

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

Alberto Chersi, Christiane Greger,
and Richard A. Houghten

Regina Elena Institute for Cancer Research, Lab. of Biochemistry, Viale R. Elena 291, 00161 Rome, Italy, and Scripps Clinic and Research Foundation, La Jolla

Z. Naturforsch. **40c**, 148–149 (1985);
received August 20, 1984

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 cross-reactivity 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-Pept2 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.

Reprint requests to Dr. A. Chersi.

0341-0382/85/0100-0148 \$ 01.30/0



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

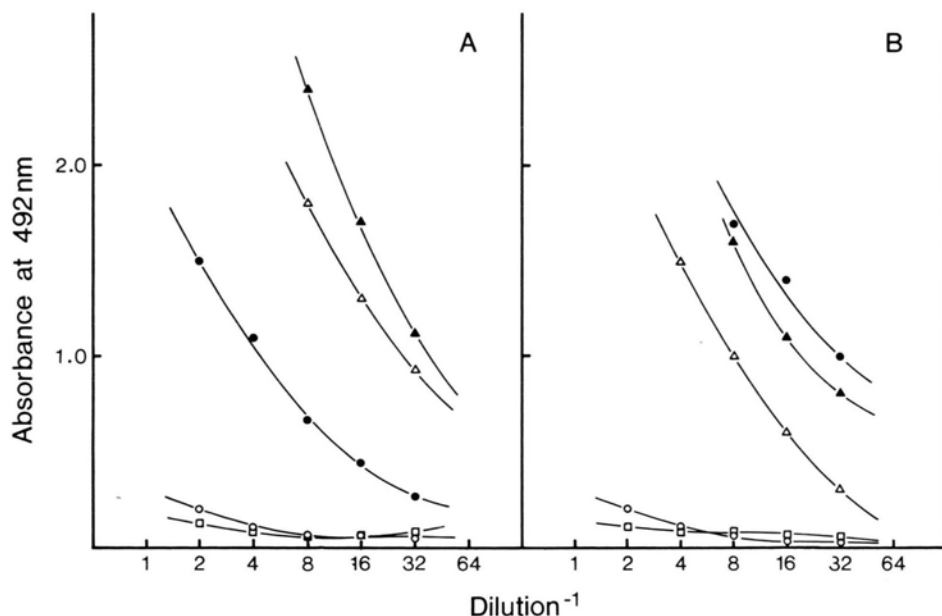


Fig. 1 A. Binding of antibody 6148/A and 6148/E, obtained by chromatography on immunoadsorbents, to peptides 1 and 2 in ELISA. 6148/A recognizes both the immunizing peptide (▲) and the related peptide (Δ); 6148/E reacts only with peptide 1 (●), but not with the "variant" fragment 2 (○). Values obtained 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 anti-peptide 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 anti-peptide antibodies may contain subpopulations with different specificities, it is obvious that is possible to minimize the frequent crossreactivity of anti-peptide 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.

The authors thank Miss Cristina Cenciarelli for skillful assistance.

- [1] N. Green, H. Alexander, A. Olson, S. Alexander, T. M. Shinnick, J. G. Sutcliffe, and R. A. Lerner, *Cell* **28**, 477 (1982).
- [2] A. R. Neurath, S. B. H. Kent, and N. Strick, *Proc. Natl. Acad. Sci. USA* **78**, 7871 (1982).
- [3] W. R. Church, L. E. Walker, R. A. Houghten, and R. A. Reisfeld, *Proc. Natl. Acad. Sci. USA* **80**, 255 (1983).

- [4] A. Chersi and R. A. Houghten, *Z. Naturforsch.* **39c**, 646 (1984).
- [5] A. Chersi, G. Schulz, and R. A. Houghten, *Mol. Immun.*, submitted.