using cat allergic patient peripheral blood lymphocytes. Here the results demonstrated no loss of reactivity with the reassociated chains as compared to *Fel d I* or the two mixed recombinant chains. To biochemically characterize the products of the reassociation reaction we have performed reverse phase HPLC and then analysed the isolated fractions by mass spectrometry. It was clear from these results that like the native *Fel d I*, the products of the reassociation reaction favored heterodimer formation, with no homodimer being detected. This implies that the reassociated protein chains had preferentially adopted a native-like conformation.

Key words: heterodimer formation, mass spectral analysis.

INTRODUCTION

Fel d I is the major allergen produced by domestic cats and, as such, it is the predominant human IgE binding component in cat hair extracts (Ohman *et al.*, 1974). It is this IgE reactivity that causes allergic and asthmatic symptoms in cat sensitive individuals, who comprise approximately 10% of the population in the United States (Friedhoff *et al.*, 1984). *Fel d I* is a stable glycoprotein with an apparent molecular weight of 35 kD under non-reducing conditions (Leitermann *et al.*, 1984). From recent sequencing data, generated at both the protein and cDNA levels, *Fel d I* has been shown to be a heterodimer formed from two separate gene products of 8 and 10 kD in an anti-parallel configuration (Morgenstern *et al.*, 1991). At present, *Fel d I* is the only allergen known to have this type of structure.

The two recombinant chains of *Fel d I* have been expressed separately in *E. coli* and purified (Rogers *et al.*, 1993). The immunochemical properties of these proteins were compared to the native molecule. The initial

analyses demonstrated a significant IgE binding capacity for both chains and a T cell stimulation profile similar to that of the affinity purified form from cat dander (Rogers *et al.*, 1993). The separate rchains, however, did have less IgE reactivity as compared to the native form of the protein (Bond *et al.*, 1993). We have undertaken experiments to improve this IgE reactivity by reassociating the purified rchains 1 and 2 to generate r*Fel d I*. In addition, we have begun delineating the potential use of this reagent for analytical and diagnostic purposes.

Though the optimal conditions for refolding proteins can vary significantly, some general approaches have emerged (Jaenicke *et al.*, 1989). A number of dimeric proteins have been reassociated and refolded *in vitro*, with the majority of them being homodimers (Jaenicke, 1987). In addition, the successful refolding of several heterodimeric proteins has also been reported in the past several years. In the present study we have mixed the two rchains of *Fel d I* in a straightforward denaturation step followed by dilution and dialysis in an attempt to recreate properties of the native structure.

Though the chain 1 sequence of *Fel d I* does share some structural homology to rabbit uteroglobin, a phospholipase A₂ inhibitor (Miele *et al.*, 1990), the function of this allergenic protein is currently unknown. For this study we have chosen to use defined parameters of allergic patients'

* Author to whom correspondence should be addressed.

Abbreviations: *Fel d I*, the major allergen from the domestic cat; rchain 1, recombinant chain 1 of *Fel d I*; rchain 2, recombinant chain 2 of *Fel d I*; r*Fel d I*, reassociated chains of *Fel d I*; PHP, pooled human plasma.

in vitro immune response to analyse the refolded material in comparison with native *Fel d I*. The first method of examination was a quantitative capture ELISA for comparison of the refolded product to native *Fel d I*. Allergic patient IgE reactivity and T cell proliferative responses comprise the analyses of *in vitro* immunoreactivity. Mass spectral properties on HPLC fractions of the reaction products were determined to better define the structural changes concomitant with the refolding of *rFel d I*.

MATERIALS AND METHODS

Protein purification and reassociation

Native *Fel d I* was purified by immunoaffinity chromatography essentially as described previously (Chapman *et al.*, 1988), except that the allergen source was cat hair extract and 100 mM glycine (pH 2.5) was used for eluting the column in place of 4 mM hydrochloric acid. Purification of the recombinant protein chains was performed as detailed by Rogers *et al.* (1993). The proteins were lyophilized after the final reverse phase HPLC purification step. *Lol p* (rye grass) pollen (Greer Laboratories, Lenoir, NC) was extracted with PBS (10 mM phosphate buffer with 0.15 M NaCl, pH 7.2) and filtered through a 0.45 μ m filter. *Amb a I* was purified from ragweed pollen (Greer Laboratories, Lenoir, NC) essentially by the procedure described by King *et al.* (1964). These pollen allergens were used as non-*Fel d I* cross-reactive controls for IgE binding.

For the reassociation reaction, the recombinant protein chains were mixed together at 1 mg/ml each in 6.6 M urea, 50 mM 1,4-dithiothreitol, 0.03 M NaCl and 2 mM phosphate buffer (pH 7.2). This solution was heated to 55°C for 3 min with shaking in order to completely unfold and disassociate the protein monomers. The protein solution was diluted to twice its original volume with water and allowed to cool to room temperature. The solution was then extensively dialysed for at least 24 hr with a 3500 Dalton cutoff membrane (Spectrum, Houston, TX) against PBS at 25°C. The protein preparations were then used in the ELISA assays to assess the degree of the reassociation.

Capture ELISA with monoclonal and polyclonal antibodies

Immulon II 96-well microtitre plates (Dynatech, Chantilly, VA) were coated at 50 ng/well with the anti-*Fel d I* chain 1 specific mouse monoclonal antibody, 1G9. Plates were washed three times with PBS containing 0.05% Tween-20 (PBS/Tween) at this point and between all subsequent incubation steps. Non-specific binding to the plates was blocked with 0.5% gelatin in PBS containing 1 mg/ml polyvinylpyrrolidone (Sigma, St Louis, MO) for 1 hr. The protein solutions, diluted in PBS as indicated, were added at 100 μ l/well for 30 min. The captured protein was detected by the addition of rabbit no. 426 serum (diluted 1:10,000 in PBS/Tween) for 30 min, followed by HRPO anti-rabbit IgG (Kirkegaard-Perry Laboratories (KPL), Gaithersburg, MD) at 25 ng/well. Rabbit serum no. 426, a high titer polyclonal antiserum, was generated by one CFA and one IFA immunization with native affinity purified *Fel*

d I. Four post-boost bleeds combined after screening had determined similar antibody titers. The ELISA assay was developed with the addition of TMB reagent mix (KPL) and color development stopped with 1 M phosphoric acid. The absorbance of the wells at 450 nm was read on a Dynatech plate reader. Purified ragweed allergen, *Amb a I*, was included as a negative control to demonstrate *Fel d I* specificity.

ELISAs with pooled human plasma

Plasma samples from 18 cat extract skin test positive patients with reactivity to multiple allergens were pooled, denoted PHP. Direct IgE binding assays were performed on Immulon II plates coated with 500 ng/well of the indicated antigen, except for the mixture of chains 1 and 2, which was coated at 500 ng each/well. Plates were incubated overnight at 4°C. The plates were washed with PBS/Tween three times between each step. The unbound antigen was removed and the wells blocked with 300 μ l/well 1% BSA (Pierce, Rockford, IL) in PBS/Tween for 1 hr at room temperature. Serially diluted PHP was added in duplicate at 100 μ l/well and incubated overnight at 4°C. The plates were then incubated with biotinylated goat anti-human IgE (KPL) at a 1:2000 dilution for 1 hr at room temperature. The assay was developed as described for the capture ELISA above except that an extra incubation with streptavidin-HRPO, at 1:10,000, was required. For the depletion assay, PHP was pre-incubated overnight on microtiter wells coated with the same antigen set used in the direct assay (as shown in Fig. 2A). Following this incubation the samples in the individual wells were collected and re-incubated, again overnight, on a *Fel d I* coated plate. For the competition assay, fresh PHP samples were mixed with equal volumes of serially diluted antigen or just PBS/Tween and incubated overnight before addition to the *Fel d I* coated plate. The ELISAs were then performed as described for the direct assay.

T cell proliferation assays

Peripheral blood mononuclear leukocytes were isolated from cat allergic patients essentially as described previously (Rogers *et al.*, 1993), using Lymphocyte Separation Media (Organon Teknica, Durham, NC). T cell lines reactive to *Fel d I* were established by stimulation of 2×10^6 mononuclear leukocytes/ml with 0.84 μ M each of the recombinant protein chains (6.5 μ g/ml rchain 1 and 8.5 μ g/ml rchain 2) for 6 days. Cells were grown in culture 20 to 22 days before being assayed. The proliferation assays were performed by incubating cells with 0.06, 0.28 or 1.40 μ M antigens (1, 5 and 25 μ g/ml) and irradiated antigen presenting cells for 3 days. The cultures were then pulsed with tritiated thymidine (ICN, Costa Mesa, CA) overnight. The cells were harvested onto glass fiber filters and the amount of tritium incorporation was measured by scintillation counting.

Reverse phase HPLC separation

Reassociated *Fel d I* was applied to a 10 $\times$ 250 mm C4 column with a 10 μ m particle size and 300 Å pore size

(Vydac, Hesperia, CA). The column was run on a Waters 4000 HPLC system at 5 ml/min with 0.1% trifluoroacetic acid in the solvents. Solvent A was HPLC grade water and solvent B was HPLC grade acetonitrile (both from Baxter, Miami, FL). The column was run isocratically at 5% B for 8 min followed by a 19 min linear gradient to 80% B and then held at 80% B for an additional 3 min. Fractions were collected manually.

Mass spectral analysis

Native *Fel d* I was at 0.3 mg/ml in PBS. Aliquots of lyophilized fractions (< 100 µg each) from the chromatography of the reassociated *Fel d* I were dissolved in 100 µl of 60% acetonitrile in 0.1% trifluoroacetic acid. These stocks were diluted 10- and 100-fold. Aliquots of each sample and dilution were loaded onto spots on the sample disk and mixed with sinipinic acid (Sigma, St Louis, MO) for the native protein and alpha-cyanohydroxycinnamic acid (Sigma) for the recombinant fractions. They were then analysed on a VG ToFSpec matrix-assisted laser-desorption mass spectrometer (Fisons Instruments, Manchester, U.K.). The instrument was calibrated using an internal standard of trypsinogen (Sigma) for the native protein and an external standard of cytochrome C from horse heart (Sigma) for the recombinant proteins.

RESULTS

Capture ELISA quantitation and IgE binding analysis

Our first examination of the reassociation reaction products was a comparison with the native protein, the individual recombinant chains, and a mixture of the two recombinant chains using a quantitative sandwich ELISA (Fig. 1). As expected, the mixture of the two chains shows a binding signal equivalent to the chain 1 alone. However, the reassociation reaction products gave a signal comparable to the native protein and this was much greater than that of the mixture.

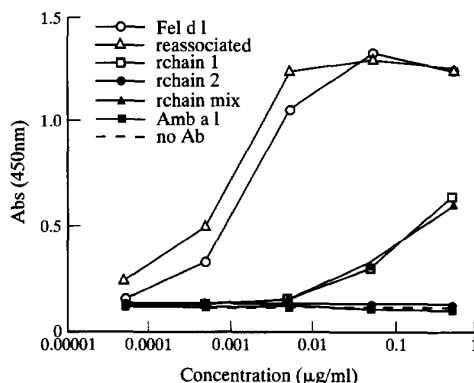


Fig. 1. Quantitative capture ELISA assay. The preparations of native and recombinant *Fel d* I were captured by mouse monoclonal antibody 1G9 coated wells, specific for chain 1. The added protein quantitation shown for the mix is for each chain. The detection of the captured antigen was dependent upon anti-*Fel d* I polyclonal rabbit antiserum, no. 426, followed by incubation with HRPO anti-rabbit IgG second antibody.

We analysed the human IgE immunoreactivity to the reassociated r*Fel d* I using several different ELISA based formats. Figure 2A shows the results of a direct binding ELISA using a pool of plasma samples from cat allergic patients, PHP, as the first antibody. Knowing that some of the patients comprising the PHP are skin test positive for grass allergens we have used the grass allergen, *Lol p* I, as a control in these assays. With this direct assay there was no apparent difference between the native protein, the mixed recombinant proteins, or the putatively refolded proteins. Nevertheless, the results of assays such as this one, wherein the antigen is attached to a solid matrix, can mask underlying reactivity differences in affinity or avidity and we feel that they are only reliable for relative measures of positive or negative binding.

Using *Fel d* I as the coating antigen (Fig. 2B) we measured the proportion of *Fel d* I specific IgE remaining in the PHP after depletion on the same antigen set used for Fig. 2A. Unlike the direct binding ELISA, here we do see a difference in the capacity of the various antigens for the depletion of *Fel d* I reactive IgE. Native *Fel d* I, as expected, removes the greatest amount of reactivity against itself followed by the reassociated preparation and then the mixture of the two chains. There is no decrease seen with depletion on either the PBS or negative control *Lol p* extract coated wells. Two aspects of this type of depletion assay relate to a relative measure of the affinity of this polyclonal IgE preparation for *Fel d* I. The first aspect is that the reactivity shown in Fig. 2B was analysed following IgE equilibration of bound versus unbound antibody. The second aspect is that in both the first and second incubation the antigen is in the same solid phase configuration (i.e. coated on ELISA wells). By comparing the curves in terms of PHP dilution needed for 50% maximal signal one can get a rough estimate of relative reactivity. In this manner we calculate that approximately 85, 80 and 65% of *Fel d* I specific IgE is removed following depletion on native *Fel d* I, reassociated r*Fel d* I, or the chains mixed, respectively.

A competition ELISA was performed as a solution phase analysis of IgE antibody reactivity and the results are shown in Fig. 2C. A different preparation of the same PHP applied in the two former assays was incubated at a constant concentration with increasing amounts of the various competing antigens. These incubation solutions were then added to ELISA wells coated with native *Fel d* I and the binding signals were measured. In agreement with the depletion assay format, the antigen which competed most effectively was the native protein. The results in Fig. 2C show that the reassociated chains gave significantly better competitive inhibition than the mixture of the two chains. These competition assays are also carried out at antibody/antigen binding equilibrium, however, affinity calculations based on this solution versus solid phase recognition can be misleading. These three ELISA based assays for human IgE reactivity to the *Fel d* I proteins have been performed several times with the same or different PHP lots

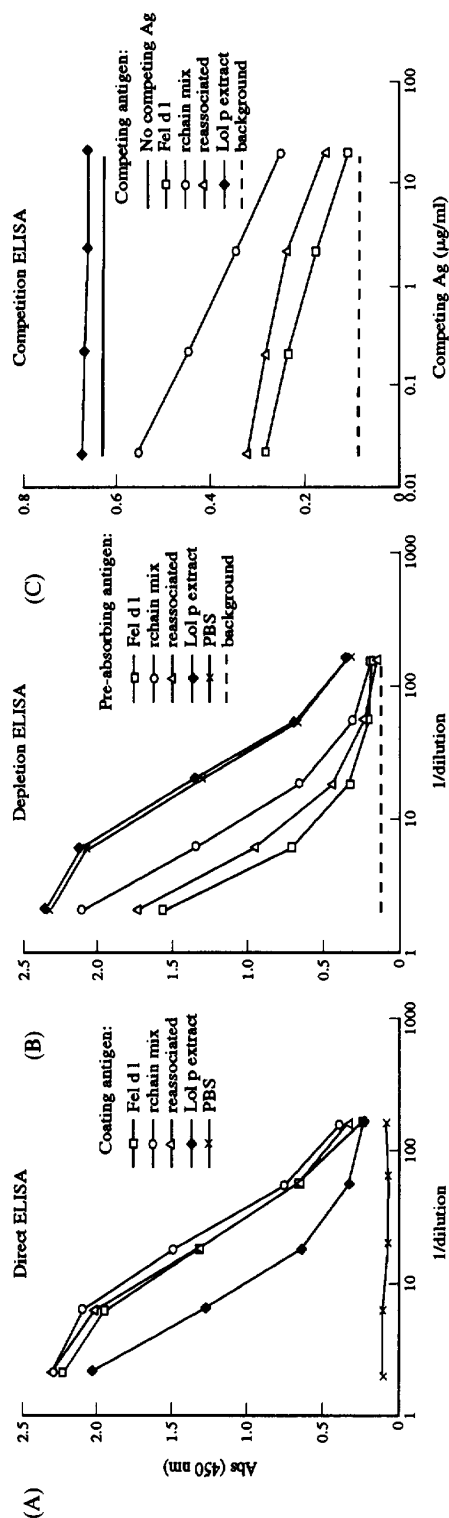


Fig. 2. Immunoreactivity of human IgE to the native and recombinant forms of *Fel d I* using (A) direct; (B) depletion; and (C) competition ELISA. For the direct and depletion ELISAs, wells were coated with the various antigens as indicated. The human IgE remaining in the wells following the first depletion incubations were all reanalysed by direct ELISA on *Fel d I* to generate the results shown in B. The competition analysis included a pre-incubation step with the various antigens in solution prior to direct binding with *Fel d I* coated wells. The grass allergen preparation, *Lol p* extract, was included as an IgE reactive but not *Fel d I* cross-reactive control. Incubations on coated wells with no first antibody are indicated as background.

showing similar profiles. Twenty-four individual patient plasma samples have also been tested in the competition assay. In all cases the reassociated r*Fel d I* was better at competing IgE binding to native *Fel d I* than the mix of recombinant chains (data not shown).

T cell proliferation data

Figure 3 shows the results of human cat allergic patient T cell responses in secondary cultures that had been primed with recombinant *Fel d I*. The secondary cultures were stimulated with the antigens as indicated in Fig. 3, and then tested for ³H-thymidine uptake as a measure of proliferation. These two representative assays show that there was little difference in response to these proteins whether recombinant or native *Fel d I* is used as the priming antigen. This is the corollary of the previously published result which demonstrated responses to the individual chains after priming with the native allergen (Rogers *et al.*, 1993). Figure 3 also shows that there was no significant difference in the response to the mixture of the two recombinant chains versus the reassociated recombinant protein. In some cases, native *Fel d I* in a secondary assay does not stimulate the cells as well as the recombinant protein and the reason for this is unknown. However, the fact that the native molecule is glycosylated and partially proteolyzed (Bond *et al.*, 1993) may play a role in how it is recognized and processed at the level of antigen presentation.

Biochemical analysis

Having established that the r*Fel d I* has greatly increased the specific IgE reactivity without affecting the T cell epitopes, we then undertook biochemical characterization of this reaction product. The reverse phase HPLC elution profiles of the individual rchains has been well established (Rogers *et al.*, 1993) and this procedure is the method of choice for their purification. We therefore applied this method for fractionating and analysing the products of the r*Fel d I* reassociation reaction. The chromatogram in Fig. 4 shows three major HPLC peaks in the reassociated material and the eluted fractions, which were analysed further, and are denoted 4, 7 and 10. The large peak in the middle, eluted as fraction 7, is not observed on HPLC with just a mixture of the individual chains. The three eluted peaks were then separately analysed by mass spectrometry in comparison to native *Fel d I*. Figure 5, panel A, shows the mass spectrum of native *Fel d I* as a broad peak with an average molecular weight of 19,600. The lack of a sharp, discrete peak is consistent with that expected for a glycosylated protein (Chait and Kent, 1992) and the mass is equivalent to a heterodimer of the two gene products. The other panels (B–D) compare mass spectral analyses of the three HPLC fractions. By this method fractions 4, 7 and 10 were shown to have the representative masses of rchain 1, reassociated rchain 1 and 2, and rchain 2, respectively. Fraction 7, containing the reassociated rchains, comprised 33% of the area in the chromatogram and the mass is specific for the heterodimeric form. Mass analysis of the fractions between 7 and 10 (Fig. 4) contained free chain 2 and some

heterodimer (data not shown). The accuracy of this mass spectral analysis was within 0.4% of the theoretical masses of rchain 1 (8016), rchain 2 (10,277) and the heterodimer (18,338) (Fig. 5). In panel C the doubly and triply charged species are observed and in panel D only the doubly charged form is seen (Fig. 5). Although the conditions used in the reverse phase chromatography (Fig. 4) might be expected to dissociate most non-covalent interactions (see Materials and Methods) further experimentation is necessary for a definitive demonstration of covalent bonding. This apparent preferential heterodimer formation of the reassociated rchains of *Fel d I* has been repeated in three separate experiments (data not shown).

DISCUSSION

We have generated r*Fel d I* by combining the separate recombinant chains in a refolding protocol. A quantitative capture ELISA demonstrated comparable reactivity for the reassociated recombinant chains and the native protein that was greater than reactivity to a mixture of the two rchains. This implied that the reaction had successfully linked the two chains together because the capturing monoclonal antibody used in the assay was directed against chain 1. Furthermore, since IgE can react with both r*Fel d I* chains separately (Bond *et al.*, 1993),

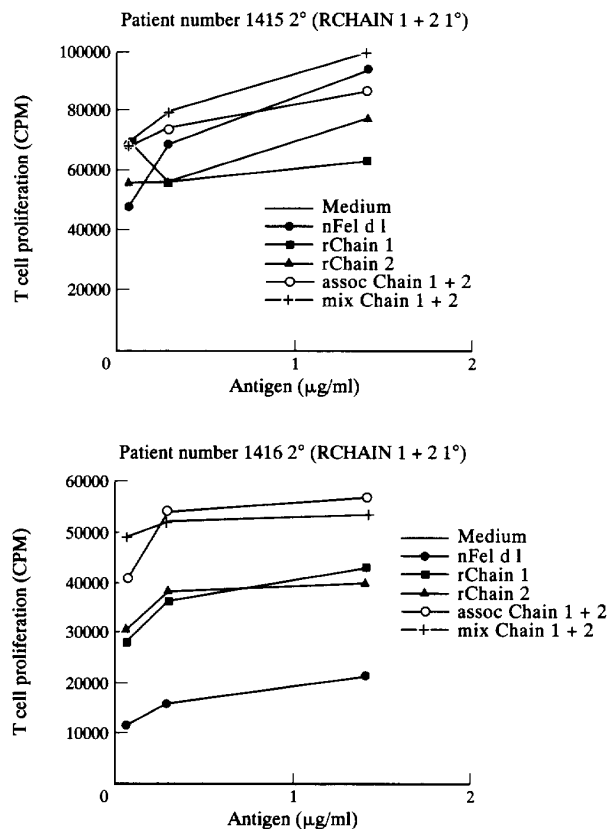


Fig. 3. Human T cell proliferation to native and recombinant *Fel d I*. Secondary T cell cultures from two patients primed with a mixture of the two chains of *Fel d I* were stimulated with either the priming antigen, native *Fel d I* or reassociated recombinant *Fel d I* and assayed by tritiated thymidine incorporation.

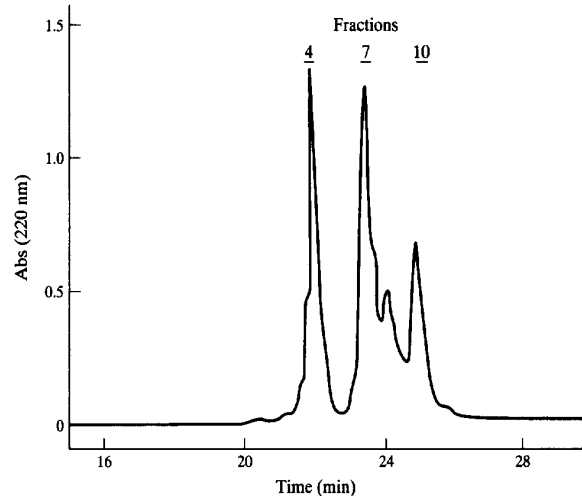


Fig. 4. Reverse phase HPLC chromatogram of the reassociation reaction products of recombinant *Fel d I*. The portion of the chromatogram shown is a linear gradient from 28 to 80% acetonitrile over 12 min followed by a 3 min isocratic gradient at 80% acetonitrile. The fractions numbered above the peaks were the ones subsequently analysed by mass spectrometry.

any increased binding of the reassociated product over a mixture of the two chains should reflect renaturation to a conformation similar to the native protein. However, the signal from our direct ELISA analysis could not distinguish any significant variation in IgE reactivity levels between the *Fel d I* preparations. We feel that the direct ELISA assay effectively tests the relative titer values of positive plasma samples but does not accurately reflect overall reactivity differences. It was with both the depletion and competition ELISA formats that we observed clear differences. The IgE reactivity results observed in the latter two assays suggested that the reassociated rchains gave a structure more similar to the native *Fel d I* molecule than a mixture of the two chains. The relationship of structural similarity to reactivity can be explained by an increased number of IgE epitopes, an increased affinity for existing epitopes, or both. The fact that native *Fel d I* shows greater IgE reactivity with both the depletion and competition assays than the reassociated rchains indicates that there are still epitopes which have not been fully regenerated. This corresponds to the observation that the reassociation reaction was not complete, as indicated by the HPLC profile and mass spectral analyses.

We next assayed T cell reactivity with the different preparations to test whether these epitopes, previously shown to be present in the native *Fel d I* and the individual rchains (Rogers *et al.*, 1993), were affected following the reassociation reaction. T cell epitopes are known to be comprised of linear stretches of amino acids recognized by T cell receptor molecules after proteolytic processing and presentation by the major histocompatibility (MHC) antigens on the surface of APCs. Therefore, the conformation of a protein, when it is taken up by an APC, would not be expected to affect peptide presentation

unless it were to effect processing. Our results showing that all three preparations of *Fel d I* protein had similar levels of reactivity imply few differences in their processing and presentation. It should be noted however, that these results were at the level of the whole protein as opposed to peptide for both *in vitro* T cell priming and secondary culture stimulation.

Our mass spectral analysis of native *Fel d I* shows the protein to have a broad spectral peak with an average mass of about 20 kD within a 4 kD range. This mass range is indicative of differentially glycosylated proteins (Chait and Kent, 1992) and in this case gives an average degree of glycosylation as 7% by weight. One N-linkage glycosylation site on *Fel d I* has been demonstrated at Asn residue 33 on chain 2 (Morgenstern *et al.*, 1991; Duffort *et al.*, 1991). Analysis of the reassociated material in

fraction 7 (Fig. 5) showed a mass equivalent to the heterodimer of the two chains. Since no homodimers of the recombinant *Fel d I* chains were detected, we believe that disulfide bond formation between monomers does not occur randomly. This preferential heterodimer formation is not unprecedented. The two chains of class II MHC protein have been shown to reassociate with very little homodimer formation (Dornmair and McConnell, 1990). It has also been shown that tropomyosin heterodimer formation is thermodynamically favored over the formation of the homodimers (Leher and Stafford, 1991). Other examples include coiled-coil proteins such as keratin (Hatzfeld and Weber, 1990) and leucine zipper proteins *fos* and *jun*, that can preferentially form heterodimers over homodimers by at least 1000-fold and in the latter case the configuration was anti-parallel

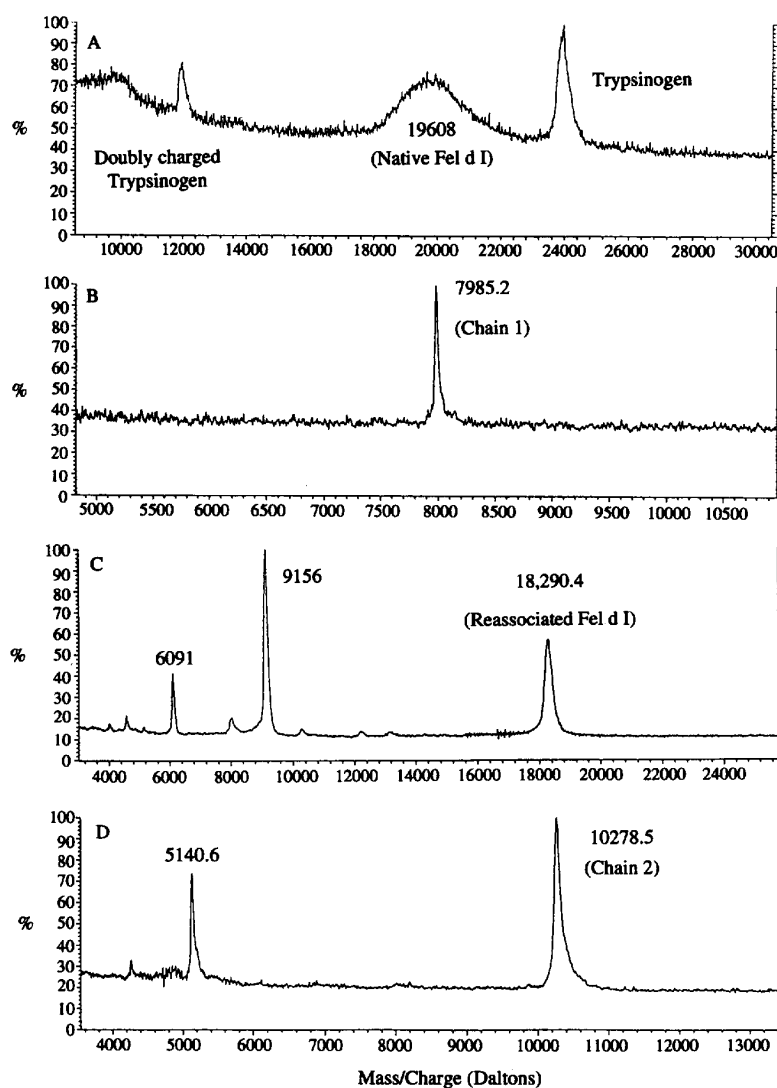


Fig. 5. Mass spectrometry of native (A) and reassociated recombinant *Fel d I* fractions (B–D). Panel A shows the mass spectra of native *Fel d I* with internal standards. Panels B, C and D show the mass spectra of fractions 4, 7 and 10 from the chromatogram shown in Fig. 4. Fraction 7 has a mass equivalent to the theoretical mass of a heterodimer of the two chains. Note that the ranges on the abscissae are different for each mass determination.

(O'Shea *et al.*, 1989). Native *Fel d I* appears to be a relatively stable protein with the two chains present in essentially equimolar ratios (Morgenstern *et al.*, 1991). The data here suggest that the free energy of r*Fel d I* folding preferentially favors the formation of a heterodimeric structure similar to that of the native molecule. This thermodynamically preferred structure may relate to the inherent stability of *Fel d I*. Comparative stability studies are underway in conjunction with determination of disulfide bonding. Further experimentation is also needed to optimize the conditions for reassociation and to improve a scale-up of the chromatographic purification step. We will then be able to further characterize the structure and activity of the product with affinity measurements of antibodies and epitope identification of IgE antibody reactivity. Our intent is to also map *in vitro* T cell responses to *Fel d I* peptides after priming with the native versus the reassociated recombinant chains. Any difference in T cell response profile to peptides in a secondary assay would imply that two separate priming antigens had unique structural properties which could relate to protein processing and presentation. Also, secondary structural analyses, including disulfide bonding as mentioned above, would provide a basis for the comparison of the structures of the native and reassociated recombinant proteins. If the product proves to be refolded into the same conformation as the native protein, as suggested by the immunoassay, it is possible that it could then replace the native protein in both functional and antibody binding assays.

Acknowledgements—We are grateful to Joyce Ho, Jeff Socolow and Meei-song Chen for supplying the native and recombinant *Fel d I* proteins and Dr Martin Chapman for contribution of the monoclonal antibody. We also acknowledge Dr Saryan and Dr Condemni for the supply of the clinical samples. We appreciate the contributions of Drs Mei-chang Kuo and Bruce Rogers to this work. We also wish to thank Drs Barbara Wallner, Gary Lazarus, Stephen Powers and Marion Kasaian for their critical review of this manuscript.

REFERENCES

- Bond J. F., Brauer A. W., Segal D. B., Nault A. K., Rogers B. L. and Kuo M.-c. (1993) Native and recombinant *Fel d I* as probes into the relationship of allergen structure to human IgE immunoreactivity. *Molec. Immun.* **30**, 1529–1541.
- Chapman M. D., Aalherse R. C., Brown M. J. and Platts-Mills T. A. E. (1988) Monoclonal antibodies to the major feline allergen *Fel d I*, II. Single step affinity purification of *Fel d I*, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess *Fel d I* exposure. *J. Immun.* **140**, 812–818.
- Chait B. T. and Kent S. B. (1992) Weighing naked proteins: Practical, high-accuracy mass measurement of peptides and proteins. *Science* **257**, 1885–1894.
- Dornmair K. and McConnell H. M. (1990) Refolding and reassembly of separate alpha and beta chains of class II molecules of the major histocompatibility complex leads to increased peptide binding capacity. *Proc. natn. Acad. Sci. U.S.A.* **87**, 4134–4138.
- Duffort O. A., Carreira J., Nitti G., Polo F. and Lombardero M. (1991) Studies on the biochemical structure of the major cat allergen *Felis domesticus*. *Molec. Immun.* **28**, 301–309.
- Friedhoff L. R., Meyers D. A. and Marsh D. G. (1984) A genetic-epidemiologic study of human immune responsiveness to allergens in an industrial population. *J. Allergy clin. Immun.* **73**, 490–499.
- Hatzfeld M. and Weber K. (1990) The coiled coil of *in vitro* assembled keratin filaments is a heterodimer of type I and II keratins: Use of a site-specific mutagenesis and recombinant protein expression. *J. cell. Biol.* **110**, 1199–1210.
- Jaenicke R. and Rudolf R. (1989) Folding proteins In *Protein Structure: A Practical Approach* (Edited by Creighton T. E.) IRL Press, Oxford, U.K.
- Jaenicke R. (1987) Folding and association of proteins. *Prog. Biophys. Mol. Biol.* **49**, 185–187.
- King T. P., Norman P. S. and Connell J. T. (1964) Isolation and characterization of allergens from ragweed pollen II. *Biochemistry* **3**, 458–468.
- Leher S. S. and Stafford W. F. III (1991) Preferential assembly of the tropomyosin heterodimer: Equilibrium studies. *Biochemistry* **30**, 5682–5688.
- Leitermann K. M. and Ohman J. L. (1984) Cat allergen I: Biochemical, antigenic, and allergenic properties. *J. Allergy clin. Immun.* **74**, 147.
- Miele L., Cordella-Miele E. and Mukherjee A. B. (1990) High level bacterial expression of uteroglobin, a dimeric eukaryotic protein with two interchain disulfide bridges, in its natural quaternary structure. *J. biol. Chem.* **265**, 6427–6435.
- Morgenstern J. P., Griffith I. J., Brauer A. W., Rogers B. L., Bond J. F., Chapman M. D. and Kuo M.-c. (1991) Determination of the amino acid sequence of *Fel d I*, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. *Proc. natn. Acad. Sci. U.S.A.* **88**, 9690–9694.
- Ohman J. L. Jr, Lowell F. C. and Bloch K. J. (1974) Allergens of mammalian origin III. Properties of a major feline allergen *J. Immun.* **113**, 1668–1677.
- O'Shea E. K., Rutkowski R., Stafford W. F. III and Kim P. S. (1989) Preferential heterodimer formation by isolated leucine zippers from Fos and Jun. *Science* **245**, 646–648.
- Rogers B. L., Morgenstern J. P., Garman R. D., Bond J. F. and Kuo M.-c. (1993) Recombinant *Fel d I*: expression, purification, IgE binding and reaction with cat allergic human T cells. *Molec. Immun.* **30**, 559–568.
- Vailes L. D., Li Y., Bao Y., DeGroot H., Aalherse R. C. and Chapman M. D. (1994) Fine specificity of B-cell epitopes on *Felis domesticus* allergen I (*Fel d I*): Effect of reduction and alkylation or deglycosylation on *Fel d I* structure. *J. Allergy clin. Immun.* **93**, 22–33.