

Epitope mapping of the cat (*Felis domesticus*) major allergen *Fel d* I by overlapping synthetic peptides and monoclonal antibodies against native and denatured *Fel d* I

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The major cat allergen *Fel d* I is a homodimer of which each monomer consists of two disulfide-linked polypeptide chains: chain 1 (70 amino acid residues) and chain 2 (92 amino acid residues). Twenty-one synthetic peptides of 14 amino acid residues length, overlapping by seven residues and spanning the entire sequence of both chains, were synthesized. These peptides were coupled to CNBr-activated Sepharose-4B and used as solid-phase antigens in epitope-mapping studies with monoclonal antibodies against native and reduced/alkylated *Fel d* I.

Two monoclonal antibodies directed against reduced/alkylated chain 1 bound to the overlapping peptides 53-66 and 60-70 of chain 1. The monoclonal antibody directed against reduced/alkylated chain 2 bound to the overlapping peptides 36-49 and 43-56 of chain 2. Binding specificity was demonstrated by inhibition by reduced/alkylated *Fel d* I for all three monoclonal antibodies.

Another monoclonal antibody against reduced/alkylated *Fel d* I had been found to bind predominantly to reduced/alkylated chain 2 on immunoblot in previous studies (27). It bound to peptides 1-16 and 60-70 of chain 1 and peptides 1-14 and 50-63 of chain 2; it is therefore probably directed against a conformational epitope formed by these four regions. Possibly because of low affinity of this monoclonal antibody, specificity of its binding could not be verified by inhibition studies.

A panel of monoclonal antibodies directed against native *Fel d* I bound to peptides 1-16 and 60-70 of chain 1 and peptides 1-14 and 43-56 of chain 2. For two monoclonal antibodies, binding to each peptide was investigated and shown to be inhibitable by native *Fel d* I. These antibodies are therefore probably directed against a conformational epitope formed by these four regions.

These studies give us substantial information about the quaternary structure of *Fel d* I.

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Epitope mapping with synthetic peptides has been demonstrated to be a suitable tool to locate some antigenic determinants on allergens. For the allergens *Gad c* I (12), *Amb a* III (3), *Chi t* I (20), *Cor a* I, and *Bet v* I (13), 0.28 wheat α -amylase inhibitor (30) and *Der p* II (29) epitopes were found by this technique.

The most important allergen from the house cat (*Felis domesticus*) is the major allergen *Fel d* I (9, 19,

22). It is found in cat saliva (1, 4, 6, 8), pelt (4, 8), and lacrimal fluid (26). The physiologic function of *Fel d* I is unknown. It has been isolated from house dust (7) and cat dander (11, 16, 17) by affinity chromatography and has an apparent mol. wt. of approximately 37000. This native *Fel d* I is a homodimer: on SDS-PAGE under nonreducing conditions *Fel d* I is an 18000-mol.-wt. protein; under reducing conditions it dissociates into two chains:

chain 1 with an apparent mol. wt. of 5000 and chain 2 with an apparent mol. wt. of 13000 (11, 27). Amino acid sequence analysis of *Fel d I* (14, 21) shows that these two polypeptide chains, respectively, consist of 70 amino acid residues (chain 1) and 92 residues (chain 2) with derived mol. wt. of 7863 and 10121. Each chain contains three cysteine residues, and preliminary analysis indicates that chains 1 and 2 are connected by disulfide bonds in an antiparallel topology (21). Chain 2 is glycosylated, probably at asparagine 33 (the sequence $^{33}\text{N}-^{34}\text{A}-^{35}\text{T}$ is a predicted glycosylation site), and treatment of the allergen with N-glycosidase shows only a slight reduction in the inhibition capacity of the RAST, as compared with native *Fel d I* (11), implying that the carbohydrate moieties do not play a major role in antibody binding. Denaturing conditions, such as heating or treatment with 6 mol/l guanidine, cause only a modest reduction of *Fel d I* activity. After cysteine reduction and blocking renaturation by alkylation, more than 95% loss of activity in RAST inhibition experiments and allergen-binding experiments (11, 16, 27) was found, suggesting that the native conformation of *Fel d I* is important for most IgE antibodies. Detection of epitopes for antibodies to native *Fel d I*, by means of synthetic peptides, therefore might seem unexpected. However, in our previous studies, an epitope of the *Dermatophagoides pteronyssinus* allergen *Der p II*, comprising residues 65–78, was found for a number of IgE sera (29), whereas group II mite allergens, like *Fel d I*, almost completely lose their IgE-binding capacity after reduction and alkylation (18).

In the present study we synthesized a panel of overlapping peptides taken from the sequence of *Fel d I*. Because of the importance of the native conformation for IgE antibody binding, we expected to find epitopes more easily with monoclonal antibodies against reduced/alkylated *Fel d I* (27). With three different monoclonal antibodies against reduced/

alkylated *Fel d I*, three different epitopes were found, one of which seemed to be a conformational epitope. With monoclonal antibodies against native *Fel d I* (7, 9, 11) a conformational epitope was also found. The antibodies bound to other peptides than human IgE sera (28). Comparison of the epitopes found with the monoclonal antibodies gives us information about the quaternary structure of *Fel d I* and the effect of reduction and alkylation upon this quaternary structure.

Material and methods

Peptides

Synthetic peptides were made by the T-bag method (2, 15, 23), adapted for Fmoc (9-fluorenylmethoxycarbonyl)-chemistry (5, 29). All peptides were synthesized with an additional lysine at the C-terminus in order to facilitate coupling to CNBr-activated Sepharose (Fig. 1).

On reversed-phase HPLC (Delta Pak C-18, gradient from 0.1% TFA (trifluoroacetic acid) in water to 0.1% TFA in acetonitrile, UV detection at 214 and 280 nm), 14 peptides, including peptides 1–16 and 60–70 of chain 1 and peptide 43–56 of chain 2, showed only one main peak. Four peptides, including peptide 50–63 of chain 2, showed a second peak, varying from 13 to 80% of the main peak (40% for peptide 50–63). Two peptides showed two secondary peaks, varying from 10 to 55% of the main peak (peptide 1–14 of chain 2 showed two secondary peaks of 10% of the main peak). Only peptide 78–92 of chain 2 gave a poor HPLC profile, probably because of poor solubility in the mobile phase.

Preparation of solid-phase antigens and testing

Lyophilized peptides were dissolved in water (the poorly soluble peptides were dissolved in 0.5 ml of fresh DMF (*N,N*-dimethylformamide), from which

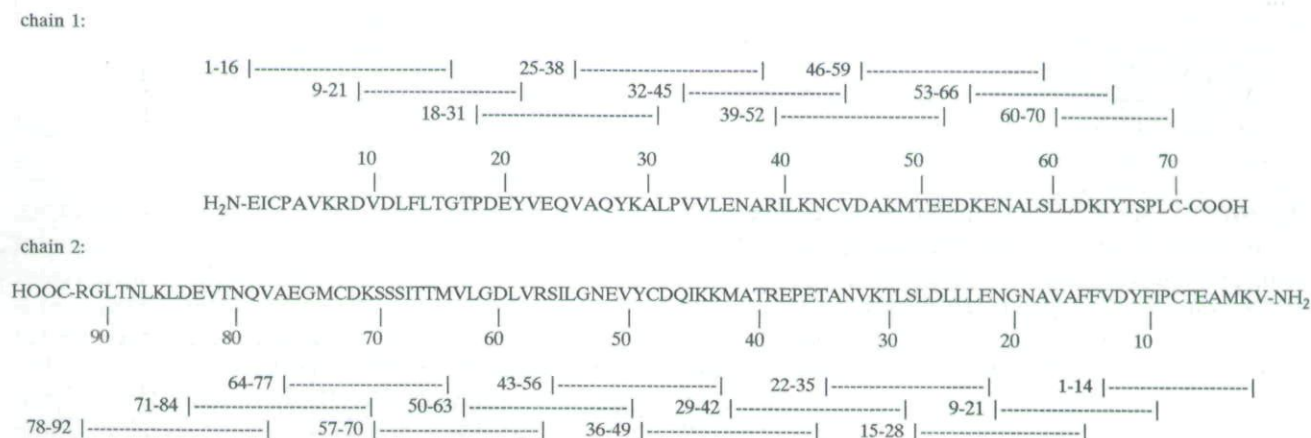


Fig. 1. Amino acid sequence of both chains of *Fel d I*, indicating the 21 overlapping synthetic peptides.

residual dimethylamine was removed by purging with nitrogen) and coupled to CNBr-activated Sepharose-4B (Pharmacia) without further purification. Of each peptide, 4 mg was coupled to 500 mg of Sepharose in 0.1 mol/l bicarbonate, pH 8.5. After a 16-h incubation at 4°C, the remaining binding sites were blocked with 0.5 mol/l glycine, pH 8.5, for 2 h. After extensive washing, the Sepharose was suspended in 250 ml PBS-AT (phosphate-buffered saline with 0.3% bovine serum albumin and 0.1% Tween-20). In the radioimmunoassay (RIA) test the monoclonal antibody was incubated head over head with 0.5 mg Sepharose-coupled peptide in an end volume of 550 µl PBS-AT for 16 h at room temperature. After washing the Sepharose five times with PBS-T (PBS with 0.1% Tween-20), we added anti-IgG reagent (1 ng), radiolabeled with 400 Bq ¹²⁵I, in PBS-ATS (PBS-AT with 4.5% normal bovine serum and 0.5% normal sheep serum) (500 µl). After a 16-h incubation the Sepharose was washed four times with PBS-T, and bound anti-IgG reagent was measured by counting the gamma radiation. Results are given as percentage of added label bound by the solid-phase antigen.

Murine monoclonal antibodies

Two monoclonal antibodies directed against different epitopes on *Fel d I*, Fd1-a, and Fd1-b, were obtained and described (9). In addition, the monoclonal antibody C5/24.6 (10) and the monoclonal antibodies 1G9, 2H4, 3E4, 5E3, and 6F9 (7) against *Fel d I* were tested. Monoclonal antibodies FdR-1a, FdR-1b, FdR-2a, and FdR-2b against reduced/alkylated *Fel d I* were obtained and described (27). Monoclonal antibody against the house-dust mite major allergen *Der p II* (25) was used as negative control.

Inhibition studies

The specificity of binding to Sepharose-coupled peptides 1–16, 32–45, 39–52, 53–66, and 60–70 of chain 1, and to Sepharose-coupled peptides 1–14, 36–49, 43–56, 50–63, and 64–77 of chain 2, was studied by competitive inhibition experiments (24). The monoclonal antibody dilutions (50 µl) were preincubated with 50 µl of a dilution series of 10% (w/v) cat dander extract, containing 13 µmol/l (1 U = 110 pmol) of *Fel d I* (Haarlems Allergenen Laboratorium, Haarlem, The Netherlands), reduced and alkylated *Fel d I* (11 µmol/l (27)), or the corresponding peptides (1 mmol/l). As negative control, an irrelevant peptide (GVTHDQLNNFRAGFD), containing a linear epitope from the nonbiting midge allergen *Chi t I* (20), was used as inhibitor. After 2 h, 250 µl of PBS-AT and 250 µl of Sepharose-coupled peptide

suspended in PBS-AT were added, and the rest of the procedure was performed as described above. With the monoclonal antibodies C5/24.6 and FdR-2b cross-inhibition studies were also performed with the four Sepharose-coupled peptides that they bound to and there four peptides as inhibitor.

Results

Monoclonal antibodies against reduced and alkylated *Fel d I*. Monoclonal antibodies FdR-1a and FdR-1b, directed against chain 1 of reduced/alkylated *Fel d I*, bound predominantly to the Sepharose-coupled overlapping peptides 53–66 and 60–70 of chain 1 (Fig. 2A). As they showed the same specificity in the peptide RAST, only monoclonal antibody FdR-1a was more closely investigated. Inhibition studies with reduced/alkylated *Fel d I* demonstrated that binding to these two peptides was specific (Table 1).

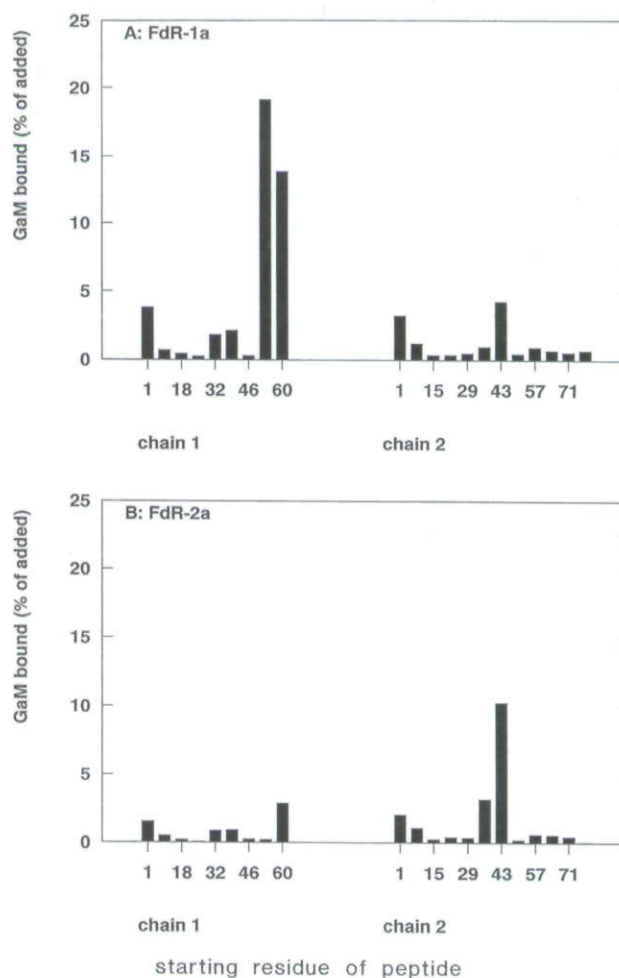


Fig. 2. Binding of monoclonal antibody FdR-1a (2 pmol IgG/test) to the Sepharose-coupled peptides of chains 1 and 2 (A) of *Fel d I* and of monoclonal antibody FdR-2a (1 pmol IgG/test) to the Sepharose-coupled peptides of chains 1 and 2 (B). As blank Sepharose blocked by 0.5 mol/l glycine was used (0.1% binding for both).

Table 1. Inhibition studies with monoclonal antibodies FdR-1a and FdR-2a against reduced/alkylated *Fel d* I

Monoclonal antibody	Inhibitor	Sepharose-coupled Ch. 1, 53-66 1 pmol IgG/test	Sepharose-coupled Ch. 1, 60-70 2 pmol IgG/test
FdR-1a	<i>Fel d</i> I	> 10	7
	<i>Fel d</i> I R/A	0.09	0.05
	Ch. 1, 53-66	51	
	Ch. 1, 60-70		> 200
	<i>Chi t</i> I peptide	> 200	> 200
Monoclonal antibody	Inhibitor	Sepharose-coupled Ch. 2, 36-49 4 pmol IgG/test	Sepharose-coupled Ch. 2, 43-56 1 pmol IgG/test
FdR-2a	<i>Fel d</i> I	> 10	> 10
	<i>Fel d</i> I R/A	0.11	0.41
	Ch. 2, 36-49	5	
	Ch. 2, 43-56		21
	<i>Chi t</i> I peptide	> 200	> 200

The amounts of nmoles of inhibitor that gave 50% inhibition are given.

Monoclonal antibody FdR-2a, directed against chain 2 of reduced and alkylated *Fel d* I, bound predominantly to the Sepharose-coupled overlapping peptides 36-49 and 43-56 of chain 2 (Fig. 2B). Inhibition studies with reduced/alkylated *Fel d* I demonstrated that binding to these two peptides was also specific (Table 1). Both monoclonal antibodies also bound, to some extent, to other Sepharose-coupled peptides, but as the percentage of binding did not exceed that of the monoclonal antibody against *Der p* II and binding was not inhibited by reduced/alkylated *Fel d* I or native *Fel d* I, it was considered to be irrelevant (Fig. 2A, 2B, 4D).

Monoclonal antibody FdR-2b bound to the Sepharose-coupled peptides 1-16 and 60-70 of chain 1 and 1-14, 9-21, 43-56, and 50-63 of chain 2 (Fig. 3A). No inhibition was found with reduced/alkylated *Fel d* I. To investigate whether this effect was caused by the low affinity of this monoclonal antibody to reduced/alkylated *Fel d* I, a factor which could lead to the requirement of a larger amount of inhibitor than that used in our assay, we tested dissociation of antigen-antibody complex. The solid-phase bound, antigen-antibody complex was incubated with an excess of native *Fel d* I and reduced/alkylated *Fel d* I for 25 h. This had no significant effect on the amount of monoclonal antibody bound to these Sepharose-coupled peptides (data not shown). The results of inhibition and cross-inhibition experiments are summarized in Table 2.

Monoclonal antibodies against native *Fel d* I. Eight monoclonal antibodies against native *Fel d* I were tested. These monoclonal antibodies were divided into three groups, based on their ability to inhibit

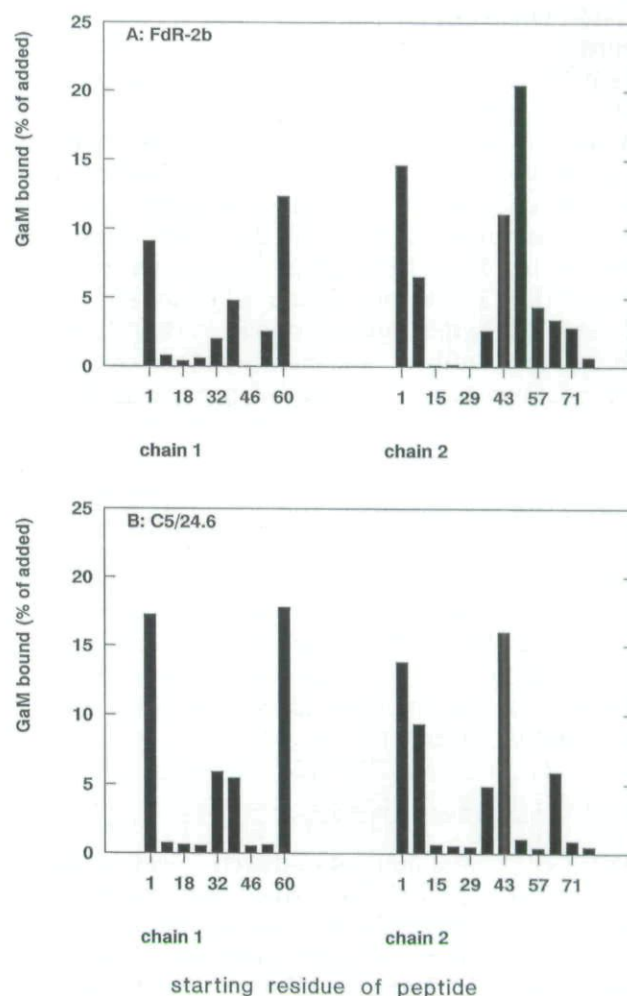


Fig. 3. Binding of monoclonal antibody FdR-2b (2 pmol IgG/test) to the Sepharose-coupled peptides of chains 1 and 2 (A) of *Fel d* I and of monoclonal antibody C5/24.6 (7 pmol IgG/test) to the Sepharose-coupled peptides of chains 1 and 2 (B). As blank Sepharose blocked by 0.5 mol/l glycine was used (0.2% and 0.7% binding, respectively).

binding of *Fel d* I to Sepharose-coupled monoclonal antibodies Fd1-a, Fd1-b, and 3F11 (Table 3). These three monoclonal antibodies only slightly inhibited each other's binding to *Fel d* I and therefore are supposed to recognize different epitopes (Chapman MD, personal communication). This is illustrated by the use of monoclonal antibody Fd1-a and monoclonal antibody Fd1-b in a two-site assay (7). All three Fd1-b-related monoclonal antibodies bound to the Sepharose-coupled peptides 1-16, 32-45, 39-52, and 60-70 of chain 1 and 1-14, 9-21, 36-49, and 43-56 of chain 2. Of the Fd1-a-related monoclonal antibodies, only 5E3, which slightly inhibited binding of Fd1-b to *Fel d* I, bound to these Sepharose-coupled peptides; the strictly Fd1-a-related antibodies, 3E4 and Fd1-a itself, did not. Both 3F11-related monoclonal antibodies, 2H4 and 6F9, which also inhibited binding of Fd1-b to *Fel d*

Epitope mapping of *Fel d I* by monoclonal antibodies

Table 2. Inhibition studies with monoclonal antibodies C5/24.6 and FdR-2b

Monoclonal antibody	Inhibitor	Sepharose-coupled Ch. 1, 1-16	Sepharose-coupled Ch. 1, 60-70	Sepharose-coupled Ch. 2, 1-14	Sepharose-coupled Ch. 2, 43-56
C5/24.6	<i>Fel d I</i>	0.02	0.06	0.04	0.01
	<i>Fel d I</i> R/A	> 10	> 10	> 10	> 10
	Ch. 1, 1-16	48	63	> 200	70
	Ch. 1, 60-70	> 200	> 200	> 200	> 200
	Ch. 2, 1-14	95	> 200	130	137
	Ch. 2, 43-56	23	14	24	8
	<i>Chit I</i> peptide	> 200	> 200	> 200	> 200
Monoclonal antibody	Inhibitor	Sepharose-coupled Ch. 1, 1-16	Sepharose-coupled Ch. 1, 60-70	Sepharose-coupled Ch. 2, 1-14	Sepharose-coupled Ch. 2, 50-63
FdR-2b	<i>Fel d I</i>	> 10	> 10	> 10	> 10
	<i>Fel d I</i> R/A	> 10	> 10	> 10	> 10
	Ch. 1, 1-16	> 200	> 200	> 200	> 200
	Ch. 1, 60-70	> 200	> 200	> 200	> 200
	Ch. 2, 1-14	110	51	94	> 200
	Ch. 2, 50-63	< 0.5	< 0.5	< 0.5	3
	<i>Chit I</i> peptide	> 200	> 200	> 200	> 200

Both monoclonal antibodies were diluted to 1 pmol/test. The amounts of nmoles of inhibitor that gave 50% inhibition are given.

I, bound to the same Sepharose-coupled peptides. In addition, 2H4 also bound to the Sepharose-coupled peptides 64-77 and 71-84 of chain 2 (Fig. 4).

For one Fd1-b-related monoclonal antibody against native *Fel d I*, C5/24.6 (Fig. 3B), specificity of binding to the Sepharose-coupled peptides 1-16 and 60-70 of chain 1 and to the Sepharose-coupled peptides 1-14 and 43-56 of chain 2 was examined with inhibition studies and cross-inhibition experiments (Fig. 5, Table 2). Binding to Sepharose-coupled peptides 32-45 and 39-52 of chain 1 and 64-77 of chain 2 was not inhibited by native *Fel d I* (data not shown).

Discussion

In epitope-mapping studies of allergenic proteins with synthetic peptides, it is very important to es-

tablish whether antibody binding to the synthetic peptides is specific for the investigated protein. Convenient methods to establish this specificity are inhibition studies with the complete protein and related and unrelated peptides (3, 12, 13, 20, 24, 29), and studies with unrelated antibodies. In our studies the monoclonal antibody to an unrelated allergen *Der p II* bound, to some extent, to the same peptides that monoclonal antibodies against reduced/alkylated *Fel d I* or native *Fel d I* bound to (Fig. 4D). For none of these peptides was binding of this unrelated antibody inhibited by native *Fel d I*. As in our epitope-mapping studies of *Der p II* with synthetic peptides (29), we found a large difference in inhibitory potency of complete protein and synthetic peptides, showing that for investigating specificity of antibody binding to synthetic peptides by inhibition studies the complete protein has to be favored over related peptides.

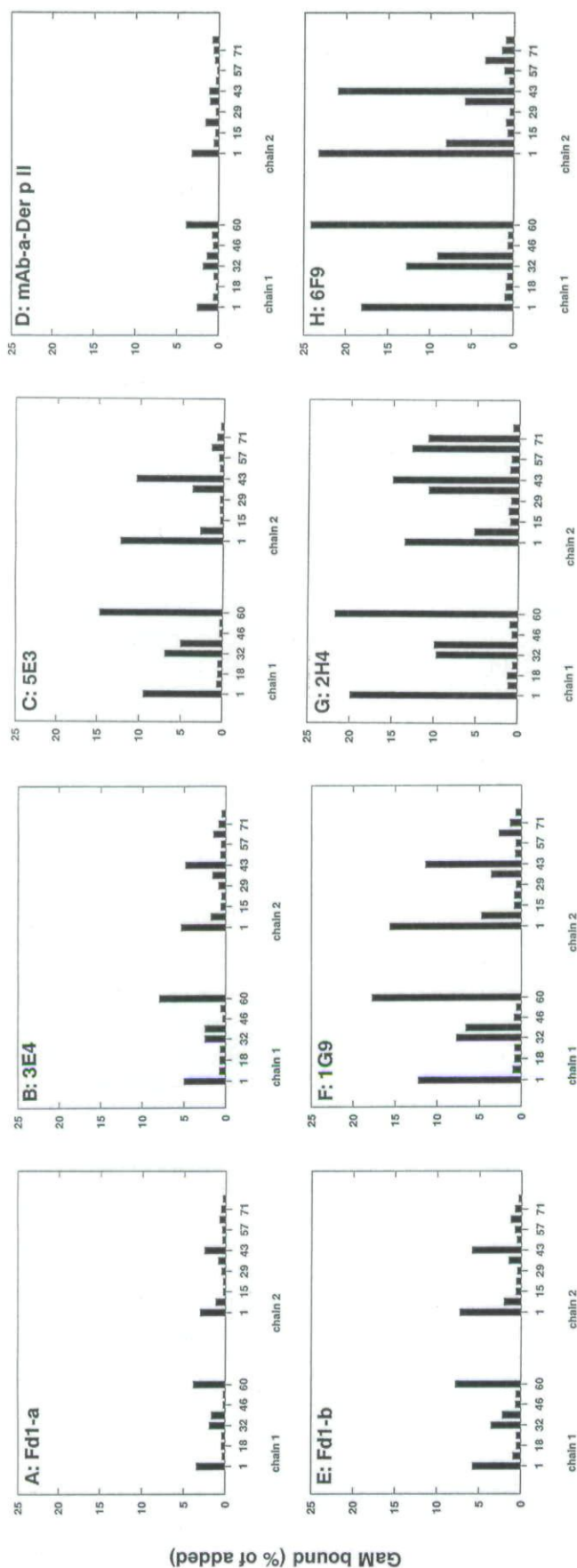
The epitopes found for monoclonal antibodies FdR-1a and FdR-2a were verified by inhibition by reduced/alkylated *Fel d I*.

The two monoclonal antibodies that did not inhibit binding of Fd1-b to native *Fel d I* did not significantly bind to any of the peptides, and for these monoclonal antibodies epitopes could not be mapped with synthetic peptides. The six monoclonal antibodies that were able to inhibit binding of Fd1-b to native *Fel d I* bound to Sepharose-coupled peptides corresponding to four regions on the *Fel d I* molecule, and inhibition studies with monoclonal antibody C5/24.6 and native *Fel d I* demonstrated that binding to these peptides was specific. The results were confirmed by inhibition studies with monoclonal antibody 1G9 and lead us to postulate that

Table 3. Cross-inhibition studies with monoclonal antibodies to native *Fel d I*

Monoclonal antibody	Sepharose-coupled Fd1-a	Sepharose-coupled Fd1-b	Sepharose-coupled 3F11	Binding to peptides
Fd1-a	+	-	-	-
3E4	+	-	-	-
5E3	+	±	-	+
Fd1-b	-	+	-	±
C5/24.6	-	+	-	+
1G9	-	+	-	+
3F11	-	-	+	Not tested
2H4	-	+	+	+
6F9	±	+	+	+

The monoclonal antibodies against native *Fel d I* were classified by their ability to inhibit binding of radiolabeled *Fel d I* to Sepharose-coupled monoclonal antibodies Fd1-a, Fd1-b, and 3F11. All monoclonal antibodies that inhibited binding to Sepharose-coupled Fd1-b bound to the Sepharose-coupled peptides.



starting residue of peptide

Fig. 4. Binding of monoclonal antibodies directed to native *Fel d I* to the Sepharose-coupled peptides. Fd1-a-related monoclonal antibodies: A-C (IgG contents [pmol IgG/test]: Fd1-a, 2; 3E4, 34; 5E3, 3), control monoclonal antibody against *Der p II*: D (6 pmol IgG/test), Fd1-b-related monoclonal antibodies: E-H (IgG contents [pmol IgG/test]: Fd1-b, 9; 1G9, 3), and 3F11-related monoclonal antibodies: G-H (IgG contents [pmol IgG/test]: 2H4, 35; 6F9, 34). As blank Sepharose blocked by 0.5 mol/l glycine was used (Fd1-a, 0.4%; 3E4, 0.8%; 5E3, 0.7%; mAb-a-Der p II, 0.4%; Fd1-b, 0.5%; 1G9, 0.6%; 2H4, 0.5%; 6F9, 0.8% binding).

these antibodies are directed against a conformational epitope on *Fel d I* formed by these four regions. Cross-inhibition studies with C5/24.6 showed that three of the four peptides inhibited binding to at

least two other Sepharose-coupled peptides. As there is no sequence homology between these four peptides, we assume that this cross-inhibition should be explained in terms of steric hindrance by free

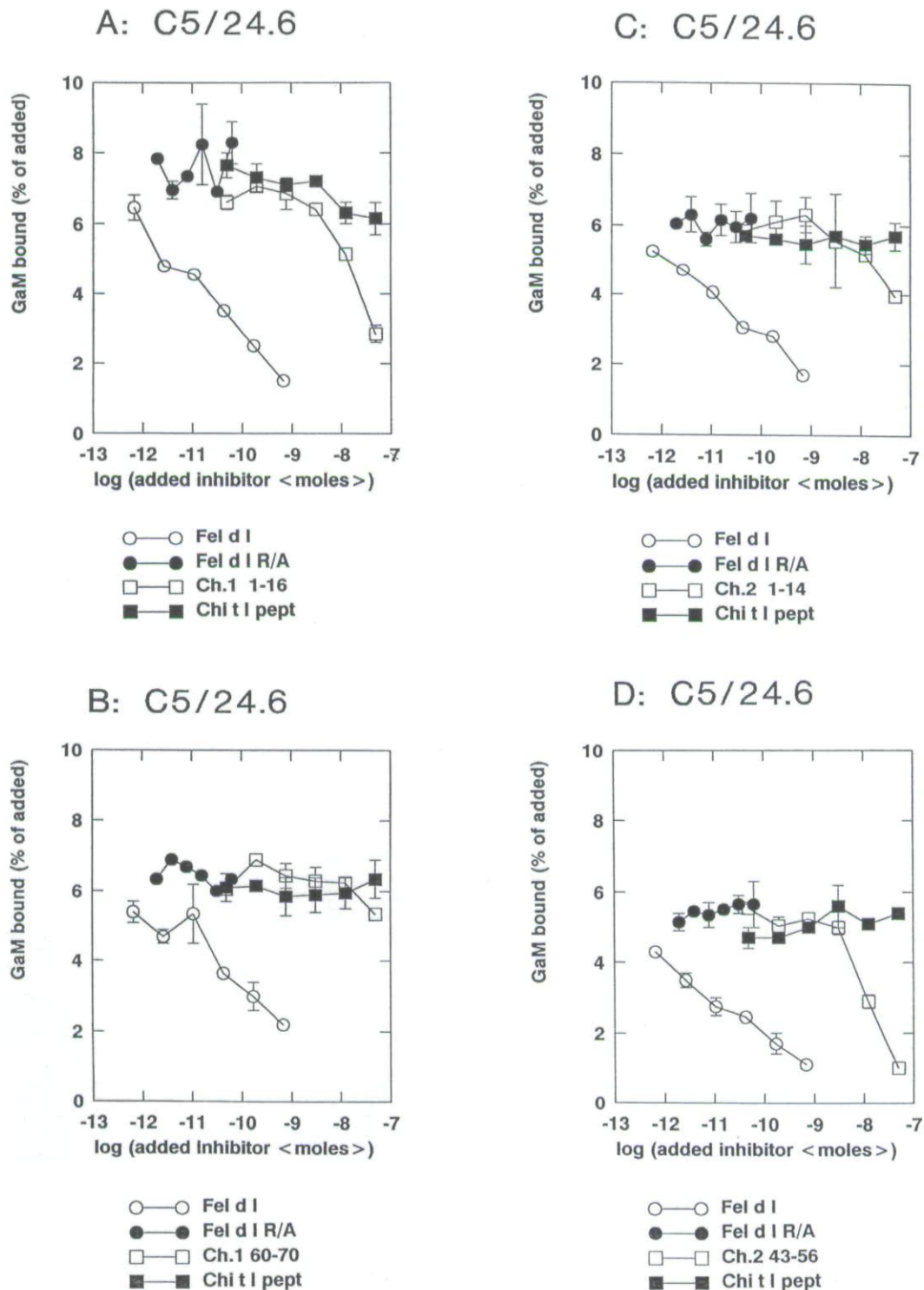


Fig. 5. Inhibition of binding of monoclonal antibody C5/24.6 (1 pmol IgG/test) to Sepharose-coupled peptides 1–16 (A) and 60–70 (B) of chain 1 and to Sepharose-coupled peptides 1–14 (C) and 43–56 (D) of chain 2 by native *Fel d I*, reduced and alkylated *Fel d I*, and the corresponding peptide. The effect of the *Chi t I* peptide, as negative control, was studied. The bars represent duplicate assays.

peptides having a much greater degree of conformational freedom than the corresponding parts of the postulated conformational epitope. In order to form a conformational epitope, these four antigenic regions have to be spatially arranged in close proximity. Such an arrangement could be effected by two disulfide-bonds, as each of these four peptides contains one cysteine residue: residues 3 and 70 of chain 1 and residues 7 and 48 of chain 2.

The most intriguing monoclonal antibody we studied has been monoclonal antibody FdR-2b. On immunoblot it binds almost exclusively to reduced/alkylated chain 2; only prolonged autoradiography shows some minor binding to reduced/alkylated chain 1 (27). Binding of reduced/alkylated *Fel d I* to this monoclonal antibody, however, is inhibited by monoclonal antibody FdR-1a against reduced/alkylated chain 1. This behavior is reflected in the pattern of binding to the Sepharose-coupled peptides. Four antigenic regions were found (Fig. 3A), suggesting a conformational epitope. However, specificity for reduced/alkylated *Fel d I* could not be demonstrated by inhibition studies, as reduced/alkylated *Fel d I* is not a potent inhibitor for this monoclonal antibody. Binding to Sepharose-coupled reduced/alkylated *Fel d I* of the same amount of FdR-2b as used in our inhibition studies with Sepharose-coupled peptides was reduced by 50% by 60 pmol of reduced/alkylated *Fel d I*, whereas binding of FdR-1a and FdR-1b was reduced by 50% by 30 pmol and 20 pmol, respectively. There are two reasons to consider that a conformational epitope is formed by these four antigenic regions. Firstly, the peptide 50–63 of chain 2 is not obviously "sticky"; i.e., it does not tend to bind monoclonal antibodies nonspecifically like some other peptides: of the other 12 monoclonal antibodies investigated, none bound to this peptide, not even if their IgG content was 35 times as high as that of FdR-2b (Figs. 1, 3B, 4). A more convincing reason is obtained by cross-inhibition studies. Peptide 50–63 of chain 2 inhibited binding of FdR-2b to all four peptides under study, whereas peptide 1–14 of chain 2 inhibited binding to three peptides. These data indicate that a conformational epitope is formed by these four antigenic regions for the same reasons as for monoclonal antibodies C5/24.6 and 1G9.

This leads us to the following model for *Fel d I* and the effect of reduction/alkylation: the two chains of *Fel d I* are aligned in an antiparallel topology (21). Our studies showed that monoclonal antibody C5/24.6 is directed against a conformational epitope stretched out over both chains in which the four regions around the cysteine residues 3 and 70 of chain 1 and 7 and 48 of chain 2 are spatially close together. After reduction/alkylation two monoclonal antibodies were raised that bound to chain 1 (FdR-1a

and FdR-1b), one that bound to chain 2 (FdR-2a), and one that bound to both chains (FdR-2b). Radioimmunoprecipitation assays with these Sepharose-coupled monoclonal antibodies followed by SDS-PAGE and immunoblotting of the precipitated materials showed that FdR-1a precipitated only reduced/alkylated chain 1, FdR-2a precipitated only reduced/alkylated chain 2, but FdR-2b precipitated both chains (27).

This suggests that after reduction/alkylation chains 1 and 2 are dissociated and FdR-2b is directed against a minor residue of not totally reduced/alkylated *Fel d I* in which at least one interchain disulfide bridge is intact. The pattern of peptide binding makes us consider a conformational epitope for FdR-2b in which the disulfide bridge between the cysteine residues 70 of chain 1 and 7 of chain 2 is intact. The quaternary structure around this disulfide bond would be distorted in such a manner that the recognition site of FdR-2b on chain 2 shifts from residue 43–56 towards 50–63, as compared with the FdR-1b-related monoclonal antibodies, and that the FdR-1a- and FdR-1b-related monoclonal antibodies no longer bind to this partially reduced/alkylated *Fel d I*. The dissociated reduced/alkylated chains 1 and 2 also have a three-dimensional form different from what they have in native *Fel d I*, resulting in a strong reduction of binding capacity for monoclonal antibodies against native *Fel d I*. The suggested conformational epitope for FdR-2b implies that we can assign the three disulfide bridges in native *Fel d I* as follows: $^3\text{cys}(1)\text{--}^{48}\text{cys}(2)$, $^{44}\text{cys}(1)\text{--}^{73}\text{cys}(2)$, and $^{70}\text{cys}(1)\text{--}^7\text{cys}(2)$. Confirmation of these assignments by conventional physicochemical techniques is necessary. The three-dimensional folding of both chains

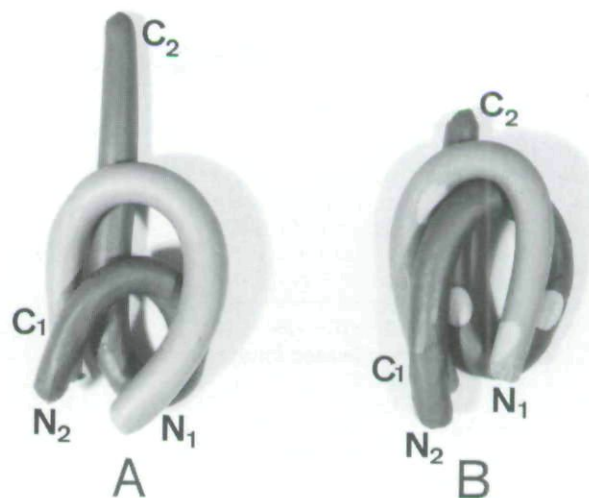


Fig. 6. Molecular models of native *Fel d I* (A) and incompletely reduced/alkylated *Fel d I* (B). The antibody-binding pattern of FdR-2b indicates that partial reduction/alkylation relieves the conformational strain.

that would allow these three disulfide bridges is depicted in Fig. 6A. In the partially reduced/alkylated *Fel d I*, as deduced from the peptide-binding pattern of FdR-2b, the conformational strain on chain 2 is relieved, leading to a three-dimensional structure in which chain 2 is less compact (Fig. 6B).

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