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Chemistry

Specific binding of antigenic peptides to separate α and β chains of class II molecules of the major histocompatibility complex

(antigen presentation/T-helper lymphocytes/reaction mechanism/protein structure)

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ABSTRACT **Class II molecules of the major histocompatibility complex bind antigenic peptides and present them to T-helper cells. Class II molecules are heterodimers consisting of one α and one β chain. Here we report that each isolated α and β chain binds antigenic peptides and that this binding is specific. The specificity of peptide binding was investigated by employing the murine major histocompatibility complex haplotypes I-A^d and I-E^k and fluorescence-labeled peptides of chicken ovalbumin and pigeon cytochrome *c*, respectively, which are known to be specific for these haplotypes. The major histocompatibility complex molecules were incubated with these peptides and subjected to SDS/PAGE under nondenaturing conditions. The gels were then scanned for the fluorescent peptides and, after silver staining, for proteins. We found that the fluorescence-labeled peptide fragment of ovalbumin bound preferentially to the isolated α and β chains of I-A^d, whereas the fluorescence-labeled peptide fragment of pigeon cytochrome *c* bound preferentially to the isolated α and β chains of I-E^k. The α and β chains of each haplotype bound their specific peptides about equally well, suggesting comparable affinities. Our results indicate that *in vivo* the kinetic pathway for the formation of antigenic peptide complexes with the α/β heterodimers may involve the initial formation of complexes of the α and/or β chains with the specific antigenic peptides.**

Affinity-purified molecules of the class II major histocompatibility complex (MHC) are known to bind antigenic peptides (1–3). Planar lipid membranes containing purified MHC molecules with bound peptide can mimic antigen-presenting cells in the triggering of specific T-helper cells (4). However, it has been found that purified MHC α/β heterodimers are not stable but may form intermediate structures and subsequently dissociate into α and β chains (5). In these experiments, antigenic peptides tagged with a fluorescein label were found to be associated with two conformations of the class II α/β heterodimers ("compact" and "floppy") and also with monomeric α and β chains (5). The significance of this result is clarified in the present work, where it is shown that the binding of the peptides to the isolated α and β chains is specific. Monomeric α and β chains from the murine class II molecules I-A^d and I-E^k preferentially bind the fluorescein-labeled peptide fragments of chicken ovalbumin [FOva(323–339)] and pigeon cytochrome *c* [FpCytc(88–104)], respectively. Involvement of the α/β heterodimers is not required since α and β chains isolated separately in detergent micelles react with the antigenic peptides. Thus the structural features of α and β chains that are involved in peptide binding and in peptide–MHC specificity need not depend on the formation of the α/β heterodimer.

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MATERIALS AND METHODS

I-A^d and I-E^k were affinity purified from A20.1.11 and CH27 cells as described (5). Briefly, the cells were grown in RPMI medium supplemented with fetal calf serum and were lysed with Nonidet P-40. The lysate was centrifuged for 90 min at 100,000 × *g* and, from the supernatant, a pool of glycoproteins was obtained by chromatography on lentil lectin-Sepharose 4B. I-A^d and I-E^k were finally purified by affinity chromatography with the antibodies MKD6 and 14-4-4S, respectively. The pool of glycoproteins was applied to the antibody column, Nonidet P-40 was replaced by octyl β-D-glucopyranoside (OG), and I-A^d and I-E^k were eluted with sodium carbonate buffer at pH 11.0 or 11.5, respectively. The fractions containing MHC molecules were neutralized immediately.

I-A^d was incubated with the fluorescein-labeled peptides FOva(323–339) and FpCytc(88–104) for 2 hr at 37°C in 10 mM potassium phosphate, pH 7.4/150 mM NaCl/30 mM OG/0.01% merthiolate/1 mM phenylmethylsulfonyl fluoride. Immediately after incubation, the samples were cooled to 4°C and subjected to SDS/PAGE. I-E^k was incubated for 3 hr at 37°C in 10 mM Tris·HCl, pH 8.3/150 mM NaCl/30 mM OG/0.02% NaN_3 and subjected to SDS gel electrophoresis. The total protein concentration of I-A^d was 1.4 μM and that of I-E^k was 0.3 μM. I-A^d and I-E^k were each incubated with 140 μM FOva(323–339) and with 117 μM FpCytc(88–104).

For the measurements on isolated, separated chains, the α/β heterodimers and the separated chains of I-E^k and I-A^d were separated by using nonreducing SDS/PAGE, omitting boiling the samples (5, 6). The bands containing monomeric α and β of I-E^k were cut out and electroeluted (7) in 25 mM Tris/0.2 M glycine, pH 8.2/0.1% SDS. Monomeric α and β chains of I-A^d were not electroeluted but were eluted by centrifugation in 10 mM potassium phosphate, pH 7.4/150 mM NaCl/30 mM OG in the cold. As the α and β chains of I-E^k differ from one another only slightly in molecular weight, they could not be separated by electrophoresis and were isolated together. In contrast, the α and β chains of I-A^d were isolated separately. The α and β chains of I-E^k were incubated at 37°C for 3 hr with 100 μM FpCytc(88–104) in electroelution buffer. The separated α and β chains of I-A^d were incubated at 37°C for 3 hr with 225 μM FOva(323–339) in 10 mM potassium phosphate, pH 7.4/150 mM NaCl/30 mM OG.

For analysis, the samples were subjected to nonreducing SDS/PAGE (5, 6). Again the samples were not boiled before applying them to the gel. Gels were scanned for fluorescent peptides associated with class II molecules on a fluorescence microscope (5) prior to silver staining. The relative protein

Abbreviations: MHC, major histocompatibility complex; FOva(323–339), synthetic peptide representing amino acids 323–339 of chicken ovalbumin labeled at its amino terminus with fluorescein isothiocyanate; FpCytc(88–104), synthetic peptide representing amino acids 88–104 of pigeon cytochrome *c* labeled at its amino terminus with carboxyfluorescein succimidyl ester; OG, octyl β-D-glucopyranoside.

concentrations of α/β heterodimers and α and β monomers were determined by scanning the silver-stained gels on an LKB UltroScan XL laser scanner.

RESULTS

I-A^d and I-E^k were incubated with fluorescein-labeled synthetic peptide fragments FOva(323–339) and FpCytc(88–104) for 2 or 3 hr at 37°C. The complexes formed upon binding were separated by using nonreducing SDS/PAGE and were assayed for bound peptides with a fluorescence scanner prior to silver staining, as described earlier (5). Both FOva(323–339) and FpCytc(88–104) bind preferentially to their known restriction haplotypes I-A^d and I-E^k (Fig. 1). The peptides bind to the α/β heterodimers (both compact and floppy; ref. 5) and to the monomeric α and β chains (5). Several other unrelated proteins, when incubated with the same peptides, show no detectable binding (data not shown), indicating that the small observed crossreactivity of FOva(323–339) and FpCytc(88–104) with I-E^k and I-A^d, respectively, may be significant.

With the incubation times employed, the binding of FOva(323–339) to the α/β heterodimers of I-A^d (Fig. 1*a*) was significantly higher than the binding to the α and β monomers, each of which showed comparable net binding to the peptide. When I-A^d was incubated with FpCytc(88–104), no binding to the monomers was observed, and binding to the α/β heterodimers was significantly smaller than that with FOva(323–339). The situation is different in the case of I-E^k (Fig. 1*b*). Upon incubation with FpCytc(88–104), binding to the individual α and β chains dominates. Furthermore, the weaker interaction with FOva(323–339) can be found for both the single chains of I-E^k as well as for the α/β heterodimers. The differences observed in the binding patterns of I-A^d and I-E^k may reflect differences in the kinetic mechanisms of the peptide recognition.

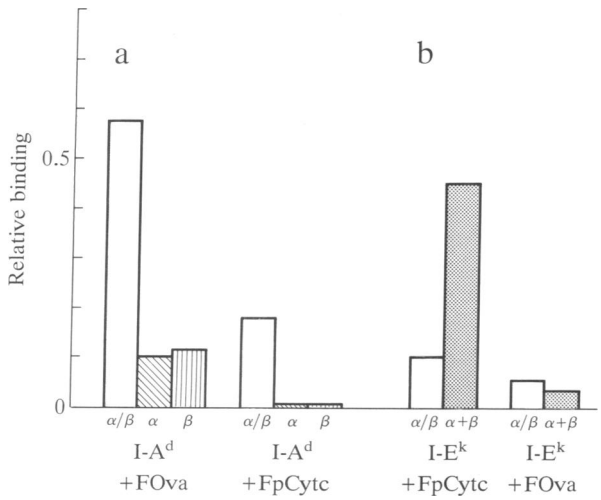

FIG. 1. Specific binding of fluorescence-labeled peptides to class II α/β heterodimers and to the separated α and β chains of I-A^d and I-E^k analyzed by one-dimensional SDS/PAGE. Vertical bars give fluorescence intensities proportional to the amount of bound peptide relative to the intensity of silver staining of α/β heterodimers and α and β chains of the same gels (in arbitrary units). Two bands are usually seen on the gels for the α/β heterodimer. The more rapidly migrating band is referred to as compact, and the more slowly migrating band is referred to as floppy. I-A^d (*a*) at a total protein concentration of 1.4 μM and I-E^k (*b*) at a total protein concentration of 0.3 μM were both incubated with 140 μM FOva(323–339) and with 117 μM FpCytc(88–104). Monomeric α and β chains of I-E^k could not be resolved because they differ only slightly in molecular weight. The vertical bars for the α/β heterodimers (compact plus floppy conformation) indicate the total fluorescence intensity per unit of protein concentration. Data labeled $\alpha + \beta$ refer to the pool of α and β chains of I-E^k. In control experiments, no fluorescent peptides were found associated with phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin (Pharmacia).

The data in Fig. 1 give no clue as to whether binding of a peptide to monomeric α and β monomeric chains is direct or requires an initial step of binding to the appropriate α/β heterodimers. To address this question, we isolated α and β monomers of I-A^d and incubated them with FOva(323–339) for 2 hr at 37°C. In Fig. 2 we show silver-stained SDS/polyacrylamide gels together with the appropriate fluorescence scans. As can be seen from Fig. 2 *a* and *b*, isolated monomeric α and β chains bind FOva(323–339). This provides evidence for a direct "on" reaction of peptide to α and β chains.

The α and β chains of I-E^k differ only slightly in molecular weight. We therefore could only isolate a pool of α and β monomers, which were incubated with FpCytc(88–104) and again scanned for fluorescence and protein (Fig. 2*d*). As is evident from the silver-stained gel, no α/β heterodimer is present in this preparation. FpCytc(88–104) bound to the separated chains. As both the peak maximum and the halfwidth of the fluorescence peak were equal to the maximum and the halfwidth of the silver-stained gel, we conclude that here again the α and β chains bind the peptide to approximately an equal extent.

DISCUSSION

Based on the results given in Figs. 1 and 2, we suggest that association of antigenic peptides with the monomeric α and β chains of MHC class II molecules may occur by two pathways. These are (*i*) direct binding of peptides to isolated monomeric chains and (*ii*) binding of peptides to the α/β heterodimers with subsequent dissociation of the complex

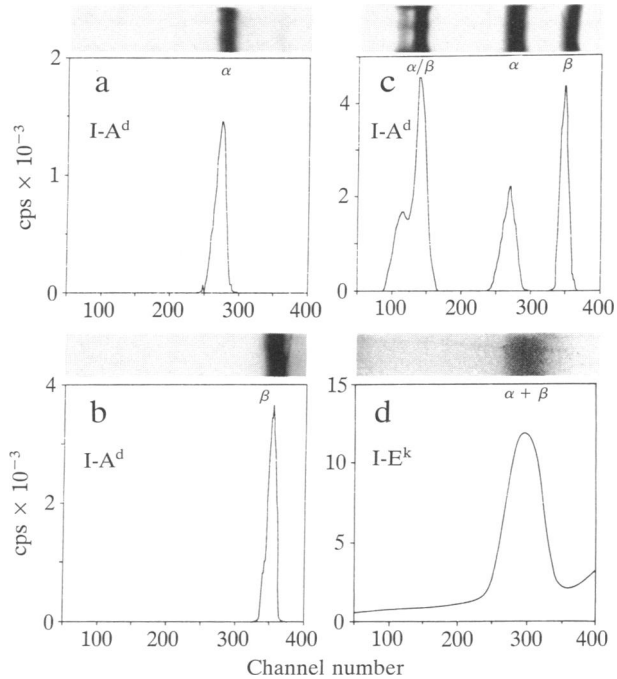

FIG. 2. Peptide binding to isolated monomeric α and β chains in detergent micelles analyzed by nonreducing SDS/PAGE. (*Upper*) Silver-stained gels. (*Lower*) Fluorescence scans of gels. Isolated α chain (*a*) and isolated β chain (*b*) of I-A^d incubated with 225 μM FOva(323–339). (*c*) I-A^d incubated with FOva(323–339). Bands due to floppy and compact α/β heterodimers and to the separated α and β chains are shown. (*d*) Pool of α and β chains of I-E^k incubated with FpCytc(88–104) (100 μM). Incubations were at 37°C for 3 hr. Data are corrected for nonspecific background fluorescence.

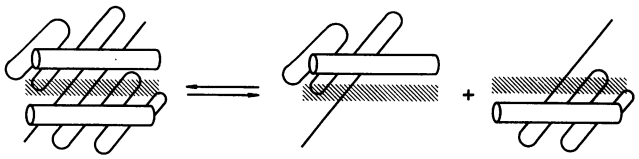

FIG. 3. Schematic representation of possible peptide binding site regions for antigenic peptides in the α/β heterodimer of a class II MHC molecule and in the separate α and β chains. The peptide binding sites are hatched. Peptides bind specifically to the α/β heterodimer as well as to the separated α and β chains. The sketch of the α and β chains is adapted from Brown *et al.* (8).

into a peptide-free monomeric chain and another chain carrying the peptide. Both possibilities imply that α and β chains undergo no major structural changes in the region of the binding site during dissociation or reassembly of the α/β heterodimer (Fig. 3). Involvement of monomeric α and β chains may prove to be necessary to account for the observed kinetics of peptide binding to and dissociation from class II MHC molecules (9, 10).

In preliminary experiments, we have observed that peptides also bind to separated chains of I-E^k reconstituted into lipid vesicles upon incubation for 3 hr at pH 5.4 (data not shown). This is of particular interest, as a low pH is found in lysosomes where processing of nonself proteins is known to take place (11) and where peptides might be bound to newly synthesized or to recycled MHC molecules. Our results raise the possibility that *in vivo* separated chains have a conformation that recognizes antigenic peptides in lysosomes. It is known that low pH favors disassembly of α/β heterodimers (5) and peptide turnover (9, 11). Upon transport of these molecules to the cell surface where the pH is higher, assembly of the isolated chains may occur. This hypothesis is supported by the finding that separated chains show peptide discrimination that is as high or even higher than that observed in direct binding to the α/β heterodimer (Fig. 1).

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