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The use of T bag synthesis with paper discs as the solid phase in epitope mapping studies

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Epitope mapping with synthetic peptides bound to derivatized paper discs has been investigated using the discs both as a solid-phase matrix for peptide synthesis as well as the solid phase in immunologic testing procedures without detachment of the peptides from the paper discs. Using the T bag (tea bag) method the simultaneous synthesis and subsequent immunologic testing of large numbers of peptides was demonstrated for the feline major allergen *Fel d* I. A total of 15,000 paper disc-bound peptides, comprising the 146 nonapeptides overlapping by eight amino acid residues on both chains, were synthesized simultaneously with 100 paper discs per T bag. Using these paper disc-bound peptides as the solid phase in radioimmunoassays the binding sites found coincided with those detected in the PEPSCAN with the commercially available epitope mapping kit and with the binding sites that had been found with Sepharose-coupled peptides. The signal to background-ratio in the paper disc-RIA was comparable to that in the PEPSCAN and the reproducibility was good. The bound antibodies could be eluted from the paper disc-bound peptides, permitting regeneration and repeated use of the paper discs for immunologic testing. This method was shown to be a useful alternative to the PEPSCAN and to have the major advantage that large numbers of antibodies could be tested with large numbers of peptides simultaneously.

Key words: Simultaneous multiple peptide synthesis; Epitope mapping; PEPSCAN; Paper disc; *Fel d* I

Introduction

In epitope mapping studies of allergens with synthetic peptides two methods have been used. In the first method synthetic peptides are made via solid-phase synthesis, isolated and used for mapping studies. For the allergens *Gad c* I

Abbreviations: ABTS, 2,2'-azinobis(3-ethylbisthiazoline-6-sulfonic acid), diammonium salt; BOP, benzotriazol-1-yl-oxy-tris(dimethylamino) phosphonium hexafluorophosphate; DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; HObt, 1-hydroxybenzotriazol; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; PBS-AT, phosphate-buffered saline with 0.3% bovine serum albumin and 0.1% Tween 20; PBS-ATS, PBS-AT with 4.5% normal bovine serum and 0.5% normal sheep serum; PBS-T, phosphate-buffered saline with 0.1% Tween 20; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; RAST, radioallergosorbent test; RIA, radioimmunoassay; SDS, sodium dodecylsulfate; SPDP, *N*-succinimidyl-3-(2-pyridyldithio)-propionate; *t*Boc, *t*-butoxycarbonyl; *t*Bu, *t*-butyl; Trt, trityl (triphenyl-methyl).

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(Elsayed et al., 1980), *Amb a* III (Atassi and Atassi, 1986), *Chi t* I (Mazur et al., 1988), *Cor a* I and *Bet v* I (Elsayed et al., 1989), *Der p* II (Van 't Hof et al., 1991) and *Fel d* I (Van Milligen et al., 1992b; Van 't Hof et al., 1992) epitopes were found using this technique. The second method is the PEPSCAN (Geysen et al., 1987), in which the same solid phase, the tips of polyethylene pins, is used for the synthesis of short peptides as well as for the subsequent immunologic testing. Recently this second method has been adapted for epitope mapping of allergens with sera containing IgE antibodies (Walsh and Howden, 1989).

Methods of simultaneous multiple peptide synthesis have been reported in which simple materials such as paper (Frank and Döring, 1988; Eichler et al., 1989) or cotton (Lebl and Eichler, 1989; Berg et al., 1989; Daniels et al., 1989) are used as solid-phase synthesis matrix. Peptide synthesis on paper is a particularly interesting technique for epitope mapping of allergens, since paper discs are often used as the solid phase in the in vitro diagnosis of allergy (Pharmacia, Phadebas-RAST). Krchňák et al. (1990) described a simple method of multiple peptide synthesis in which paper discs were used as solid-phase synthesis matrix in polypropylene reactor columns coupled to a manually operated peptide synthesizer. After completion of the synthesis the paper disc-bound peptides were incubated with antiserum in a paper disc-ELISA in plastic syringes equipped with polypropylene sinters. After development of the color these syringes were emptied in ELISA plates and the absorbance was measured. The authors described the synthesis of four peptides with ten paper discs, which were cut in four pieces for subsequent use as the solid phase in an ELISA (Krchňák et al., 1990). However, both the method of synthesis as well as the determination of antibody binding are not practical for large numbers of peptides or antisera. For epitope mapping both issues are important: the method must allow the parallel synthesis of a large number of peptides in order to map a complete protein and offer a convenient method for large-scale testing. Different methods of simultaneous multiple peptide synthesis have been described that permit the parallel solid-phase synthesis of large numbers of peptides (Houghten, 1985; Ruggeri et al.,

1986; Schnorrenberg et al., 1989; Appel et al., 1990; Gausepohl et al., 1990). We found that with the peptides made by simultaneous multiple peptide synthesis with the T bag (tea bag) method and subsequently coupled to Sepharose-particles a large number of sera could be tested simultaneously (Van 't Hof et al., 1991; Van Milligen et al., 1992b). The PEPSCAN method also allows the parallel synthesis of a large number of peptides, but the parallel testing of a large number of antisera is not feasible: the peptides cannot be cleaved from the polyethylene pins and only one antiserum at a time can be tested with the complete panel of peptides. In order to test 100 pin-bound peptides with 100 antisera within a reasonable time the synthesis of 10,000 pin-bound peptides would be necessary, i.e., without regeneration of the pins. We decided to perform a T bag synthesis with paper discs as the solid-phase synthesis matrix and to compare the immunologic results obtained with these paper disc-bound peptides with those obtained with the PEPSCAN method to demonstrate that a PEPSCAN-like approach with paper disc-bound peptides is possible. Using the T bag method of simultaneous multiple peptide synthesis combined with a paper disc-RIA, both the synthesis and subsequent immunologic testing of large numbers of peptides is feasible. For *Fel d* I 146 T bags were made with 15,000 paper discs, thereby permitting 100 simultaneous PEPSCAN-like epitope mapping tests. This synthesis and subsequent testing can be performed within 3 working weeks.

Materials and methods

Paper disc-coupled peptides

The tetrapeptides PLAQ and GLAQ, the hexapeptides 139–144 to 145–150 of IL-6 and all nonapeptides, overlapping by eight amino acid residues and spanning the complete amino acid sequences of chain 1 and chain 2 of *Fel d* I, were synthesized on paper discs. This length and overlap were chosen to make a good comparison between paper disc-RIA and PEPSCAN possible. 62 nonapeptides were synthesized for chain 1 of 70 amino acid residues length, and 84 nonapep-

tides were synthesized for chain 2 of 92 amino acid residues length.

Discs of filter paper (Schleicher & Schüll, Dassel, Germany) having a diameter of 0.6 cm were punched out with a standard perforator. The paper discs were activated with 1,1'-carbonyldiimidazole (CDI: Bethell et al., 1981), after which they were treated with an excess of 1,2-diaminoethane to obtain free amino groups on the discs. To 30 g ($n = 15,000$) of paper discs 15 g of CDI were added in 750 ml DMF. After reaction for 1 h the paper discs were washed twice with DMF, twice with water and a solution of 112 g of ethylenediammoniumdichloride and 36 g of sodiumhydroxide in 500 ml water was added (pH 8.5). After 2 h the liquid was decanted, after which 500 ml 0.5 M glycine, pH 8.5, were added to block any remaining activated carboxy groups. After 1 h the discs were washed with 0.1 M acetate buffer, pH 4.0, and 0.1 M borate buffer, pH 8.0 (three times each), twice with water, twice with methanol and air-dried.

The number of free amino-groups on the paper discs was determined by acylation by SPDP,

followed by the spectrophotometric determination of the subsequently released pyridine-2-thione ($\epsilon_{343} = 8080 \text{ liter mol}^{-1} \text{ cm}^{-1}$) upon treatment with dithiothreitol (Ngo et al., 1986).

T bags made of polypropylene mesh (Spectrum Medical Industries, Los Angeles, CA) with a mesh opening of $75 \mu\text{m}$ were filled with 200 mg (about 100) paper discs. Each T bag was labeled by the insertion of a number from a transfer sheet in between the seals (Fig. 1). In each coupling step each T bag was allowed to react for 1 h with 2.5 ml of a solution containing 0.2 M amino acid (Novabiochem, Läufelfingen, Switzerland), 0.2 M BOP (Richelieu Biotechnologies, Quebec, Canada), 0.2 M HOBT (Fluka, Buchs, Switzerland) and 0.26 M DIPEA in DMF. Dimethylamine was removed from the DMF by purging a freshly opened bottle of PA-grade DMF (Aldrich, Brussels, Belgium) with nitrogen gas until no more amine-like odor could be detected. The N termini of the amino acids were protected by the Fmoc group, semi-permanent side chain protection of arginine was achieved with the Pmc group (Ramage and Green, 1987), of histidine (Sieber

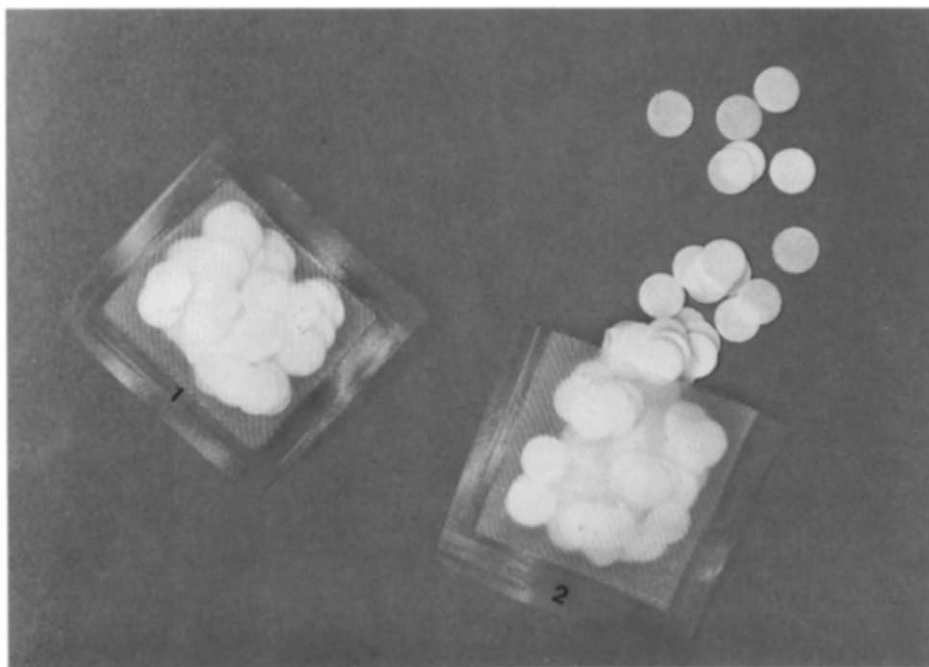


Fig. 1. T bags containing derivatized paper discs. The T bags were indelibly labeled by inserting numbers from a transfer sheet between the bottom seals.

and Riniker, 1987) and cysteine (McCurdy, 1989) with the Trt group and of other amino acids with the *t*Boc group or *t*Bu group. Deprotection of the collected T bags was performed with 30% piperidine in DMF for 15 min. After each coupling and deprotection step the collected T bags were extensively washed with 200 ml DMF, dichloromethane and 2-propanol. For deprotection and washings LAB-quality solvents were used (Merck, Darmstadt, Germany). Deprotection of the side chains was performed with 200 ml trifluoroacetic acid containing 5% thioanisole at room temperature for 4 h.

PEPSCAN

The same peptides as were synthesized on derivatized paper discs were synthesized on derivatized polyethylene pins (Cambridge Research Biochemicals, Cambridge, UK). In each coupling step each pin was allowed to react for 1 h with 150 μ l of the reaction mixture described above. The extensive washing after each coupling and deprotection step was performed in standard polypropylene boxes. To reduce the amount of trifluoroacetic acid necessary for the final side-chain deprotection to about 0.5 ml for each pin this reaction was not performed in the polypropylene boxes, but in polypropylene deep-well ELISA plates (Beckman Instruments, Fullerton, CA) onto which the polyethylene reaction blocks could be snapped as a lid. After the final side-chain deprotection the pins were washed successively with dichloromethane, dichloromethane containing DIPEA and dichloromethane, then air-dried and washed with water, according to the manufacturer's instructions. After immersion in methanol for 16 h and air-drying the pins were ready for ELISA testing.

Antisera

In the case of the sperm-whale myoglobin peptide we studied binding of the monoclonal antibody which is directed towards the cryptotope, the antigenic determinant recognized by an antibody directed against a denatured antigen (Laver et al., 1990), PLA_E in denatured sperm-whale myoglobin (Geysen, 1985). It is included in the epitope mapping kit (Cambridge Research Bio-

chemicals, Cambridge, UK) as a control antibody towards the control peptide with the related sequence PLA_Q and does not bind to the negative control peptide with the sequence GLA_Q. The monoclonal antibody mAb 7 against denatured interleukin-6 that binds to the hexapeptides 141–146 to 143–148 of IL-6 was a gift from Dr. J. Brakenhoff from the Department of Auto-immune Diseases of the CLB (Brakenhoff et al., 1992). Monoclonal antibodies against native (Chapman et al., 1988; De Groot et al., 1988; Duffort et al., 1988) and reduced/alkylated *Fel d* I (Van Milligen et al., 1992) were also tested and as control for non-specific binding a monoclonal antibody against *Der p* II was used (Van der Zee et al., 1988).

In addition we tested a panel of patients' sera that bound more than 20% of the added anti-IgE reagent in the cat-RAST (for RAST procedures see Aalberse et al., 1981) and that bound to the Sepharose-coupled peptides (Van Milligen et al., 1992b) with the paper disc-bound peptides. As a control for non-specific binding serum with a high total IgE (238 IE/ml) from a non cat-allergic subject was used.

Radioimmunoassays

Paper disc-coupled peptides. Each paper disc was incubated end over end with 50 μ l of diluted serum and 500 μ l PBS-AT for 16 h at room temperature. After washing the paper discs five times with PBS-T, anti-IgG or anti-IgE reagent (1 ng) radiolabelled with 400 Bq ¹²⁵I was added in PBS-ATS (500 μ l). After 16 h end-over-end incubation the paper discs were washed four times with PBS-T and bound anti-IgG or anti-IgE was measured by counting the gamma radiation. Results were expressed as percent of added label bound by the solid-phase antigen. The paper disc-RIA were performed as single tests, as each serum was also tested in a PEPSCAN. Binding to paper disc-bound peptides was verified with inhibition experiments. The paper disc-RIA with monoclonal antibody FdR-2b was repeated since binding of this antibody was not inhibited by reduced/alkylated *Fel d* I.

Sepharose-coupled peptides. In the RIA test a suspension of 500 mg of Sepharose in 250 ml PBS-AT (250 μ l) was incubated head over head

with 50 μ l of diluted serum and an additional 250 μ l PBS-AT according to the above procedure.

To investigate whether the paper disc-bound peptides could be regenerated a batch of 28 paper discs with covalently bound peptide 143–148 of IL-6, used for inhibition studies, was treated with 500 μ l/disc 0.5 M glycine/HCl pH 2.5 with 10% dioxane for 0.5 h. After washing with PBS-T five times the remaining percentage binding was measured. The paper disc-bound peptides were incubated with mAb 7, followed by radiolabeling as described above. After measuring the percentage binding the discs were treated with 0.5 M glycine/HCl pH 2.5 with 10% dioxane again. The remaining percentage binding was then measured, after which the procedure was repeated.

ELISA procedure

According to the manufacturer's directions the pins were precoated with 200 μ l of PBS-T containing 1% (w/v) bovine serum albumin (de-mineralized BoSerAl powder, Organon Technika, Turnhout, Belgium) and 1% (w/v) ovalbumin (Sigma, St. Louis, MO) for 1 h. The pins were incubated for 16 h with a 1/1000 dilution of the monoclonal antibody in the same buffer. After washing with PBS-T the pins were incubated for 1 h with a 1/2000 diluted HRP-conjugated goat anti-mouse Ig reagent (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands) in the same buffer. After washing with PBS-T the pins were incubated with a mixture of 0.1 M citrate buffer, pH 4.0, a 40 mM solution of ABTS and a stock solution of 0.6% hydrogen peroxide in the proportions 100:2:1 in the dark. After evolution of the green color the reaction was stopped by removing the pins from the ELISA plates and the extinction value at 405 nm was then measured directly.

After testing the antibodies were removed from the pins by extensive washing with 0.1 M glycine/HCl, pH 2.5. After washing with PBS, water and methanol and air-drying the pins were ready for the next ELISA test.

Inhibition studies

In the inhibition studies 50 μ l of antibodies, diluted in PBS-AT, were preincubated with 50 μ l

of various concentrations of inhibitor. After 2 h 250 μ l of PBS-AT and 250 μ l of Sepharose-coupled peptide suspended in PBS-AT, or 500 μ l PBS-AT and a paper disc containing a covalently bound peptide, were added. The rest of the procedure was performed as described above.

Results

Amino group content

The number of free amino groups per paper disc was determined to be 45 nmol by spectrophotometric analysis after treatment with SPDP followed by pyridine-2-thione release by reduction with dithiothreitol. The number of free amino groups on each of four pins from the epitope mapping kit was also determined with the SPDP procedure after deprotection of the Fmoc- β -alanine spacer with 20% piperidine. As background correction four unprotected pins were used. This amount ranged from 255–340 nmol/pin with an average of 295 nmol/pin, which agrees well with the manufacturer's specification of 150–300 nmol/pin.

Signal-background ratio and reproducibility

Monoclonal antibody against denatured sperm-whale myoglobin. This monoclonal antibody bound strongly to pin-coupled peptide PLAQ and poorly to pin-coupled peptide GLAQ (E_{405} 1.328 and 0.138 in the pin-ELISA, compared to 1.655 and 0.119 for the control pins included in the epitope mapping kit). In the paper disc-RIA we observed 21.7% binding to PLAQ compared to 3.0% binding to GLAQ. In order to test reproducibility and the signal-background ratio both the paper disc-RIA and the pin-ELISA were performed again in six-fold replicates. In the paper disc-RIA the mean percentage binding to PLAQ was 22.5 (standard deviation 0.9) and to GLAQ 2.6 (standard deviation 0.4), which corresponded with a signal-background ratio of 8.8. In the pin-ELISA a mean absorbance at 405 nm of 1.44 (standard deviation 0.19) was observed for PLAQ and of 0.13 (standard deviation 0.01) for GLAQ, corresponding with a signal-background ratio of 10.9.

The peptides PLAQK and GLAQK, with an

additional lysine to facilitate coupling to Sepharose, were also synthesized on a solid-phase resin (Van 't Hof et al., 1991) and coupled to Sepharose (8 mg peptide per gram Sepharose). The maximum binding that we found for the monoclonal antibody was 5.0% for Sepharose-coupled PLAQK compared to 1.1% for Sepharose-coupled GLAQK (16 nmol/test).

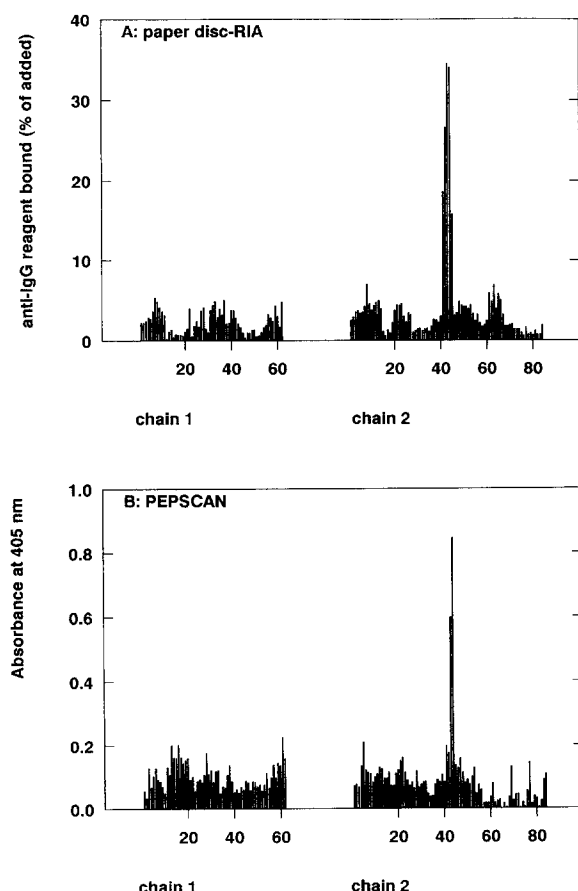


Fig. 2. Results of paper disc-RIA (A) and pin-ELISA (B) with FdR-2a for both chains of *Fel d I*. In the paper disc-RIA peptides 41–49 to 45–53 of chain 2 showed binding, in the PEPSCAN peptides 43–51 and 44–52. In our epitope mapping studies with Sepharose-coupled peptides we had previously shown binding to the overlapping peptides 36–49 and 43–56 of chain 2 (Van 't Hof et al., 1992). Specificity of binding in the paper disc-RIA was demonstrated by inhibition with reduced/alkylated *Fel d I* and peptides 36–49 and 43–56.

Reusability / regeneration of paper disc-bound peptides

Brakenhof et al. (1992) found moderate binding of monoclonal antibody mAb 7 raised against denatured interleukin-6 to pin-coupled peptides 141–146, 142–147 and 143–148. We found strong binding to paper disc-bound peptide 143–148 and the same epitope was found in our pin-ELISA. Reproducibility was tested in nine-fold replicates in the paper disc-RIA: the mean percentage binding to peptide 143–148 was 11.1 (standard deviation 0.5) and to peptide 141–146 1.0 (standard deviation 0.3), corresponding with a signal-background ratio of 10.7.

In regeneration studies with 28 paper disc replicates with covalently bound peptide 143–148 and mAb 7 we also found good reproducibility (Table II). Occasionally, however, an aberrant disc was found, which underlines the importance of duplicate testing.

Epitope mapping

For monoclonal antibody Fd1-b and related monoclonal antibodies against *Fel d I* a conformational epitope had previously been found in our epitope mapping studies with Sepharose-coupled peptides (Van 't Hof et al., 1992). Neither of these monoclonal antibodies nor the monoclonal antibody Fd1-a bound to any of the 146 nonapeptides of *Fel d I* in the paper disc-RIA or PEPSCAN.

Monoclonal antibodies against reduced / alkylated Fel d I. Monoclonal anti-bodies FdR-1a and FdR-2a bound to the same regions in the

TABLE I
SIGNAL-BACKGROUND RATIO IN PAPER DISC-RIA AND PEPSCAN FOR THE BINDING REGIONS FOUND WITH MONOCLONAL ANTIBODIES AGAINST REDUCED/ALKYLATED *Fel d I*

	Peptide	Paper disc-RIA	PEPSCAN
FdR-1a	Ch.1: 13–21	4.1	5.8
	Ch.1: 59–67	5.8	6.0
FdR-2a	Ch.2: 43–51	13.2	9.5
FdR-2b	Ch.1: 12–20	3.3	
	Ch.1: 13–21		4.4
	Ch.1: 60–68	2.9	4.6
	Ch.2: 50–58	8.2	9.9

paper disc-RIA as in the PEPSCAN. In Fig. 2 the results of both methods are plotted for monoclonal antibody FdR-2a against reduced/alkylated chain 2 of *Fel d* I. For another antibody, FdR-2b, we observed a shift from peptide 13–21 to 12–20 of chain 1 in the paper disc-RIA. The signal-background ratios are listed in Table I. The negative control antibody against *Der p* II did not bind significantly to any of the paper disc-bound, pin-bound or Sepharose-coupled

peptides of sperm-whale myoglobin, interleukin-6 or *Fel d* I.

IgE antibody containing sera of cat-allergic subjects. Five IgE-rich sera that bound strongly to Sepharose-coupled peptides of *Fel d* I (Van Milligen et al., 1992b) were selected for epitope mapping studies with paper disc-RIAs. They bound most strongly with Sepharose-coupled peptide 25–38 of chain 1 (Van Milligen et al., 1992b). Only 2/5 sera bound satisfactorily strong to the six paper disc-bound nonapeptides related to this peptide, whereas the negative control serum did not bind to these peptides. With these two sera, pf 139 and pf 151, a paper disc-RIA was performed with all 146 nonapeptides.

For pf 139 we found IgE binding by peptide 29–37 of chain 1 and peptide 43–51 of chain 2, for pf 151 we found IgE binding by peptide 28–36 of chain 1 (see Fig. 3). IgE binding found with two Sepharose-coupled peptides (peptides 1–14 and 78–92 of chain 2) was not observed in the paper disc-RIA.

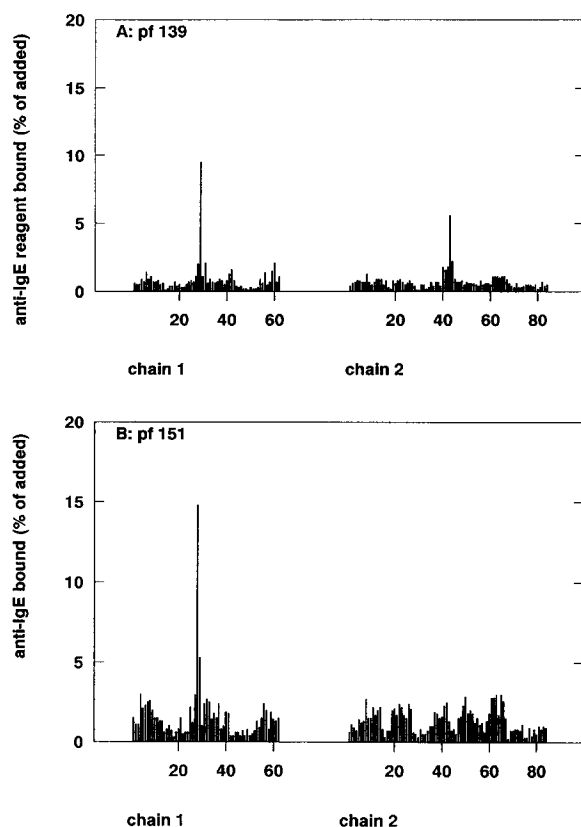


Fig. 3. Results of paper disc peptide-RAST with pf 139 (A) and pf 151 (B). The binding to paper disc-bound peptides 28–36 (B) and 29–37 (A) of chain 1 corresponded with the binding to Sepharose-coupled peptide 25–38 that had been previously found (Van Milligen et al., 1992b) for these IgE rich sera and was inhibited by *Fel d* I and this longer peptide. The binding of pf 139 to paper disc-bound peptide 43–51 of chain 2 (A) corresponded with the binding to Sepharose-coupled peptides 36–49 and 43–56 and was not inhibited by *Fel d* I and peptide 43–56. Binding of pf 139 to Sepharose-coupled peptide 1–14 of chain 2 and of both sera to Sepharose-coupled peptide 78–92 of chain 2 was not reflected in the paper disc-RIA.

Discussion

We have found that T bag synthesis with paper discs as solid-phase synthesis matrix followed by immunologic testing with the same paper discs without detachment of the synthetic peptides can be used for epitope mapping studies. The loading of peptides was relatively low: 3–6 times lower than the polyethylene pins of the epitope mapping kit and five times lower than the quarter discs used by Krchňák et al., who used quarter discs with a diameter of 1.3 cm and a loading of 750 nmol/cm² resulting in 248 nmol peptide per test. We found that in general the loading was high enough to obtain good results in the paper disc-RIA. However, for IgE antibody assays, the amount of peptide on each paper disc may not be sufficient. This problem may be solved to some extent by using more than one paper disc per test in the paper disc-RAST or by using another type of paper: Jaggi and co-workers found large variations in the number of reactive groups CNBr-activated paper discs for different kinds of paper (Jaggi et al., 1989).

The signal-background ratio in the paper disc-

RIA was good and of the same order of magnitude as in the PEPSCAN. For the monoclonal antibody against denatured sperm-whale myoglobin the signal-background ratio was 9 in the paper disc-RIA compared to 11 in the PEPSCAN, which makes this commercially available monoclonal antibody (Cambridge Research Biochemicals, Cambridge, UK) a good antibody for monitoring the peptide synthesis of the control peptides PLAQ and GLAQ. However, this monoclonal antibody bound poorly to the Sepharose-coupled peptides PLAQK and GLAQK, although we coupled four times as much peptide as we did in other epitope mapping studies: 16 nmol/test instead of 4 nmol/test (Van 't Hof et al., 1991, 1992). Perhaps these small molecules can penetrate into the pores of the agarose beads more easily than the longer peptides, making them inaccessible for antibodies. Both derivatized polyethylene pins (Geysen et al., 1987) and derivatized paper discs (Pasteka, 1984) have their reactive sites exposed on the hydrophilic outside which presumably renders even these very small peptides accessible for antibodies.

Assay reproducibility was good as demonstrated by binding of a monoclonal antibody against peptide 143–148 of interleukin-6. In our regeneration studies with 28 paper disc-bound peptide 143–148 of interleukin-6 we also found good reproducibility, although one paper disc yielded extreme high binding. This demonstrates that one has to beware of false positive results. The paper disc-RIA should be performed as duplicate assays. After elution of the antibodies from the paper discs with glycine/HCl, pH 2.5, with 10% dioxane the paper discs could be used again. However after each elution the residual binding was increased (Table II), limiting the number of times the paper discs could be used.

The two monoclonal antibodies against native *Fel d* I did not bind to any of the paper disc or pin-bound peptides, whereas for the Fd1-b related monoclonal antibodies binding has been observed with Sepharose-coupled peptides (Van 't Hof et al., 1992). We can only speculate about why this conformational epitope is not found with the paper disc-RIA or the pin-ELISA. Perhaps these antibodies have a low affinity towards short peptides, or possibly the combination of a large

TABLE II

RESULTS OF THE REGENERATION EXPERIMENTS USING 28 PAPER DISCS WITH COVALENTLY BOUND PEPTIDE 143–148 OF INTERLEUKIN-6 AND mAb 7

	Mean	Standard deviation
First elution	0.7	0.3
Second incubation	11.1	1.0
Second elution	1.7	0.4
Third incubation	11.8	0.6
Third elution	3.5	0.3

and deep paratope and short peptides makes binding to the solid phase-bound nonapeptides unfavorable by steric hindrance. In the example of both IgE rich sera we also found that binding to several Sepharose-coupled peptides was not reflected in the paper disc-RIA. On the other hand we found in the paper disc-RIA and the PEPSCAN a binding region for FdR-1a that had not been found with Sepharose-coupled peptides, peptide 13–21 of chain 1 (Van 't Hof et al., 1992).

Our results demonstrate that paper disc-bound peptides made by T bag synthesis can be used for epitope mapping studies. The parallel synthesis of a large number of paper disc-bound peptides is feasible and these paper disc-bound peptides can be used as the solid phase in a paper disc-RIA without detachment of the peptides from these paper discs. The signal-background ratio is comparable to that in the PEPSCAN and the reproducibility of antibody binding is generally good. Together with the possibility of regenerating the paper discs these properties make this technique a good alternative to the PEPSCAN. If large numbers of samples and peptides are to be tested, such as a test with 100 peptides and 100 antisera, our method is more convenient than the PEPSCAN. As we demonstrated for both chains of *Fel d* I it was quite easy to synthesize 15,000 paper disc-bound peptides and test these simultaneously with several antisera.

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