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# Towards assignment of secondary structures by anti-peptide antibodies. Specificity of the immune response to a β-turn

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**In an attempt to assign secondary structure elements to protein primary structures with antibodies, we synthesized a model peptide (β-peptide: TVTVTDPGQTVTY) with a putative β-turn structure and analysed the anti-peptide antibodies for their specificity towards the turn sequence. At least 50% of the peptide fraction adopts the intended conformation of a β-turn (DPGQ) inserted between the two segments of an antiparallel β-sheet structure. The specific anti-β-peptide antibodies of the hyperimmune response bind the β-turn containing epitope of the immunogenic β-peptide with a three orders of magnitude higher affinity than the synthetic control peptide (Gly-peptide: GGGGGDPGQGGGG). The affinity of the antibodies with specificity for the β-turn region increases from the primary to the hyperimmune response. Therefore, probing of secondary structure elements, i.e., of individual β-turn regions, by anti-peptide antibodies now seems feasible for proteins of known sequence and may result in sequence assignments of secondary structures.**

*Key words:* anti-peptide antibodies/β-turn model peptide/secondary structure assignment

## Introduction

Understanding of protein function at the molecular level requires knowledge of the detailed three-dimensional structure of the proteins. Elucidation of protein folding depends on primary structure information, which is now readily available from rapid DNA cloning and sequencing techniques, and on information on the spatial arrangement of the peptide backbone and side chains, which is still very difficult to obtain. To achieve the latter, the most reliable methods, X-ray diffraction (Liljas and Rossman, 1974) and two-dimensional n.m.r. spectroscopy (Wagner and Wüthich, 1982), provide very detailed information but require mg amounts of pure crystalline proteins, or are restricted to very small proteins of 5 – 10 kd mol. wt. Therefore, the rapidly growing number of proteins of known sequence far exceeds that of known three-dimensional structures.

Clues to the function of proteins, partly provided, for example, by site-directed mutagenesis or peptide synthesis, often do not rely on the knowledge of the entire spatial structure of the protein but only a specific part of it. It would be useful to devise a simple technique for determining the secondary structure of a limited part of the protein sequence, i.e., to assign particular residues to a secondary structure.

We made a first approach to this problem by generating polyclonal antibodies against a peptide with defined secondary structure, although for the eventual use of antibodies as tools for secondary structure determination monoclonal antibodies will be necessary. Since antibody binding to a specific primary sequence, folding into two different secondary structures, will probably not result in an all-or-none response but in different affinities, structure determination will require affinity measurement of monoclonal antibodies to the respective sequence. We describe experiments employing anti-peptide antibodies designed as probes for the β-turn conformation, this being the most frequently occurring secondary structure in proteins (Rose and Wetlaufer, 1977). A model peptide (β-peptide) was designed with a high probability of adopting β-sheet and β-turn conformations, according to the prediction rules of Chou and Fasman (1978). The characterization of this solid phase synthesized peptide by c.d.-analysis (circular dichroism) verified the intended structure for a large fraction of the peptide in solution. The β-peptide coupled to carrier protein was used as immunogen in rabbits to investigate the following two questions: (i) is it possible to raise antibodies against a typical β-turn structure that may be similar in sequence and structure to a large proportion of the animal's own proteins or are they tolerant to this structure? (ii) If they are capable of an immune response, do the resulting antibodies reveal specificity for the anticipated structure as well as sequence, or is the recognized epitope characterized just by the short linear peptide sequence without further structural contribution? To answer these questions we analysed the antibody specificity of the immune response to the β-turn structured peptide. Measurements of relative affinities of the antibodies we obtained towards the immunogenic and partially homologous peptides with different conformations give information about the size of the antigenic determinant, the importance of peptide structure and the participation of specific amino-acid residues in antigen-antibody binding. Furthermore, we present a system with potential for investigating the repertoire of the immune response against a defined proteinaceous antigenic determinant.

## Results

### *Design of the model peptide pair*

A pair of peptides with homology restricted to a tetrapeptide sequence with high β-turn propensity was designed. One of them should adopt β-turn conformation in the homologous region (β-peptide, Figure 1a) whereas the other should attain either no stable conformation or a different conformation (Gly-peptide, Figure 1b). To stabilize the β-turn conformation of the β-peptide the postulated β-turn sequence was inserted between two segments of an antiparallel β-sheet structure. When choosing the amino-acid sequence we took into account the prediction rules of Chou and Fasman (1978) and the observations of Brack and Orgel (1975) about the tendency of polypeptides with alternating hydrophilic and hydrophobic residues to adopt β-conformations. These considerations allowed the selection of a typical β-turn sequence (DPGQ) with a turn potential of 1.38 (Chou and Fasman, 1978) inserted between two segments of alternating Thr and Val residues with a sheet potential of 1.39 (Chou and Fasman, 1978;

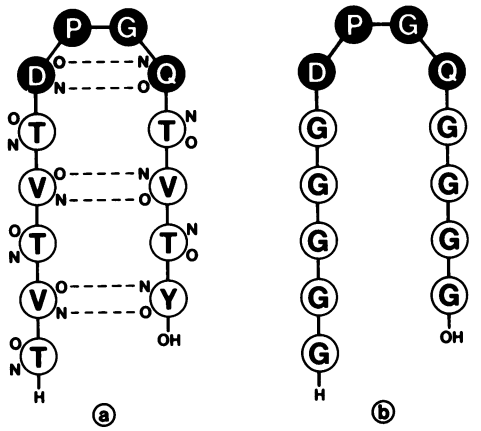

**Fig. 1.** Model peptides employed. Sequence and putative secondary structure of the synthetic β-peptide (**a**) and Gly-peptide (**b**). Dotted lines indicate hydrogen bonds between NH and CO groups of the backbone. The Gly-peptide is thought to adopt none of the known secondary structures. Therefore hydrogen bonds are not included in the structure of the Gly-peptide.

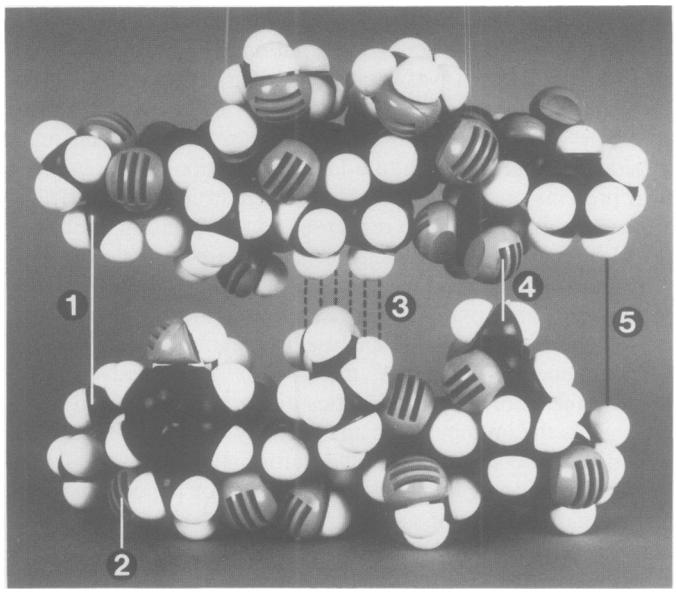


**Fig. 2.** CPK-space filling model of the β-peptide dimer. The structure of the monomer includes an antiparallel β-pleated sheet comprising residues 1–5, 10–13 and a β-turn (residues 6–9). The dimerization shown allows stacking of the C-terminal tyrosine residues, hydrophobic interactions between the valine side chains and formation of hydrogen bonds between the aspartic acid and glutamine side chains at the interface. The numbers indicate: (1) N-termini; (2) C-terminus; (3) hydrophobic interactions between valine side chains; (4) hydrogen bond between the aspartic acid (upper residue) and glutamine (lower residue) side chain; (5) β-turns.

Figure 1a). Providing that this peptide adopts the intended conformation, the Thr- and Val-side chains should point to opposite sides of the β-pleated sheet plane causing an unequal distribution of hydrophobic and hydrophilic amino acid side chains. This expected three-dimensional arrangement of the β-peptide is illustrated by a CPK- (Corey-Pauling-Kendrew) space-filling model shown in Figure 2. The Gly-peptide (Figure 1b) was designed for characterization of the putative β-turn specific antibodies with regard to the influence of sequence and secondary structure to their specificity. Therefore it is homologous with the β-peptide only in the β-turn sequence, but has a completely different sequence in the remainder. The hypothetic sheet structure of the β-peptide was replaced by stretches entirely composed of Gly-residues which should endow the peptide with high flexibility and minimize non-specific interactions between peptide and antibodies.

*Synthesis and purification of the β-peptide and Gly-peptide.*

Both peptides were synthesized by the solid-phase method (Merrifield, 1963; Gutte *et al.*, 1972) and purified by gel-permeation chromatography on Biogel P4 or P2. The purified synthetic peptides were characterized by amino acid analysis (Table I), reverse phase h.p.l.c. (Figure 3) and sequencing (data not shown) and proven to be of the desired chemical structures.

*C.d. spectroscopic studies*

The c.d.-spectra of the β-peptide and Gly-peptide in the far and in the near u.v. are depicited in Figure 4a–c. The far u.v.-spectrum of the β-peptide (Figure 4a) could be fitted satisfactorily [normalized r.m.s. (root mean square) errors $< 10\%$] with any set of standard data applied. The analysis which came closest to the 31% β-turn/59% β-sheet predicted according to the rules of Chou and Fasman (1978) was that with the standard data of Chang *et al.* (1978) which resulted in 2% α-helix, 33% β-sheet, 20% β-turn and 45% unordered structure. We cannot judge without further evidence whether less regular structure than predicted is realized in every molecule, or whether the prediction is fully realized only in one half or two thirds of the molecules, depending on reference being made to sheet or turn, respectively.

The c.d.-spectrum of the Gly-peptide (Figure 4b) differs quite strongly from that of the β-peptide, indicating a different conformation. Although the curve-fitting program proved unable to arrive at an unequivocal result, the structure of this molecule can be estimated to be mainly unordered and flexible. This is apparent from the fact that upon addition of 8 M urea/l, the spectrum of the Gly-peptide remains substantially unchanged, although measurements cannot be made below 205 nm because of the absorption of the urea. The complications encountered with the Gly-peptide are a reflexion of the inadequacy of the standard data, which are derived from protein X-ray structures or extended polypeptides, for the analysis of short peptides. The preponderance of optically inactive glycine residues may also contribute to the difficulties in interpretation.

*Aggregational behaviour of the β-peptide*

Since the design of the β-peptide took into account the tendency of polypeptides with alternating hydrophobic and hydrophilic residues to form β-sheet structures, we expected the aggregation of these peptides by building bilayer structures (Brack and Orgel, 1975). To prove that the peptides aggregate under certain conditions the approximate molecular size was determined by exclusion chromatography on Biogel P4 in 1 M acetic acid and in PBS (phosphate-buffered saline) (Figure 5a,b). The elution profiles clearly show an increase of apparent molecular weight of the β-peptide from ~1500 d (monomer) in 1 M acetic acid to 3000–3500 d in PBS (dimer or trimer). The aggregate could therefore be composed of two or three peptide monomers (mol. wt. 1379), but the most reasonable model for aggregation of alternating polypeptides (Brack and Orgel, 1975) by forming bilayer structures driven by hydrophobic interactions favours the existence of a β-peptide dimer.

*Characterization of β-peptide specific antibodies*

In the characterization of β-peptide specific antibodies we concentrated on the antibodies of the primary and hyperimmune

**Table I.** Amino-acid compositions[a] of the synthetic peptides before and after gel permeation chromatography

| | β-peptide | | | Gly-peptide | | | |
|---|---|---|---|---|---|---|---|
| | Theoretical composition | Protected resin bound | After Biogel P4 chromatography | Theoretical composition | Protected resin bound | After Biogel P2 chromatography | |
| | | | | | | Front peak | Sec. peak |
| Asp | 1 | 0.90 | 1.03 | 1 | 1.04 | 1.06 | 0.92 |
| Glu | 1 | 1.05 | 1.05 | 1 | 1.11 | 1.16 | 1.67 |
| Pro | 1 | 1.02 | 0.99 | 1 | 0.99 | 1.00 | 0.25 |
| Gly | 1 | 1.22 | 1.05 | 10 | 15.90 | 10.41 | 27.40 |
| Tyr | 1 | 1.02 | 0.92 | – | – | – | – |
| Thr | 5 | 4.90 | 4.94 | – | – | – | – |
| Val | 3 | 2.85 | 2.94 | – | – | – | – |

[a] Values are expressed as mol per mol of peptide; the background for the other residues was <5% of total. The analyses were performed on a Beckman 121 M amino-acid analyser.

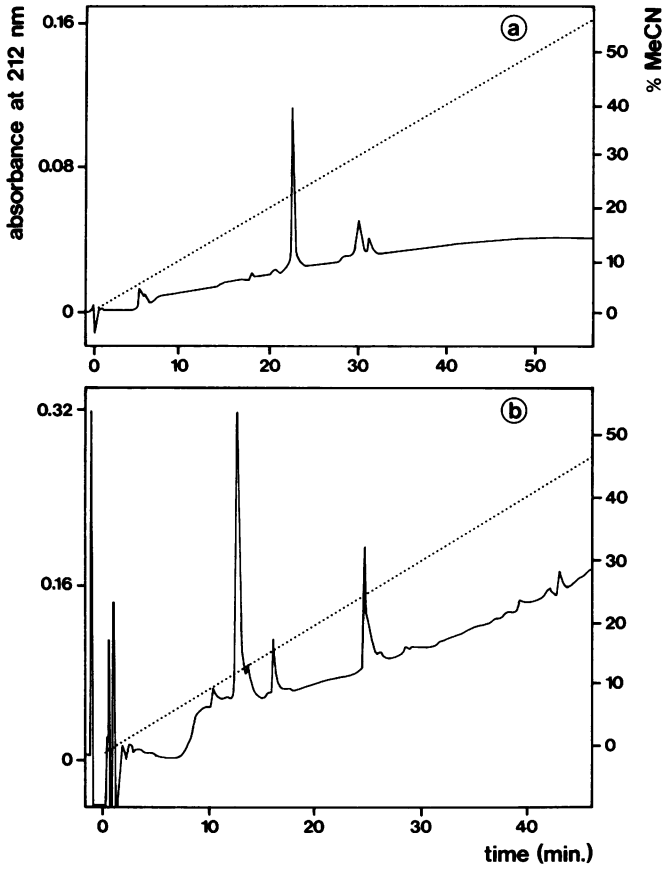


**Fig. 3.** Reverse phase h.p.l.c. of β-peptide (**a**) and Gly-peptide (**b**) after purification by gel permeation chromatography. ~3 nmol (**a**) and 10 nmol (**b**) peptide were separated in a single run on a C8-column [0.46 x 25 cm, Baker (**a**) or Du Pont (**b**)]. Solvent A is 0.1% TFA in water and solvent B is 0.1% TFA in 60% MeCN. The column was run at ambient temperature with a flow rate of 1 ml/min and a linear gradient of 1.67% B/min. Absorbance was recorded at 212 nm. The peptides eluting at 12 and 25 min in (**b**) had the same compositions after HCl-hydrolysis (data not shown). Treatment of the latter peptide at pH 9.0 for 12 h at 37°C resulted in material also eluting at 12 min.

response, presuming that this appraoch should enable us to detect potential changes during the course of the immune response. The β-peptide specific antibodies constituted 0.4% of the primary antibodies and 2.5% of the tertiary antibodies as determined by affinity chromatography on β-peptide Sepharose. The analysis of the antibody specificities was performed by inhibition experiments in the form of solid phase radioimmunoassays (Figure 6a–d). The results reveal a remarkable difference between the specificity of the primary and hyperimmune response.

Primary antibodies before affinity purification can be inhibited in binding to coated β-peptide-BSA (bovine serum albumin) by all five free peptides used in these experiments but there are two kinds of inhibition: two peptides lead to the level of inhibition achieved by free β-peptide. The remaining background was shown to be due to antibodies which are specific to glutaraldehyde-treated BSA (data not shown). The three peptides competing almost fully are the immunogenic β-peptide, the partially homologous Gly-peptide and the T14-peptide (Figure 6a). The T14-peptide does not contain a long homologous sequence stretch but does contain many Thr-residues, which are also found in the β-peptide, and Ser-residues which are known to occur frequently in β-turn structures (Chou and Fasman, 1978; Brahms *et al.*, 1977). The ability of the Gly-peptide to exhibit total inhibition in this assay implies that most of the β-peptide binding antibodies recognize the 'turn' sequence, although simultaneous specificity for additional different epitopes is not excluded.

The second kind of inhibition is caused by the β-peptide, V10- and A13-peptide which are much stronger inhibitors than the Gly-peptide and T14-peptide (concentrations for 50% inhibition: Gly-peptide, $6 \times 10^{-5}$ M; T14-peptide, $4 \times 10^{-6}$ M; V10-peptide, $10^{-7}$ M; A13-peptide, $4 \times 10^{-8}$ M; β-peptide, $2 \times 10^{-8}$ M). The V10- and A13-peptide, however, cannot inhibit the whole fraction of β-peptide binding antibodies (~85% inhibition at maximum). The three stronger inhibitor peptides of the second group, β-peptide, V10- and A13-peptide, hold in common a C-terminal tyrosine which obviously contributes to an epitope recognized by the main part of the β-peptide binding primary antibodies, although a small fraction cannot be inhibited by the V10- and A13-peptide. Since the main part of the primary β-peptide binding antibodies is inhibitable by the tyrosine-containing peptides and the 'turn' peptides as well, although by different concentrations, a simultaneous specificity of one antibody molecule for both epitopes is inferrable (multispecificity, Berzofsky and Schechter, 1981). The affinity of these antibodies to the tyrosine-containing epitope of peptides V10, A13 and the β-peptide is higher than to the 'turn' epitope of the Gly- and β-peptide, but obviously not sufficient for isolation of these antibodies by affinity chromatography on β-peptide Sepharose, where the C-terminal tyrosine containing epitope is possibly inaccessible. The binding of the putative multispecific antibody fraction to the β-turn epitope of the affinity column is not strong enough for retention. Accordingly, the affinity-purified primary antibodies can be inhibited only by the β-peptide and in much higher concentrations by the Gly-peptide and T14-peptide (Figure 6b).

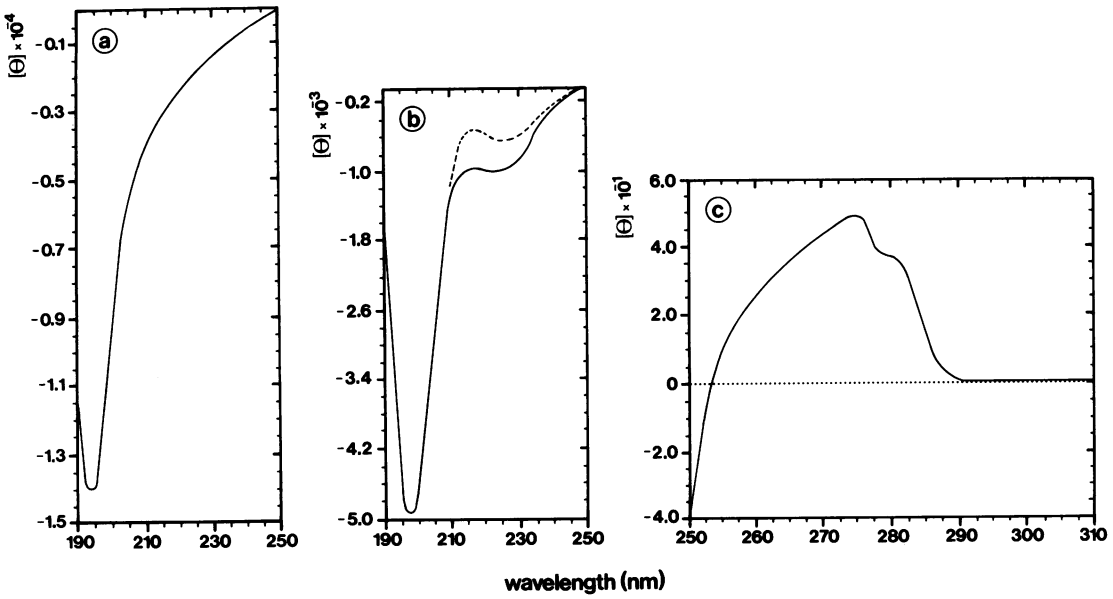


**Fig. 4.** C.d.-spectra of the β-peptide (**a,c**) and the Gly-peptide (**b**). The peptides were dissolved in 10 mM phosphate buffer pH 7 (**a, b, c**, solid line) and the same buffer containing 8 M urea (**b**, dotted line). The spectra were recorded at ambient temperature. The peptide concentrations were ~0.3 mg/ml (**a,c**) and 1.3 mg/ml (**b**), respectively. C.d.-band intensities are expressed as the mean residue ellipticity, $\theta$ (in units of degrees cm² per dmol).

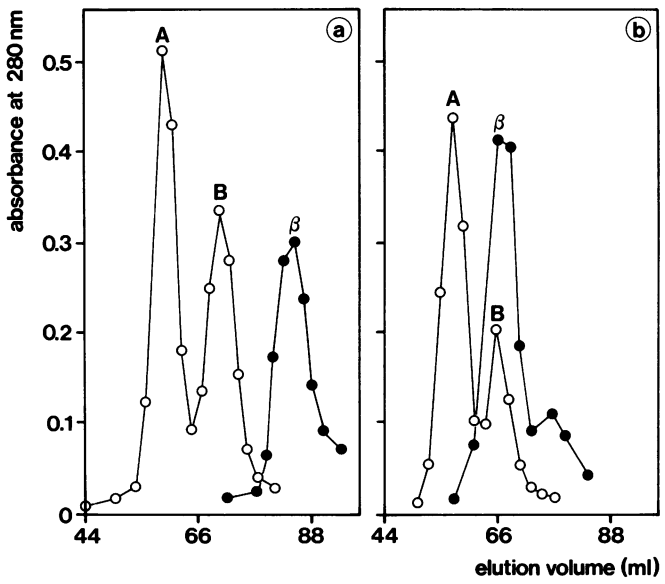


**Fig. 5.** Chromatography of the synthetic β-peptide (filled circles) and molecular weight standards (open circles) on Biogel P4 (column: 180 x 1 cm) in 1 M acetic acid (**a**) and PBS (**b**). Marker proteins employed are: (A) aprotinin, mol. wt. 6500; (B) insulin B, mol. wt. 3500; (β) β-peptide monomer, mol. wt. 1379.

The specificity of the tertiary antibodies (after three immunizations, hyperimmune response) before and after affinity purification conforms to that of the primary affinity-purified antibodies. The tertiary antibodies do not bind the tyrosine-containing, but otherwise unrelated, peptides V10 and A13 (Figure 6c,d) even before affinity purification. The amount of specific antibodies in the hyperimmune response is increased by a factor of ~6 giving rise to 2.5% tertiary antibodies with β-peptide specificity compared to the 0.4% of the primary response. The affinity to the inhibiting peptides, β-peptide, Gly-peptide, and T14-peptide seems also to be changed as illustrated in Figure 6b,d and revealed by one order of magnitude difference in antibody concentration employed for the competition experiments. Interestingly, competition is exerted by the same concentrations of free β-peptide and free Gly- and T14-peptide for both antibody preparations, as measured at half saturation of anti β-peptide antibody binding to β-peptide conjugates. We conclude that the specific primary and hyperimmune response to β-peptide as hapten does not lead to antibodies of altered relative affinities for the three peptides employed for the competition studies.

## Discussion

We presented experiments on the synthesis of a short peptide composed of 13 amino acid residues which adopts at least 50% of the anticipated combination of β-turn and β-sheet structure. This result is quite surprising since theoretical considerations (Anfinsen and Scheraga, 1975) and practical experience (Sachs *et al.*, 1972; Green *et al.*, 1982) have shown that short peptides very rarely attain a stable secondary structure. The shortest polypeptides supposed to adopt one of the classical secondary structures are 20–40 residues long (Pfaff *et al.*, 1982; Moser *et al.*, 1983; Kullmann, 1984), just the range of size for the observed β-peptide aggregate. So the intrinsic conformational propensities of the β-peptide seem to be realized by intermolecular long-range interactions which stabilize the sequence-favoured β-sheet, β-turn structure. The Gly-peptide is inferred to be of unordered or random structure like most peptides of similar size.

The results of immunizations with β-peptide-KLH-conjugate clearly demonstrate the possibility of raising antibodies against the specific sequence adopting a β-turn structure but there is a remarkable change of antibody specificity in the course of the immune response. Whereas the main fraction of the primary immunoglobulins seems to be multispecific, i.e., that it recognizes the β-turn sequence and a tyrosine-containing epitope as well, the tertiary antibodies only bind to the turn sequence or to another similar sequence. This finding may have several reasons, none of which can be unequivocally proven by the experiments done so far, but will be the subject of future experiments. Nevertheless, a potential relation between the probably poor immunogen and

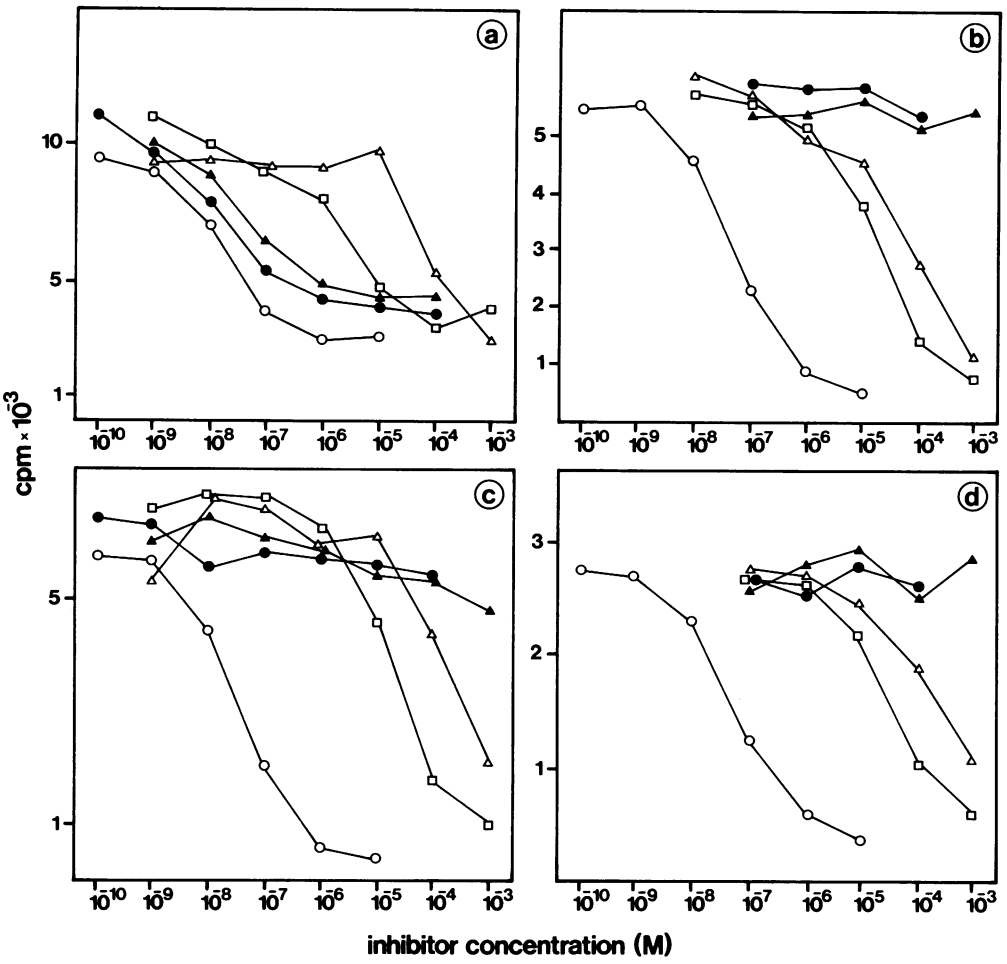


**Fig. 6.** Analyses of the antibody specificity by solid-phase radioimmunoassay. The presented data show the characteristic features of the immune response of one rabbit to the immunogenic β-peptide, coupled to KLH *via* glutaraldehyde, by comparing the inhibitory effect of five synthetic peptides on antibodies of the primary immune response before (**a**) and after (**b**) and of the hyperimmune response before (**c**) and after (**d**) affinity chromatography. The following antibody concentrations were employed in the assay to give half-maximal binding to the coated β-peptide-BSA without inhibition: a: 85 μg/ml; b: 130 ng/ml; c: 6 ng/ml; d: 12 ng/ml. The sequences of the five tested peptides are: ○—○, β-peptide: TVTVTDPGQTVTY; △—△, Gly-peptide: GGGGGDPGQGGGG; □—□, T14-peptide: TTSSGTTSSTTSSG; ▲—▲, V10-peptide: VFGDEKASFY; ●—●, A13-peptide: AKYDYYGSSYFDY. (degenerated) T, R(NO₂)

the characteristics of the primary immune response are indicated. Providing that the rabbits are tolerant to β-turns existing in their own proteins they will show little or no reactivity to antigens similar to these structures unless the immune system is capable of developing specific antibodies during the progression of the immune response. Starting with B-cell clones originally specific to a determinant different from the immunogenic one, but with additional low affinity to the immunogenic peptide, these clones could be stimulated for immunoglobulin production in our case leading to the fraction of tyrosine- and β-turn binding antibodies. After several immunizations those clones expressing antibodies with relatively high affinity to the immunogenic peptide should expand selectively according to the clonal selection theory (Burnet, 1959), thereby changing the specificity of the polyclonal antiserum. The final antibodies could either originate from few incipient B-cell clones which already expressed high affinity antibodies and expanded preferentially during the immune response, or from B-cell clones which synthesized low affinity antibodies in the beginning but became more specific in the later immune response, presumably by somatic mutations. In any case the low affinity multispecific antibodies of the primary immune response could perhaps represent some kind of 'non-specific immunoglobulins' which are known to be induced by antigen injection but could not yet be detected to possess antibody function (Antoine and Avrameas, 1976).

The antibodies of the hyperimmune response and those of the primary immune response after affinity purification reveal a more restricted binding pattern. They possess a relatively high affinity to the immunogenic β-peptide but a roughly three magnitudes lower affinity to the partially homologous Gly-peptide. Whereas this result was expected and intended, the antibody binding to a third peptide, a threonine-rich peptide, (T14 peptide) was quite surprising. Evaluation of possible sequence-related or structure-based reasons for the observed cross-reactions revealed the following common features: the β-peptide and Gly-peptide possess a homologous sequence of four consecutive amino acid residues selected as residues of high β-turn propensity which according to c.d.-analysis adopt different conformations in both peptides. From studies of the size of the antibody-combining site (Amzel and Poljak, 1979; Poljak *et al.*, 1976) it is known that peptides of five (β-sheet conformation) to ten (α-helix conformation) amino acid residues can be accommodated by the binding crevice, implying that the homologous tetrapeptide sequence of the Gly-peptide is incapable of specific use of the whole area

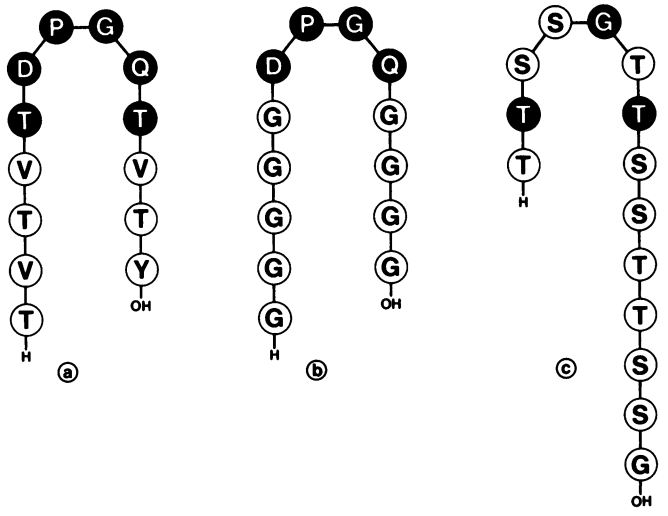


**Fig. 7.** Antigenic determinants shared by β-peptide (**a**), Gly-peptide (**b**) and postulated for the T14-peptide (**c**). The amino-acid residues in filled circles indicate regions of homology between the immunogenic β-peptide and the other two inhibiting peptides.

available for contact and binding to the antibody. Moreover, one should take into account the higher flexibility of the Gly-peptide compared with the β-peptide, which contributed additionally to a low-affinity binding (Pfaff *et al.*, 1982).

The most obvious similarity of the T14-peptide and β-peptide is the high content of Thr residues suggesting that they constitute a considerable part of the epitope consistent with the size of an antibody binding site as discussed above. In addition the remaining Ser and Gly residues confer a propensity for β-turn structure upon the T14-peptide (Brahms *et al.*, 1977; Lewis *et al.*, 1971) which may contribute to antibody binding by restriction of peptide flexibility. Figure 7 shows a comparison of the supposed antigenic determinant of the three cross-reacting peptides.

The difference in antibody affinity to the immunogenic β-peptide of possibly β-pleated sheet, β-turn structure and to the Gly-peptide of possibly unordered structure amounts to about three orders of magnitude. A similar difference in affinity is found for the T14-peptide. This encourages us to proceed towards structure examination using the anti-peptide-antibody approach. Improvements like enlargement of the homologous turn region from four to six residues, the retaining of the dimerizing Thr-Val skeleton as turn-flanking sequences and the use of monoclonal antibodies for affinity measurements should provide us with suitable tools for probing predicted turn regions in proteins of unknown three-dimensional structures.

## Materials and methods

Solvents and reagents for solid-phase peptide synthesis were of analysis grade as specified by the suppliers (Fluka, New Ulm, Merck, Darmstadt and Riedel de Haen, Seelze Hannover, FRG). Solvents used for h.p.l.c. separations and sequencing were h.p.l.c. and sequencer grade, respectively (Applied Biosystems, Forster City, USA; Baker, Deventer, Netherlands; Beckman Instruments, Palo Alto, USA). The A13-peptide was a gift of Dr. S.Zaiss. Rabbits were derived from cross-breedings between 'Deutsche Riesen, Grau' and Chinchilla-cross-breeds.

*Synthesis of peptides*

The polypeptides (10 – 14 amino-acid residues) were synthesized by the solid-phase method (Merrifield, 1963) according to Gutte *et al.* (1972). After coupling of the C-terminal and t-Boc-amino acid to chloromethylated (1.07 mM Cl/g resin) styrene-1%-divinylbenzene beads (Gutte and Merrifield, 1971) synthesis proceeded with α-amino-t-Boc protected amino acids. The side chains of aspartic acid, glutamic acid, serine, threonine and tyrosine derivatives were blocked by the benzyl group. The ε-amino group of lysine was protected by the benzyloxycarbonyl group and the guanidino function of arginine by the nitro group. Cleavage of the peptides from the resin with simultaneous removal of sidechain protection groups was performed by treatment with anhydrous HF (Gutte *et al.*, 1972) or HBr in 50% TFA/methylenechloride (Gutte and Merrifield, 1971) depending on the amino acid composition of the respective peptide. After removal of the HF, the resin was washed with ether and dried *in vacuo*. The resulting mixture of peptide and resin was extracted three times with 50% acetic acid, filtered and evaporated to dryness. After treatment with HBr the cleaved synthetic material was filtered, the resin washed with TFA and the combined filtrates were extracted with ether. The peptide-containing water phase was evaporated to dryness. The remaining peptide was dissolved in 1 M acetic acid for further purification.

*Peptide purification*

The various peptides were purified by column chromatography on Biogel P2 or P4 (200 x 2.0 cm) in 1 M acetic acid which normally results in 80 – 90% pure compounds as determined by analytical procedures. Purification of the Gly-peptide required an additional separation of two co-eluting components by chromatography on DEAE cellulose (Whatman DE 52) using a gradient of 0.01 – 1 M ammonium bicarbonate. A peptide of expected composition, charge, and size eluted in the second peak. Reversed phase h.p.l.c. showed that 90 – 95% of this material elutes as a single, symmetric peak. The major contaminant eluting as the first peak from DE 52 was chemically indistinguishable from the DE 52 peak 2 material, but was of different charge due to an alkali-labile (sensitive to treatment at pH 9.0 for 12 h at 37°C) modification of the aspartic acid or C-terminal carboxyl group. For h.p.l.c. separation of peptides a C8-column (Du Pont, 70 Å pore size, or Baker 0.46 x 25 cm, 300 Å pore size) was routinely applied and developed at 1 ml/min with a gradient system of 0.1% TFA and 0.1% TFA/60% acetonitrile. The purified peptides were characterized by amino-acid analysis, amino-acid sequencing and by thin layer electrophoresis (Offord, 1966). Automated Edman degradation was done in an updated Beckman 890 B sequencer with 10 – 20 nmol peptide using a 0.2 M Quadrolprogram (Beyreuther *et al.*, 1977) and a gas-phase sequencer (Applied Biosystems) with 1 – 2 nmol peptide. Both systems employed 1.5 – 3 mg Polybrene (Tarr *et al.*, 1978).

*Determination of peptide concentration*

The concentration of tyrosine-containing peptides for inhibition experiments and c.d. studies was determined by molar absorption coefficients (Wetlaufer, 1962). Otherwise it was measured by amino-acid analysis on a Beckman 121 M amino-acid analyser after hydrolysis in 6 M HCl for 24 h at 110°C.

*C.d. spectroscopy*

The circular dichroic measurements were carried out using a computerized version (Glatter and Szameit, in preparation) of a Cary 61 spectrometer, calibrated with 0.1% d-10-camphosulphonic acid. Peptide samples were dissolved in 10 mM phosphate buffer pH 7 and the same buffer containing 8 M urea, respectively. The spectra were recorded at 1.5 nm spectral bandwidth and were not corrected with respect to refractive index. A curve-fitting program was used to estimate the various proportions of secondary structure from standard curves (Chang *et al.*, 1978; Bolotina *et al.*, 1979; Brahms and Brahms, 1980).

*Coupling of synthetic peptides to carrier proteins*

The β-peptide was coupled to KLH (Calbiochem) and BSA (Sigma >98% pure) with glutaraldehyde (Sigma, grade I, 25% solution) according to Walter *et al.* (1980). After coupling, the protein-peptide-conjugates were exhaustively dialysed against PBS. About 4 – 9 mol peptides were conjugated per mol of BSA or KLH subunit with an assumed average mol. wt. of 55 kd (Garvey *et al.*, 1977). The coupling efficiency was determined by comparing amino-acid analyses of the carrier proteins before the conjugation procedure and the theoretical amino-acid compositions of BSA (Dayhoff, 1976) and KLH (Malley *et al.*, 1965) with amino-acid analyses of the respective peptide-protein-conjugates. The repetitive sequence of the β-peptide greatly facilitated these calculations leading to reproducible results for the conjugates.

*Preparation of antibodies against the β-peptide*

Antisera were obtained by immunizing rabbits with 700 μg of peptide-KLH-conjugate, emulsified in complete Freund's adjuvant (Difco Lab.) at a 1:1 (v/v) ratio. The s.c. injection was repeated after day 28 with 100 μg conjugate in incomplete Freund's adjuvant and followed by i.v. injection after day 49 with 50 μg peptide-KLH in 0.9% NaCl. Blood samples were taken on day 0, 28, 35, 38 and 56.

The serum was fractionated by ammonium sulfate precipitation and affinity chromatography on a 2 ml column containing 5 mg β-peptide coupled to 0.5 g CNBr activated Sepharose 4B by a procedure recommended by the manufacturer (Pharmacia). The antibody fraction of 10 ml serum was incubated with 2 ml β-peptide Sepharose overnight at 4°C and transferred onto a 0.7 x 5 cm column. The flow-through volume was saved for controls; the bound material was eluted with 0.1 M glycine/HCl pH 2.1 in 1-ml fractions, which were neutralized immediately by addition of solid Tris-base. Protein concentrations in all fractions and in the flow-through volume were determined by absorbance measurements at 280 nm

and the binding activity to β-peptide-BSA by radioimmunoassay. The pooled β-peptide specific antibodies were dialysed against PBS and stored at −20°C.

*Solid phase radioimmunoassays*

To ensure that antibodies were specific for the peptide itself and not directed against the carrier protein or the coupling reagent the following radioimmunoassay was performed. Microtiterplates (Dynatech) were coated with BSA-β-peptide or BSA treated with glutaraldehyde as control (2 μg/ml) in 15 mM $Na_2CO_3$, 30 mM $NaHCO_3$ pH 9.6 overnight at 4°C, washed with 0.05% Triton in PBS, incubated for 1 h with 1% ovalbuminin PBS at ambient temperature, washed again and incubated with 50 μl of serial dilutions of antibodies in 1% ovalbumin for 3 h. After an additional wash the specifically-bound antibodies were detected by $^{125}$I-labelled goat anti-rabbit antibodies (Biorad, affinity purified). The radioactivity was measured in a gamma counter. Pre-immune sera were used in this assay as controls.

In order to characterize the specificity of the antibodies in more detail the inhibition of antibody binding to β-peptide-BSA by various free peptides was measured. The inhibiton assay was performed as the normal binding assay with the following modifications. A β-peptide-BSA solution of 1 μg/ml was used for coating to reduce the favoured cooperative antibody binding to the fixed conjugate. The antibodies were diluted to give half-maximal binding without inhibition and were pre-incubated with serial dilutions of free peptide at 4°C overnight before addition to the sensitized microtiterplates.

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