Isolation of Populations of Antipeptide Antibodies Directed against Different Epitopes of the Same Fragment

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Synthetic Peptides, Epitopes, Antibody Populations

Rabbit antibodies against small peptides may be composed by subpopulations recognizing different epitopes made likely by few amino acids. This explains the frequent crossreactivity of antipeptide antibodies with unrelated peptides.

A suitable use of immunoadsorbents is suggested to obtain truly specific antibodies able to react with restricted amino acid sequences.

Immunization of rabbits with hapten conjugates made with synthetic peptides has been used recently in many laboratories with the aim of producing immune sera with predetermined specificity [1–3]. Since the specific antipeptide antibodies have been isolated in very few cases [4, 5], little is known about their molecular complexity. One might expect that a small peptide would present a single antigenic determinant and that the rabbit immune response against the hapten would consist of homogeneous immunoglobulin molecules. The present communication indicates on the contrary that the antibodies elicited by a small 14-residue fragment consist of IgG moieties recognizing different epitopes. A partial characterization of the specificity of those antibodies is presented. Two immune sera (6148 and 6150) were prepared by immunization of two rabbits with two very similar, but not identical peptides (Table I). The two synthetic fragments were coupled to KLH as carrier protein and then used for immunization. Details of the peptide synthesis, the coupling to the carrier protein, and the schedule of immunization have been given elsewhere [5]. Immunoglobulins was recovered from these immune sera by precipitation with ammonium sulphate at 50% saturation. The antipeptide antibodies were then isolated by chromatography on specific immunoadsorbents prepared by linking the terminal –SH group of each peptide to Sepharose-tetramethylendiamine resins with the aid of MBS, as previously described [4].

The binding of the purified antipeptide antibodies 6148 and 6150 was tested against peptides 1 and 2 by an ELISA assay and a partial crossreactivity was found. This is predictable since the two fragments share 12 of the 14 amino acid residues. When examined by SDS-PAGE, each antibody showed a polymorphic distribution of heavy and light chains. Although some of the band multiplicity could be explained in terms of allotypic heterogeneity in both heavy and light chains of the immunoglobulins in the rabbits randomly selected for immunization, this result might indicate that the immune response of the animals was polyclonal, and directed against different epitopes of the small fragment. Each antibody population could comprise a mixture of specificities, some of which could recognize determinants in the C-terminal half, some other recognize determinants in the N-terminal half of the peptide. Since those latter antigenic sites are characteristic of each fragment, some antibody populations for each peptide might not react with the "variant" fragment.

To confirm this possibility, the two purified antibody preparations were chromatographed again on immunoadsorbents, but this time antibody 6148, elicited by peptide 1, was loaded on the Se-TMD-Pept 2 immunoadsorbent, while antibody 6150, raised against peptide 2, was chromatographed on

Table I. Amino acid sequences of the two synthetic peptides used as immunogens.

		Antisera
1)	YNSQKDILEEARAC	6148
2)	YNSQMDFLEEARAC	6150

Peptide 1 was selected from the sequence of human HLA-DR2 membrane glycoproteins (positions 61 to 73). Peptide 2 is a variant.

The amino acid differences of the two peptides are both located in the amino terminal half. The two fragments could be approximately considered as formed by a variable N-terminal, and a constant C-terminal half.

Abbreviations: Se-TMD-Pept., Sepharose-tetramethylen-diamine-peptide immunoadsorbent; KLH, Keyhole Limpet Hemocyanin; MBS, *m*-maleimidobenzoyl-N-hydroxysuccinimide ester.

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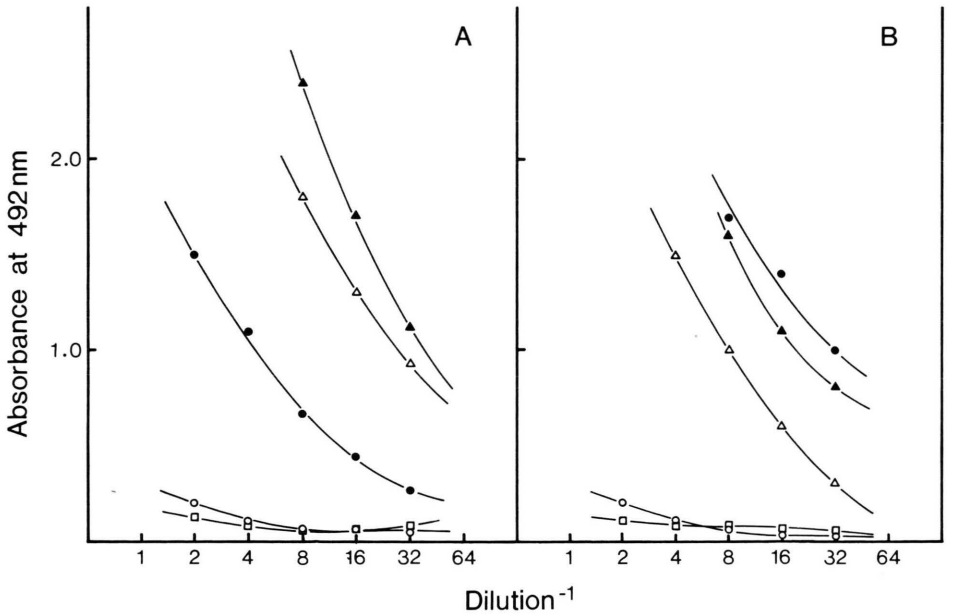

Fig. 1 A. Binding of antibody 6148/A and 6148/E, obtained by chromatography on immunoadsorbents, to peptides 1 and 2 in ELISA. 6148/A recognizes both the immunizing peptide (▲) and the related peptide (△); 6148/E reacts only with peptide 1 (●), but not with the "variant" fragment 2 (○). Values obtained with control rabbit IgG are also shown (□). B. Binding of antibody 6150/A and 6150/E to the peptides 1 and 2. 6150/E recognizes the immunizing peptide 2 (●), but not the "variant" fragment 1 (○).

Se-TMD-Pept 1 immunoadsorbent. By this technique, each antibody preparation was separated into two fractions, one promptly eluted from the resin by PBS (E), and one adsorbed (A). The latter was subsequently eluted by the use of acidic buffers.

All four fractions obtained were then tested against peptides 1 and 2 in the ELISA assay. The results are shown in Fig. 1 A and 1 B and indicate that antibodies 6148/A and 6150/A still recognize both peptides 1 and 2. In contrast, antibodies 6148/E and 6150/E react only with the eliciting peptide and do not crossreact with the "variant" peptide. This appears to confirm that the antibody populations 6148/A and 6150/A are directed against antigenic determinants common to both peptides (possibly the C-terminal half), whereas antibodies 6148/E and 6150/E are specific for antigenic sites represented in the N-terminal portion of the peptide used as immunogen.

The existence of multiple specificities in the antipeptide antibodies elicited by a small fragment is also suggested by the difference in reactivity of antibodies 6148 and 6150 against the intact proteins from which peptide 1 was derived (HLA-DR2 glycoproteins from human lymphoblastoid cells): Antibody 6148 recognizes antigenic sites on the native protein, whereas antibody 6150 does not [5]. This result would also imply the fact that the C-terminal part of the fragment, containing the "common" determinants, is buried or masked in the three dimensional folding of the native molecule.

Once demonstrated that antipeptide antibodies may contain subpopulations with different specificities, it is obvious that is possible to minimize the frequent crossreactivity of antipeptide antibodies against very similar peptides, as these selected from the primary structure of the products of allelic genes, and obtain useful reagents able to recognize specifically the sequence of few amino acid residues.

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