Volume 294, number 3, 244–246 FEBS 10473 December 1991

Binding of truncated peptides to the MHC molecule IA^d

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Received 24 September 1991

A peptide comprising amino acids 323–339 of chicken ovalbumin is known to bind to two heterodimeric conformations of the MHC molecule IA^d, and to each of its separate α- and β-chains. We report that minor C- and N-terminal truncations of the parent peptide do not alter the binding pattern. A decrease in binding activity was observed upon deletion of the histidine residues of the already truncated peptides. Peptides as short as 4 amino acids associate weakly with all four proteins.

Antigen presentation; T-Helper lymphocyte

1. INTRODUCTION

Class II molecules of the major histocompatability complex (MHC) present antigenic peptides to $CD4^+$ T-cells. They consist of an α- and β-chain of about 30 kDa each. Two variable domains of each chain shape the peptide binding site. The peptides are thought to bind in a cleft between two parallel α-helices on top of a flat platform of β-strands [1]. It has been shown that the separate α- and β-chains independently bind the same peptides, although the two chains may or may not bind identical amino acid residues in the peptides [2,3].

One given MHC molecule may bind a variety of peptides, and one given peptide may bind to different MHC molecules [4]. A well investigated system concerns the interaction of the murine class II molecule IA^d and the chicken ovalbumin fragment comprising amino acids 323–339 (Ova(323–339). A hexapeptide V^{327}-H-A-A-H-A has been identified to be essential for T-cell recognition [5], and it has been found that valine 327 and histidine 328 bind to IA^d, whereas histidine 331 is essential for T-cell activation [6]. Here we report that peptides composed of only four amino acids bind weakly to both chains. The introduction of one histidine residue is sufficient for strong binding to each of the two chains of IA^d.

Abbreviations: MHC, major histocompatability complex; FITC, fluorescein isothiocyanate; F-OVA(x-y), FITC-labeled peptide fragments of chicken ovalbumin comprising amino acids x to y; TFA, trifluoroacetic acid.

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2. MATERIALS AND METHODS

IA^d was purified by affinity chromatography as described [5]. After elution the samples were dialyzed 36 h in 2 mM dodecyl-β-D-maltoside, 10 mM Tris/HCl, pH 8.3, 150 mM NaCl, 0.02% NaN_3. This buffer was used for all further steps unless indicated otherwise. To increase the peptide binding capacity [7] the disulfide bonds of IA^d were reduced by incubating IA^d for 1 h at 37°C with 10 mM dithiothreitol to release co-purifying peptides. The samples were then re-oxidized by dialysis for 18 h in air-saturated buffer using 9DC DiaCell dialysis capsules (Instrumed, Union Bridge, MD). The reduced and re-oxidized samples were then incubated with 50 mM of the desired peptide for 2 h at 37°C. They were applied to SDS-PAGE omitting boiling and reduction [2]. Prior to fixation the gels were scanned for fluorescent peptides on a fluorescence microscope as described [2]. Gels were stained with silver and scanned for proteins on an LKB UltroScan XL laser scanner. The protein concentration was 1.0 μg/lane.

Peptide C-terminal carboxylate or carboxamide derivatives were synthesized on a Milligen 9050 peptide synthesizer using Pepsyn KA or Pepsyn KB solid support and FMOC-protected amino acids. Peptides were de-protected by TFA and reverse phase HPLC-purified using linear gradient elution (Buffer A: 0.1% TFA; Buffer B: 0.1% TFA in 70% aqueous acetonitrile). Lyophilized peptides were labeled with fluorescein on the N-terminal amino group or the epsilon amino group of lysin residues by reaction with an excess of FITC in dimethylsulfoxide in the presence of diisopropylethylamine. Fluoresceinated peptides were precipitated with ethyl acetate, washed, dried, redissolved in 0.1% TFA and HPLC purified, as described above.

3. RESULTS AND DISCUSSION

The top panel of Fig. 1 shows a silver stained SDS gel of the IA^d preparation used for all experiments described here. The relative concentrations of the heterodimeric conformations, floppy and compact, and of the separate α- and β-chains were floppy:compact:α-chain:β-chain = 0.7:1:0.3:0.25. The lower panels of Fig.

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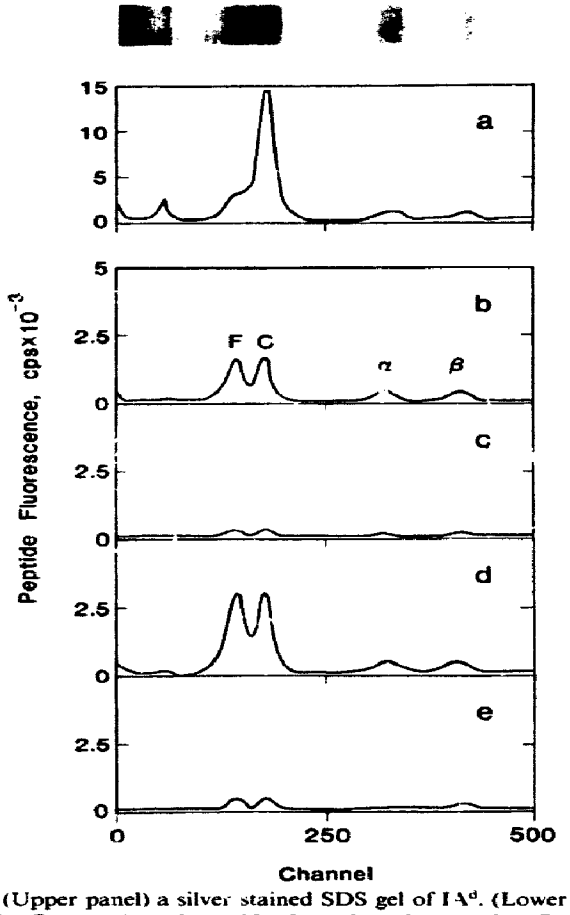

Fig. 1. (Upper panel) a silver stained SDS gel of IA^d. (Lower panel) scans for fluoresceinated peptides bound to the proteins. Scans are shown for (a) the parent peptide F-Ova(323–339) and (b) the truncated peptides F-Ova(323–328), (c) F-Ova(323–326), (d) F-GA_2HA and (e) F-GA_4. See Table I for detailed sequences of the peptides. The heterodimeric conformations floppy (F), compact (C) and the separate α- and β-chains are indicated. Their apparent molecular weights are 55, 64, 33 and 27.5 kDa, respectively.

I shows scans of this gel for the following fluorescent peptides bound to these conformations: the parent peptide, F-OVA(323–339) (a) see Table I, first line for the sequence), the truncated peptides F-OVA(323–328) (b) and F-OVA(323–326) (c), and the peptides F-G-A-A-H-A (d), and F-G-$(A)_4$ (e). Table I lists all peptides employed and the relative peptide capacities of floppy, compact and the separated chains. The relative binding capacity is defined by the quotient of the relative fluorescence intensity of a particular peptide associated with one of the conformations of IA^d, and of the relative concentration of this particular conformation. The binding capacity of compact-to-F-Ova(323–339) was set at 1.0.

It is evident that long peptides bind better to the compact conformation as compared to the floppy conformation or the separate chains. Compact binds the parent peptide F-Ova(323–339), the N-terminally truncated peptide F-Ova(325–339) and the C-terminally truncated peptide F-Ova(323–333) about 3–5-fold better, as compared to floppy or the separate chains. Further truncations on both the C- and N-terminus led to almost equal binding to all conformations (see F-Ova(323–330) and F-Ova(329–339) and further truncations). One possible explanation is that floppy might be a conformation where the conserved α_2 and β_2 domains are still assembled, but with disassembled α_1 and β_1 domains which form the binding site. Compact might be a conformation with assembled α_1 and β_1 domains, thus the two histidine binding sites of the α- and the β-chain would be in sufficiently close distance allowing for binding of two histidine residues from one peptide. This should yield slower off-rates and consequently show higher amounts of associated peptide. Floppy, with distant binding sites, would bind two histidine residues from different peptides independently, and thus kinetically behave like the separate chains.

Truncations down to the peptide F-Ova(331–339) and F-Ova(323–326) did not completely abrogate binding to both heterodimeric conformations or to both separated chains although these peptides lack the amino acids valine 327 and histidine 328 found to be necessary for binding peptides to IA^d in competition experiments [6]. However, in competition experiments a strong binding peptide may displace a weak binding peptide quantitatively depending on the ratio of their dissociation constants. In contrast, in our experiments all peptides are offered empty MHC molecules in the absence of competitors. Thus even weakly binding peptides may be detected associated with IA^d, as long as their off-rates are slow enough, irrespective of their dissociation constant. It could be argued that the fluorescein group is responsible for the observed binding of labeled peptides to IA^d. This can be excluded because all peptides employed here are fluorescein labeled but show distinct binding patterns. Even the shortest peptides employed here therefore dissociate with slow off-rates as they could be detected by a technique of considerable slow time resolution. We therefore surmise that they interact similarly with IA^d as the parent peptide. Thus, if fluorescein would bind, one would expect all peptides to exhibit the same binding pattern.

Even the four amino acid-peptide F-Ova(323–326) and F-GA_4 bound to all four conformations. This result suggests that either the polypeptide backbone of peptide interacts with the MHC binding site [8], or that both chains bear weak alanine binding sites, as all peptides investigated here share only one alanine residue. Major contributions of A^{332} and V^{327} beside histidine in the interaction of Ova(323–339) and IA^d have been proposed [6]. The presence of a histidine residue increases the amount of peptide associated with all heterodimeric

Table I

Relative binding capacities of peptides to different conformations of IA^d

	F	C	α	β
FITC-[illegible]ISQAVHAAHAEINEA[illegible]R[illegible]-OH	0.22	1.0	0.21	0.26
FITC-[illegible]ISQAVHAAHAE[illegible]-NH$_2$	0.11	0.31	0.05	0.06
FITC-[illegible]ISQAVHAA[illegible]-NH$_2$	0.09	0.08	0.08	0.08
FITC-[illegible]ISQAVH[illegible]-NH$_2$	0.14	0.15	0.14	0.13
FITC-[illegible]ISQA[illegible]-NH$_2$	0.03	0.03	0.06	0.04
Ac-[illegible]QAVHAAHAEINEAGK[illegible](FITC)-OH	0.08	0.51	0.13	0.14
Ac-[illegible]AAHAEINEAGK[illegible](FITC)-OH	0.06	0.08	0.12	0.13
Ac-[illegible]HAEINEAGK[illegible](FITC)-OH	0.13	0.08	0.05	0.06
FITC-GAAHA-OH	0.34	0.23	0.19	0.18
FITC-GAAHA-OH	0.05	0.04	0.02	0.06

The sequences of the parent peptide F-Ova (323–339) and its derivatives employed here are shown. Listed next to the sequences is their relative binding capacity to the different conformations of IA^d.

conformations and separate chains of IA^d by an average factor of 5 (Table I). The two histidine residues 328 and 331 have already been shown to play crucial roles in peptide binding to IA^d molecules and in triggering T-cell activation [5,6]. Here we found enhanced binding of the histidine-containing peptides to both heterodimeric conformations and to each of the separate chains. This suggests that each of the separate chains contains a histidine binding site. The peptide F-Ova(331–339) showed a binding pattern comparable to such peptides lacking histidine, but has a histidine residue as its N-terminal end. Therefore we suppose that histidine with a free or acetylated amino terminus may not be sufficient for strong binding, but a sequence 'X-His' is necessary.

In summary, we tentatively suggest a three-stage model for the binding of peptides to MHC molecules. The first stage is the recognition of either the polypeptide backbone or of not very prominent amino acid side chains, such as the methyl group of alanine. In addition there are specific binding sites for side chains which are more characteristic, such as X-His in our case or tyrosine for HLA-DR1 [8]. Such distinct binding sites may contribute considerably to specificity and to the binding energy, and lower the off-rates. Specificity is further enhanced by binding of long peptides to the compact conformation. This type of binding may result from a summation over many strong and weak contributions to the binding free energy. This interpretation may explain the well-documented cross-reactivity of peptides binding to MHC molecules [4] by assuming that not all possible interaction sites of peptide and MHC have to be occupied for observing binding or competition. The complex binding pattern allows binding of merely related peptides which may or may not induce stimulation of T-cells.

Acknowledgements: This work was supported by the EMBO through Grant ALTFF 113-1988 and by the NIH Grant 5R37 AI 13587-16.

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