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Competitor Analogs for Defined T Cell Antigens: Peptides Incorporating a Putative Binding Motif and Polyproline or Polyglycine Spacers

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Summary

We describe a new approach for modeling antigenic peptides recognized by T cells. Peptide A24 170–182 can compete with other antigenic peptides that are recognized by H-2k^d-restricted cytolytic T cells, presumably by binding to the K^d molecule. By comparing substituted A24 peptides as competitors in a functional competition assay, the A24 residues Tyr-171, Thr-178, and Leu-179 were identified as possible contact residues for K^d. A highly active competitor peptide analog was synthesized in which Tyr was separated from the Thr-Leu pair by a pentaproline spacer. The choice of proline allowed the prediction of a probable conformation for the analog when bound to the K^d molecule. The simplest conformation of the A24 peptide that allows the same spacing and orientation of the motif as in the analog would be a nearly extended polypeptide chain incorporating a single 3_{10} helical turn or similar structural kink.

Introduction

T lymphocytes recognize antigen in the context of class I or class II major histocompatibility complex (MHC) molecules expressed at the cell surface (reviewed by Zinkernagel and Doherty, 1979; Schwartz, 1987). Within recent years, a few of the molecular details of the interaction between antigen and MHC molecules have begun to emerge. In many cases, defined antigens can be mimicked by synthetic peptides or protein cleavage products, provided they are presented by cells expressing the appropriate MHC phenotype (Barcinski and Rosenthal, 1977; Corradin and Chiller, 1979; Townsend et al., 1986). It has been further demonstrated that defined antigenic peptides can bind in vitro directly and specifically to purified MHC class II molecules (Babbitt et al., 1985; Buus et al., 1986). Moreover, peptides recognized in the context of the same MHC class II restriction element were found to be capable of competing with each other both in functional assays and in direct binding studies (Babbitt et al., 1986; Buus et al., 1986; Guillet et al., 1986, 1987). These results form the basis for current models of antigen recognition by T cells whereby antigen fragments interact specifically with MHC molecules.

Analysis of the structure of the HLA-A2 class I MHC molecule further supports such a model (Bjorkman et al., 1987a, 1987b). The two most external domains of the molecule form a groove composed of two α helices overlying an eight-strand, antiparallel β sheet. The most polymorphic residues among different class I MHC molecules are located within the groove which was therefore suggested to be the site for peptide binding. A similar structure has been proposed for class II MHC molecules (Brown et al., 1988).

We have recently developed a model system for antigen recognition by MHC class I–restricted cytolytic T cells (CTLs) (Maryanski et al., 1986a, 1986b, 1988; Pala et al., 1988a). H-2K^d-restricted CTLs derived from DBA/2 (H-2^d) mice immunized with syngeneic P815 transfectants expressing HLA-CW3 or HLA-A24 class I molecules can lyse untransfected P815 target cells in the presence of synthetic peptides corresponding to amino acid residues 170–182 of the appropriate HLA molecules. The two HLA peptides differ only at residue 173 (Lys for CW3 and Glu for A24). For CTL clones that recognize mutually exclusively either the CW3 or the A24 peptide, we found that the alternative HLA peptide could compete with the antigenic peptide for recognition on P815 target cells (Maryanski et al., 1988). Homologous peptides from the endogenous H-2^d class I MHC molecules could also compete with the HLA peptides (Maryanski et al., 1988). Competition might result from the binding of the peptides to a common site on the K^d restriction molecule. In support of this mechanism, we have demonstrated that an unrelated K^d-restricted peptide from region 147–158 of the influenza nucleoprotein (NP) can also compete efficiently with the HLA peptides for recognition by K^d-restricted CTLs (Pala et al., 1988b). Moreover, the HLA peptides can also compete with the NP peptide. In contrast, other NP peptides that have different restriction specificities either failed to compete or were relatively inefficient competitors for the K^d-restricted HLA or NP peptides.

The identification of the peptide residues required for functional competition might provide clues about the molecular details of peptide–MHC interaction. In the present study, we have analyzed HLA peptides modified by truncation or by amino acid substitution to determine which residues might allow the peptide to interact with the K^d restriction element. The analysis identified three such HLA residues (Tyr-171, Thr-178, and Leu-179). Based on the possibility that these residues form a binding motif, we designed competitor peptide analogs that contain the three HLA residues separated by short stretches of homo-

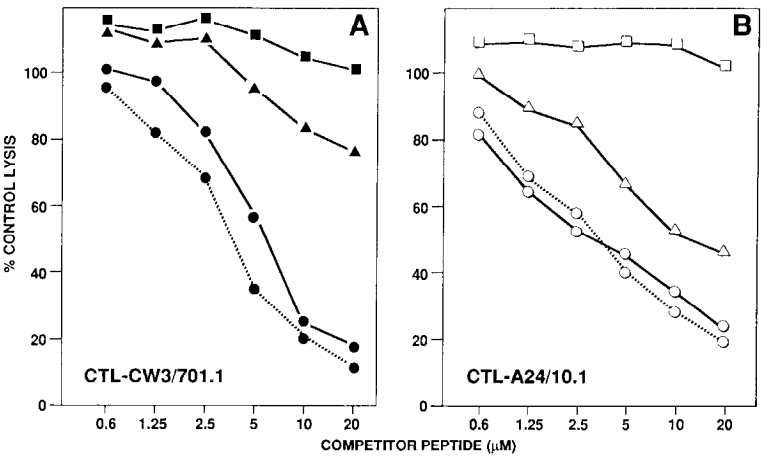

Figure 1. Comparison of Truncated A24 and CW3 Peptides as Competitors

P815 target cells were incubated with the indicated concentration (final) of competitor peptides corresponding in (A) to A24 170–182 (●–●), A24 171–182 (▲–▲), A24 172–182 (■–■), or A24 170–180 (●- -●) and in (B) to CW3 170–182 (○–○), CW3 171–182 (△–△), CW3 172–182 (□–□), or CW3 170–180 (○- -○). The antigenic peptide CW3 170–182 (A) or A24 170–182 (B) was added at a final concentration of 0.1 μM. CTLs from clone CW3/701.1 (A) or A24/10.1 (B) were then added at a CTL to target ratio of 3:1. The assay was terminated after 4 hr of incubation at 37°C. The control lysis in the absence of competitor peptides was 63% for clone CW3/701.1 and 57% for clone A24/10.1, and the percentage of control lysis was calculated as described in the Experimental Procedures.

oligo-amino acids. Our demonstration that conformationally constrained peptide analogs containing polyproline were functionally active competitors allows us to predict a probable conformation for the HLA peptides when bound to the K^d molecule.

Results

Truncated and Substituted HLA Peptides Define Three Residues Critical for Competition

We showed previously (Maryanski et al., 1988) that lysis of P815 target cells by CTL clones CW3/701.1 and A24/10.1 in the presence of antigenic peptides CW3 170–182 and A24 170–182, respectively, could be inhibited by an excess of peptide corresponding to the same region of the nonrecognized (A24 or CW3) HLA allele. As shown in Figure 1, it appears that all of the residues critical for this inhibition are contained within region 170–180 for both HLA alleles. Thus, truncated HLA peptides corresponding to region 170–180 were as active as the full-length (170–182) peptides as competitors, whereas other 11-residue peptides truncated by two N-terminal residues (172–182) were at least 20-fold less active (Figure 1, and data not shown). Intermediate results were obtained with peptides truncated by only one residue at the N terminus (171–182).

The decrease in activity on truncation of N-terminal residues 170 and 171 could simply reflect a requirement for a minimal peptide length. Alternatively, the deleted residues might be directly involved in peptide binding. To assess more directly the contribution of Tyr-171, we synthesized modified full-length CW3 and A24 peptides (region 170–182) that contained an Ala or Phe substitution at position 171. For both HLA alleles, replacement of Tyr-171 with Ala reduced by over 100-fold the efficiency of the peptides as competitors (Figure 2). Moreover, the Tyr-substituted CW3 and A24 peptides were not recognized as antigens by CTL clones that recognize the unsubstituted HLA peptides (Maryanski et al., 1989; unpublished data). For the more conservative substitution of Tyr with Phe, the reduction was 10- to 50-fold (Figure 2; Table 1).

We found previously (Maryanski et al., 1988) that a peptide corresponding to region 170–182 of HLA-B7 was a poor competitor for the CW3 and A24 peptides. Within this region, B7 differs from A24 by three residues (see Figure 3). As demonstrated by the experiment in Figure 3, a single B7 substitution at position 178 (Thr to Lys) within the A24 sequence was sufficient to reduce the efficiency of the peptide as a competitor to that of B7. In contrast, B7-specific substitutions at positions 177 and 180 had no effect.

Additional single-substitution peptides that otherwise correspond to the A24 170–182 sequence were synthesized and analyzed as competitors. The relative efficiency of the substituted peptides compared with peptide A24 170–182 was calculated for each experiment, and the

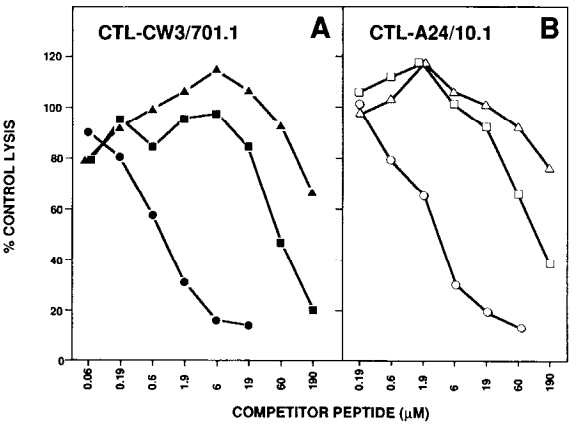

Figure 2. A Critical Role for Tyr-171 in the HLA Peptides

The experiment was carried out as described in the legend to Figure 1. Control competitor peptides corresponded to the natural sequence of region 170–182 of A24 (●) or CW3 (○). Single-substitution peptides corresponded to A24 (solid) or CW3 (open) 170–182, but contained Phe (■,□) or Ala (▲, △) in place of Tyr at position 171. Control lysis (without competitor) for CTL clone CW3/701.1 (A) in the presence of peptide CW3 170–182 at 0.1 μM was 56%, and that of CTL clone A24/10.1 (B) in the presence of peptide A24 170–182 at 0.03 μM was 40%.

Table 1. Substituted A24 Peptides Define Three Residues Critical for Competition

Peptide	Sequence	Competitor Efficiency		# of Experiments
A24	R Y L E N G K E T L Q R A	(1.0)		9
186C1	- A - - - - - - - - - - -	<0.01[a]		2
186C2	- F - - - - - - - - - - -	0.075	(± 0.06)	3
B7	- - - - - - - D K - E - -	0.12	(± 0.05)	8
99	- - - - - - - D - - - - -	0.66	(± 0.23)	9
100	- - - - - - - - K - - - -	0.12	(± 0.03)	9
AV	- - - - - - - - - - E - -	0.91	(± 0.13)	2
136	- - - - - - - - - - A - - -	0.08	(± 0.04)	3
137	- - - - - - - - - - K - - -	0.16	(± 0.09)	4
132B	- - - - - - - - D - - - -	0.15	(± 0.05)	5
162	- - - - - - - - K A - - -	0.11	(± 0.04)	3
135A	- - A - - - - - - - - - -	0.85	(± 0.31)	3
145A	- - - - - T - - - - - - -	1.14	(± 0.20)	2
145C	- - - - - - R - - - - - -	6.13	(± 4.4)	3
K^d	- - - - L - N - - - L - T	3.91	(± 3.88)	9
D^d/L^d	- - - K - - N A - - L - T	1.55	(± 1.56)	4

Competition experiments were performed with CTL clone CW3/701.1 and antigenic peptide CW3 170–182 (0.05–0.1 μM). The relative efficiency of each peptide as a competitor relative to peptide A24 170–182 was calculated as described in the Experimental Procedures by comparing the concentration required to inhibit lysis by 50%. The values are given as the mean relative competitor efficiency (± standard deviation).
[a] Actual values were 0.009 and <0.005.

results are compiled in Table 1. The analysis confirms the importance of residue Thr-178, in that peptides for which residue 178 was replaced with Lys (corresponding to B7) or Asp were more than 5-fold less efficient as competitors, compared with the unsubstituted A24 peptide. The adjacent residue, Leu-179, also appears critical in that substitutions with either Ala or Lys decreased competitor potency by at least 5-fold. However, no further reduction in competitor activity could be obtained by a double substitution of residues 178 and 179 compared with the individual substitutions. Peptides with single substitutions at positions 172 (Leu to Ala), 175 (Gly to Thr), or 176 (Lys to Arg) were still potent competitors.

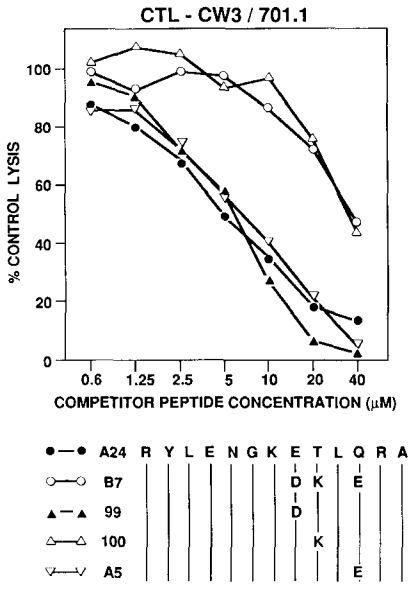

Figure 3. Identification of a B7-Specific Substitution That Alters the Competitor Efficiency of the A24 Peptide

The experiment was performed with CTL clone CW3/701.1 in the presence of 0.1 μM antigenic peptide CW3 170–182 as described in the legend to Figure 1. The sequence of each of the competitor peptides is shown. Control lysis in the absence of competitor peptides was 64%.

The experiments presented in Table 1 also confirm our previous finding (Maryanski et al., 1988) that peptides corresponding to region 170–182 of the K^d or D^d molecules are also very efficient competitors in this system. These peptides contain four substitutions compared with the A24 and CW3 sequences (see Table 1). Both contain the same replacements at residues 176 (Lys to Asn), 180 (Gln to Leu), and 182 (Ala to Thr). In addition, the K^d peptide has an Asn to Leu substitution at position 174, whereas the D^d peptide has a Glu to Ala substitution at position 177. At position 173, the residues for K^d and D^d correspond to those of A24 (Glu) and CW3 (Lys), respectively. Taken together, the results with truncated and substituted HLA peptides suggest that residues 171 (Tyr), 178 (Thr), and 179 (Leu) contribute to the capacity of the HLA peptides to compete with each other for recognition.

Competitor Peptide Analogs Containing Polyproline or Polyglycine Spacers

One interpretation of the apparent importance of HLA peptide residues 171 (Tyr), 178 (Thr), and 179 (Leu) is that they interact directly with residues on the K^d restriction element. If that were the case, we considered that it might be possible to design functional competitor peptide analogs sharing only those three residues with the original HLA sequences. As potential spacers between the Tyr and Thr residues, we inserted different-length stretches of homo-oligo-amino acids. In addition, Ala residues were added at both ends of the peptides to avoid situating the N- and C-terminal charged groups immediately adjacent to the putative binding motif.

The first series of analogs contained polyproline spacers.

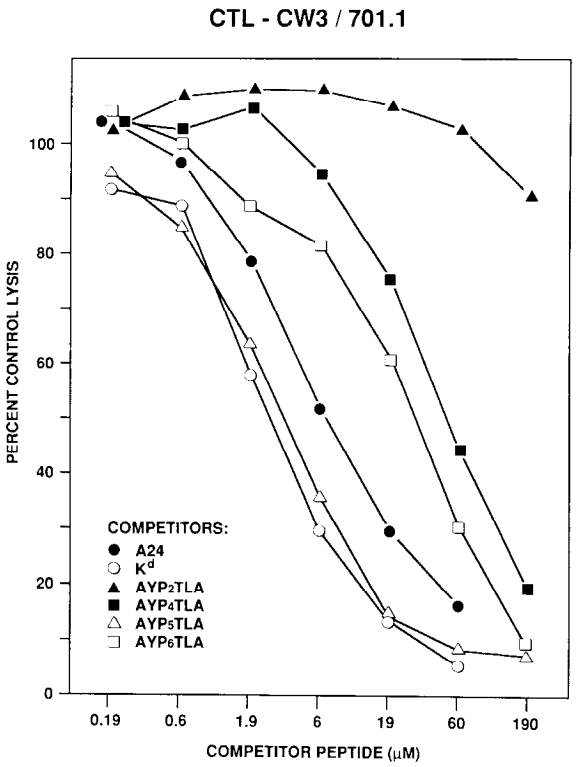

Figure 4. Competition for Peptide CW3 170–182 by Analogs Containing the Tyr . . . Thr-Leu Motif and Proline Spacer Residues

The experiment was performed with CTL clone CW3/701.1 in the presence of antigenic peptide CW3 170–182 (0.1 μM) as described in the legend to Figure 1. Control lysis in the absence of competitor peptides was 68%.

As shown in Figure 4 and Table 2, peptide analogs containing four, five, or six proline residues were efficient inhibitors for the CW3 170–182 peptide in our standard competition assay. Of these, the pentaproline analog (AYP_5TLA) was the most efficient competitor. Remarkably, it was as active as the K^d (170–182) peptide in terms of the concentration of peptide required for inhibition of lysis. In contrast, the diproline analog (AYP_2TLA) was 500-fold less active (Figure 4; Table 2). Analogs containing polyglycine spacers were also active as competitors (Table 2). As was found for proline, the optimal number of glycine residues was five. However, the pentaglycine analog was severalfold less active than its pentaproline counterpart.

Within the A24 and CW3 sequences (regions 170–182), the most critical residue for competition appeared to be Tyr-171 (Figure 2; Table 1). If the oligo-amino acid spacer peptides function as structural analogs for the HLA peptides, their Tyr residues should likewise be sensitive to substitution with Ala. As demonstrated in Table 2, replacement of Tyr with Ala rendered the pentaproline peptide inactive as a competitor, thus confirming its critical role within the analog. By further analogy with the HLA peptides, deletion of the residue (Ala) N-terminal to Tyr also decreased the activity of the pentaproline competitor peptide (Table 2). We verified (data not shown) that the lack of inhibition by the latter two peptides (204 and G6; Table 2) was not due to their recognition by the CTL clone (CW3/701.1) used in the competition assay.

A control experiment (Figure 5) showed that inhibition of lysis by the pentaproline analog could be overcome by increasing the concentration of the antigenic CW3 170–182 peptide. This result implies that the inhibition was indeed competitive. In addition, complete inhibition could be obtained by preincubation of the target cells with a mixture of antigenic and competitor peptides followed by washing (Figure 6). Thus, as we had shown previously for the HLA peptides (Maryanski et al., 1988), the competition with amino acid analogs also appears to occur at the level of the target cell.

A Critical Role for Tyrosine in Another Peptide Recognized in the Context of H-2K^d

We have shown previously (Pala et al., 1988b) that an unrelated peptide from influenza nucleoprotein (peptide NP 147–158 [R^{-}_{156}]) that is also recognized in the context of H-2K^d can compete with HLA peptides for recognition. Similarly, the HLA peptides can compete with the NP peptide for recognition by NP-specific CTLs. In experiments using CTL clones specific for HLA (Figure 2; Table 1), the

Table 2. Comparison of Competitor Analogs Containing the Tyr . . . Thr-Leu Motif and Either Proline or Glycine Spacers

Peptide	Sequence	Competitor Efficiency Relative to Peptide 194C (AYP_5TLA)		# of Experiments
A24 170–182	RYLENGKETLQRA	0.29	(± 0.13)	7
K^d/170–182	- - - - L - N - - - L - T	1.03	(± 0.38)	6
194A	AYPPTLA	0.002	(± 0.0008)	4
194B	AYPPPPTLA	0.039	(± 0.02)	6
194C	AYPPPPPTLA	(1.0)		7
194E	AYPPPPPPTLA	0.083	(± 0.03)	6
204	AAPPPPPTLA	0.002	(± 0.001)	2
G6	YPPPPPTLA	0.002	(± 0.0009)	3
G9	AYGGGGTLA	0.008	(± 0.007)	4
G10	AYGGGGGTLA	0.11	(± 0.06)	5
G11	AYGGGGGGTLA	0.03	(± 0.017)	5

Competition experiments were performed with CTL clone CW3/701.1 and antigenic peptide CW3 170–182 (0.05–0.1 μM), as described for Table 1.

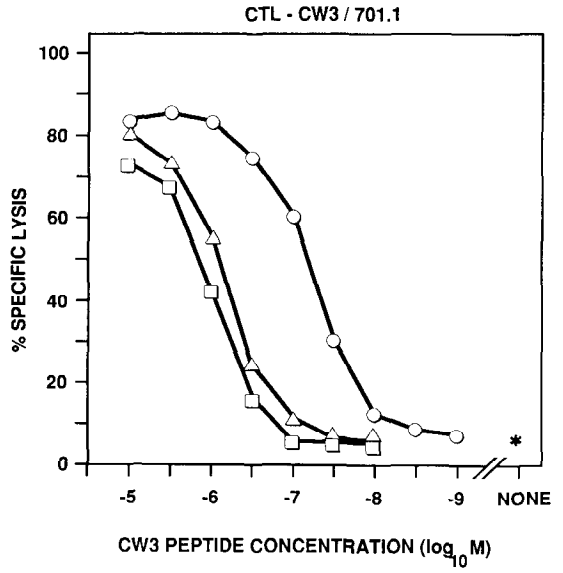

Figure 5. Inhibition of Lysis by the Pentaproline Analog Can Be Overcome by Increasing the Antigen Concentration

P815 target cells were incubated for 15 min at room temperature with peptide K^d 170–182 (△) or the analog AYP_5TLA (□) at 10^{-5} M or with medium (○) as a control, and then added (without washing) to the indicated concentrations (final) of antigenic peptide CW3 170–182. CTLs from clone CW3/701.1 were added at a CTL to target ratio of 3:1. The assay was terminated after 4 hr of incubation at 37°C.

most critical residue in the A24 and CW3 peptides (172–182) appeared to be Tyr-171. To determine whether this residue would also be important for competition against the

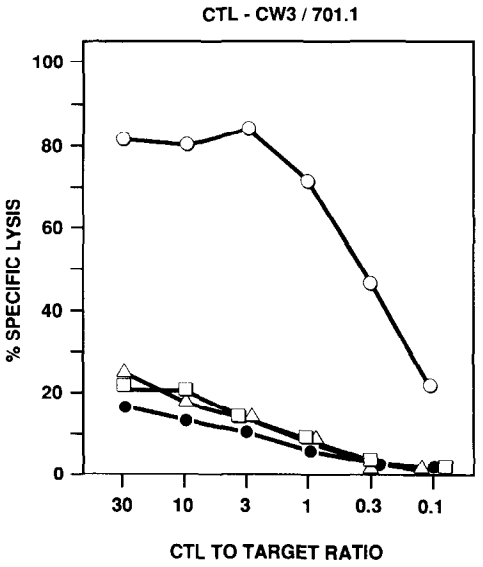

Figure 6. Competition with the Pentaproline Analog Occurs at the Level of the Target Cells

P815 target cells were incubated for 1 hr at 37°C with 5 μM antigenic peptide CW3 170–182 and 200 μM competitor peptide K^d 170–182 (△) or analog AYP_5TLA (□). Controls were incubated with the CW3 peptide alone (○) or with medium (●). After washing, the target cells were added to cells from CTL clone CW3/701.1 at the indicated ratios. The assay was terminated after a 4 hr incubation at 37°C.

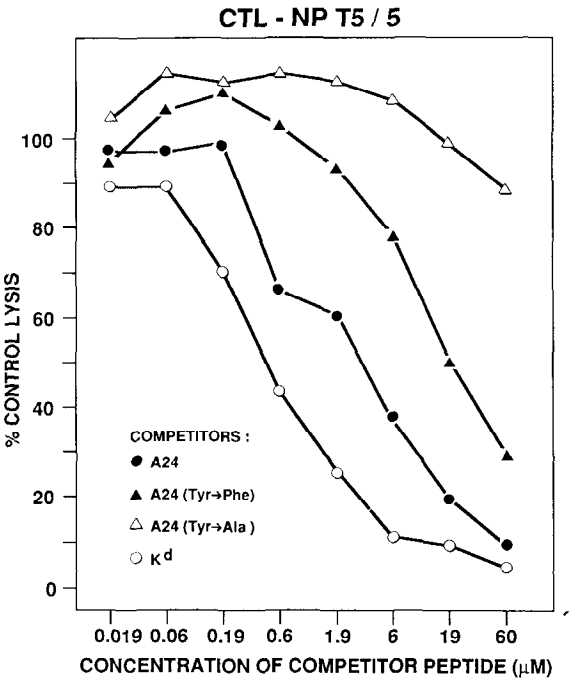

Figure 7. The Tyr-171 Residue of the A24 Peptide Is Critical for Competition with the Unrelated NP Peptide

The experiment was carried out as described in the legend to Figure 1 with anti-NP CTL clone NP T5/5 and antigenic peptide NP 147–158 (R^{-}_{156}) at 10^{-10} M. The competitor peptides were K^d 170–182 (○), A24 170–182 (●), and peptides corresponding otherwise to A24 170–182 but with Phe (▲) or Ala (△) substitutions for residue Tyr-171. Control lysis in the absence of competitors was 56%.

NP peptide, we used the K^d-restricted CTL clone NP T5/5 (Taylor et al., 1987; Bodmer et al., 1988), which recognizes peptide NP 147–158 (R^{-}_{156}). Replacement of Tyr-171 with Ala reduced by about 100-fold the capacity of the A24 peptide to compete with the NP peptide, whereas the conservative substitution of Tyr with Phe resulted in a 5- to 10-fold reduction (Figure 7; data not shown). Moreover, recognition of the NP peptide by CTL clone NP T5/5 could also be inhibited by the pentaproline-containing analog AYP_5TLA, but not by a nearly identical peptide in which the Tyr residue was replaced by Ala (Figure 8).

The sequence of peptide NP 147–158 (R^{-}_{156}) (TYQRTRALVTG) also contains a Tyr residue. It was therefore of interest to determine whether this Tyr residue would likewise be critical for competition. This indeed appears to be the case, as shown in Figure 9. Replacement of Tyr-148 with either Phe or Ala reduced by more than 100-fold the activity of the NP peptide as a competitor for peptide CW3 170–182. The activity of the Phe- or Ala-substituted NP peptide as antigen for CTL clone NP T5/5 was likewise reduced by over 100-fold (data not shown).

Discussion

Using a functional competition assay and peptides modified either by amino acid substitution or by truncation, we have identified a putative K^d binding motif for the anti-

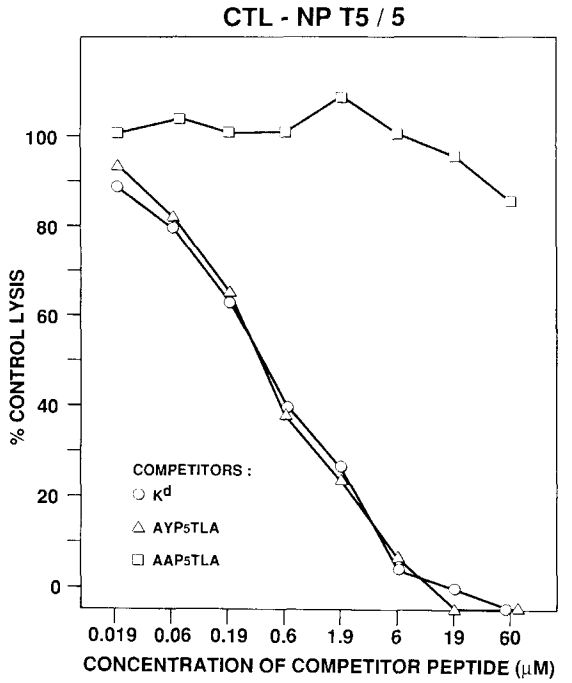

Figure 8. The Pentaproline Analog Containing the Tyr . . . Thr-Leu Motif Competes with Peptide NP 147–158 (R^{-}_{156})

The experiment was carried out as described in the legend to Figure 1 with CTL clone NP T5/5 and antigenic peptide NP 147–158 (R^{-}_{156}) at 10^{-10} M. The competitors were peptides K^d 170–182 (○), AYP_5TLA (△), AAP_5TLA (□). Control lysis in the absence of competitors was 72%.

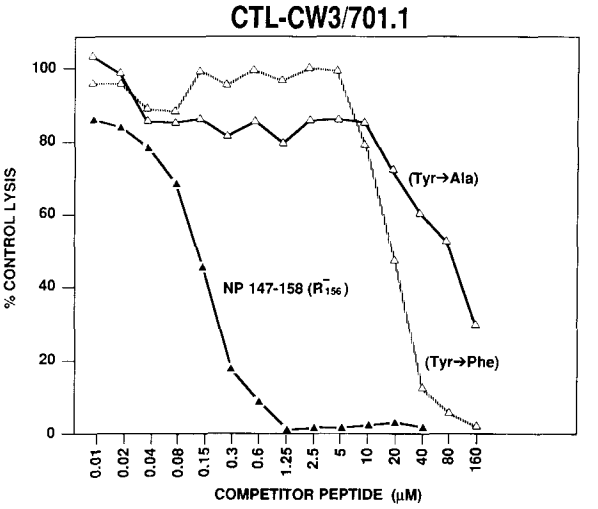

Figure 9. Peptide NP 147–158 (R^{-}_{156}) also Contains a Critical Tyr Residue

The protocol was as described in the legend to Figure 1 with CTL clone CW3/701.1 and antigenic peptide CW3 170–182 at 0.1 μM. Competitors were either peptide NP 147–158 (R^{-}_{156}) (▲—▲) with Tyr at position 148 or substituted NP peptides in which Tyr-148 was replaced with Phe (△- -△) or Ala (△—△). Control lysis in the absence of competitors was 71%.

genic peptide A24 170–182. The motif includes residues Tyr-171, Thr-178, and Leu-179. The same three residues are present in the nearly identical antigenic peptide CW3 170–182, as well as in other potent competitors corresponding to region 170–182 of the K^d and D^d molecules. The most critical residue for binding appears to be Tyr-171, since its replacement with Ala reduced by over 100-fold the efficiency of the HLA peptides as competitors. Residues 178 (Thr) and 179 (Leu) appear to contribute less than the Tyr residue to the overall affinity of the A24 peptide for K^d, since substitution at either position resulted in only a 5- to 10-fold reduction in competitor potency and no further reduction was obtained by a double substitution at these adjacent positions. Our finding that a Tyr residue in the unrelated peptide NP 147–158 (R^{-}_{156}) also appears to be critical for binding to the K^d molecule is of particular interest. Indeed, it is possible that Tyr functions as an important contact residue, perhaps as a principal anchor for peptides within the MHC antigen binding site. This may be a general feature of peptides recognized in the context of K^d, since we have recently found that Tyr residues are likewise crucial for the competitor activity of two additional peptides (Maryanski and Corradin, unpublished data).

Further evidence that the sequence Tyr . . . Thr-Leu constitutes a binding motif for the HLA peptide was provided by its expression in a completely different molecular context. For this purpose the Tyr residue was separated from the Thr–Leu pair by homo-oligo-amino acid residues, and single Ala residues were added to both ends of the peptides. Functional competitor peptides were obtained with either proline or glycine spacers. The most potent analog contained a pentaproline spacer. Remarkably, its activity was comparable to that of peptide K^d 170–182, which itself is the most potent of the competitors tested and which contains the Tyr . . . Thr-Leu motif as part of its natural sequence. As evidence that the competitor activity of the pentaproline analog was due to the same motif, we showed that its activity decreased over 100-fold on substitution of Tyr with Ala. The pentaproline analog containing the Tyr . . . Thr–Leu motif could compete efficiently with peptides recognized by K^d-restricted CTLs as shown here for peptides A24 and CW3 170–182 and NP 147–158 (R^{-}_{156}). The activity appears to be specific, in that the same analog failed to compete with other peptides recognized in the context of D^d or L^d (Maryanski et al., unpublished data).

The observation that molecules incorporating oligo-amino acid spacers act as competitors for natural peptides provides important information about the structural features that confer specificity on peptide–MHC interactions. Most notable is the ability of peptide analogs that contain the Tyr . . . Thr-Leu motif and polyproline spacers to compete with natural peptides. In contrast to other naturally occurring amino acids, the side chain of proline cycles back to join with the peptide nitrogen and form a five-member ring. As a result, backbone torsional rotations around the N-Cα bond (φ) are highly restricted relative to other amino acids. Although both *cis* and *trans* isomers of proline occur in globular proteins, polyproline sequences generally assume all *trans* conformations (Almassey and Dickerson, 1978).

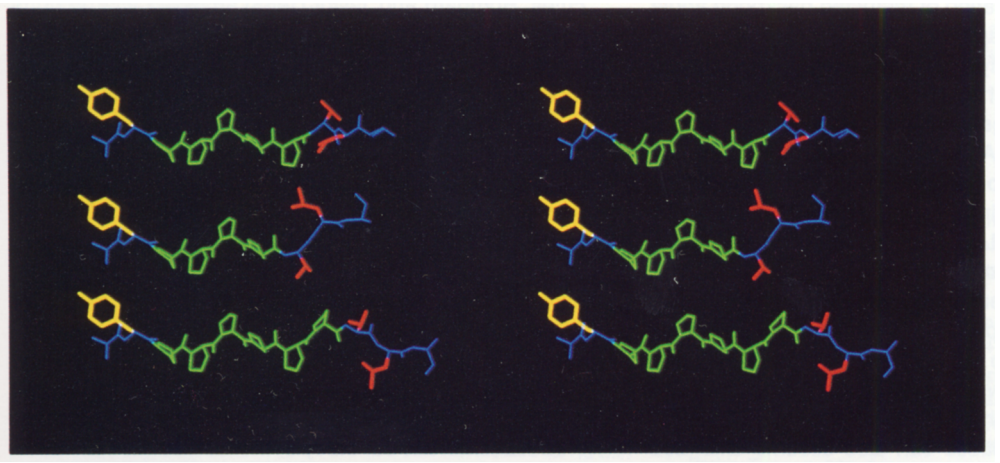

Figure 10. Distance and Orientation of Tyr . . . Thr-Leu Motif Side Chains Differ with Number of Intervening Polyproline Spacer Residues

Top stereogram shows the sequence AYP_5TLA, with the central pentaproline sequence (green) organized in the energetically preferred polyproline II conformation, and with the end residue segments AY and TLA oriented in preferred extended conformations, as described in Experimental Procedures. The residues that confer binding specificity are colored yellow (Y) and red (TL) in this and succeeding stereo pairs. The second and third stereograms, respectively, show the sequences AYP_4TLA and AYP_6TLA with the same local conformations for respective types of amino acid residues. Note that variation in the number of proline spacer residues causes differences in both the distance and the relative orientations between the specificity-conferring side chains at the ends of the rigid polyproline spacer.

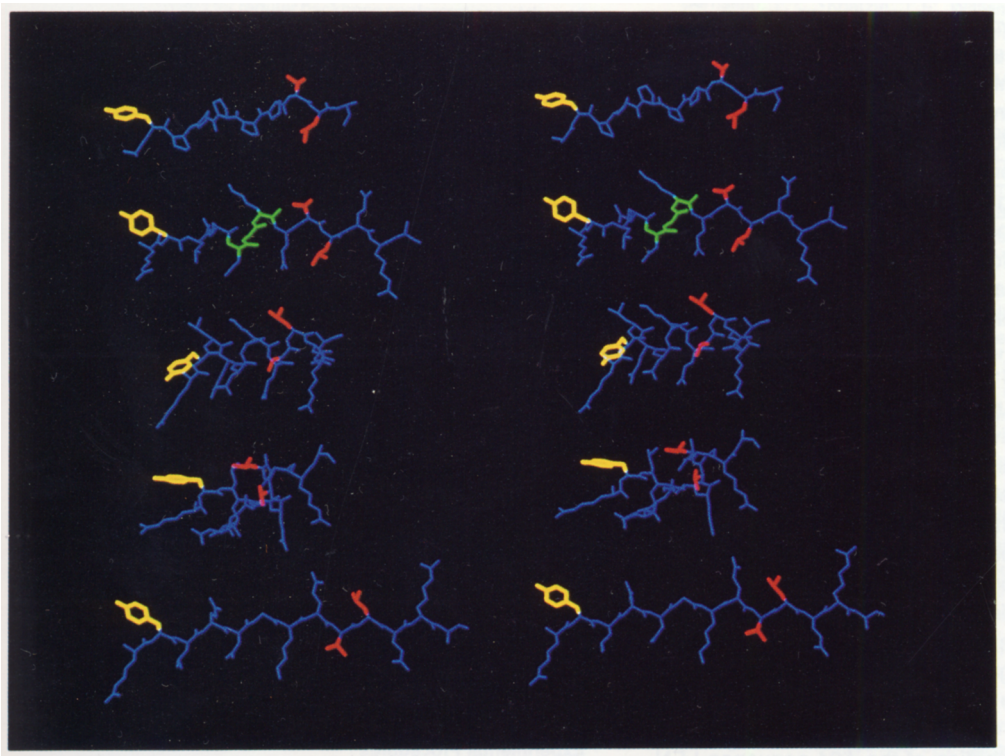

Figure 11. Comparison of Tyr . . . Thr-Leu Motifs in Alternative Polypeptide Conformations Suggests That the Binding Epitope Is Organized as an Extended Chain Incorporating a 3_{10} Helical Turn

Top stereogram shows the sequence AYP_5TLA, with the preferred conformation described in Figure 10. The residues that confer binding specificity are colored yellow (Y) and red (TL) in this and succeeding stereo pairs. The second stereogram shows the A24 peptide sequence, RYLENGKETLQRA, organized in a stable conformation that preserves the distance and approximate orientation of the specificity-conferring side chains in the sequence AYP_5TLA. This conformation consists of an essentially extended polypeptide chain interrupted by a 3_{10} helical turn (green). The conformations shown are directly derived as fragments from refined protein structures (Experimental Procedures). This accounts for slight differences in the orientations of some side chains, which have not been otherwise graphically manipulated. The third, fourth, and fifth stereograms, respectively, show the A24 peptide organized as an α helix, with the conformation experimentally observed in the crystal structure of the homologous region of the HLA-A2 histocompatibility antigen (Bjorkman et al., 1987a) and as an extended polypeptide strand.

Owing to the combination of restricted backbone angle rotation and close juxtapositioning of the proline rings in adjacent residues, polyproline sequences assume extended and stiff 3-fold helical conformations (Sasisekharan, 1959). Figure 10 shows the sequences AYP_5TLA, AYP_4TLA, and AYP_6TLA, which illustrate the central polyproline 3-fold helical conformation, and the resulting differences in both spacing and orientation of the critical residues, Tyr . . . Thr-Leu. Even allowing for deviations from the preferred conformations shown, the data of Table 2 show a marked dependence on peptide length, which strongly suggests a preference for binding extended states of the peptide to the K^d molecule. The preference is mirrored in the relative binding affinities of the polyglycine peptides, where the G5 oligomer is again the best competitor. These peptides can also assume extended 3-fold helical conformations, but might otherwise be expected to bind more weakly than the polyproline peptide owing to the conformational entropy lost from the relatively flexible polyglycine chain when it binds to the MHC in extended conformation.

In view of the strong possibility that the polyproline and polyglycine peptides bind in extended 3-fold helical conformations, it is most interesting to note that the natural peptides incorporate not five, but six residues between Tyr . . . Thr-Leu sequences. If it is reasonably assumed, given the efficacy of the polyproline competitors, that the Tyr . . . Thr-Leu motif assumes a similar orientation in the natural peptides, then the central six residues of the natural peptides must differ in conformation from the polyproline helix. Furthermore, conformational correspondence of the Tyr . . . Thr-Leu sequences necessitates near correspondence of neighboring residues, so that the difference in the interval backbone conformation is essentially restricted to the central four residues of the natural peptide. The simplest molecular model that allows a near spatial correspondence with the polyproline peptide incorporates the introduction of a 3_{10} or similar hairpin bend in the natural peptide (Figure 11). Alternative A24 peptide conformations shown in Figure 11, including the α helix, the native conformation derived from the homologous HLA-A2 major histocompatibility antigen structure (Bjorkman et al., 1987a), and fully extended chain conformation, all fail to match the Tyr . . . Thr-Leu residue spacing and orientation of the AYP_5TLA peptide. To verify that the extended-hairpin structure proposed here as a binding conformation for the A24 peptide would not violate basic principles of protein structure, we searched for similar conformations in a data base of 48 highly refined protein structures (Bernstein et al., 1977). The search produced 24 hexapeptides of variable sequence whose backbone atoms fit the extended-hairpin conformation of the A24 sequence LENGKE (Figure 11) with a root-mean-square error of less than 1.0 Å.

Diverse proposals have been made concerning the relative importance of sequence or structural features in determining interaction specificity between peptides and MHC molecules. Although relatively little is known in structural terms about peptide binding to class I MHC molecules, Rothbard and Taylor (1988) analyzed a large number of T cell epitopes recognized in the context of either class I or class II MHC molecules and identified a common sequence pattern. The general pattern found in most of the peptides was a linear stretch of residues where the first is a charged amino acid or Gly, the next two or three are hydrophobic amino acids, and the last is a polar amino acid or Gly. Allele-specific subpatterns identified for some of the peptides consist of residues 1, 4, 5, and 8, where 4 and 5 correspond to the two central hydrophobic amino acids. In that analysis, the general pattern for the CW3 171–182 peptide includes residues 177–180 (Glu-Thr-Leu-Gln) (Rothbard and Taylor, 1988). By including the Thr-Leu pair, this sequence partially overlaps the Tyr . . . Thr-Leu motif that we have identified experimentally in this study. However, whereas our study demonstrates a major contribution of Tyr residues for the competitor activity of both the A24 and CW3 peptides and the NP 147–158 (R^{-}_{156}) peptide, the Rothbard and Taylor patterns for these peptides exclude Tyr. We have not attempted to identify other NP residues involved in peptide binding.

The conformation that peptides assume as they bind MHC molecules is still the subject of considerable debate. The experiments of Allen et al. (1987) for the peptide lysozyme 46–61 were interpreted as demonstrating an α-helical conformation of the peptide as it binds to the MHC class II molecule $I\text{-}A^k$. In contrast, Sette et al. (1987) concluded that the ovalbumin peptide OVA 323–336 assumes an extended conformation for interaction with $I\text{-}A^d$ via residues 327, 328, 332, and 333. In computer modeling studies with the HLA-A2 class I molecule, Claverie et al. (1989) concluded that peptides in α-helical conformation could not be positioned deep into the MHC groove.

Our strategy of designing functional competitor analogs containing polyproline or polyglycine spacers has led to the prediction of at least one possible conformation for the A24 peptide as it binds to the K^d molecule. Although further work will be required to confirm that the bound A24 peptide is in an extended-hairpin conformation, it seems clear that such a structure could potentially bind deep in the MHC groove, as described for an endogenous peptide in the recent MHC X-ray structure analysis (Bjorkman et al., 1987a). In this context, it is most notable that the groove tapers at its ends, so that it potentially provides complementary fits with polypeptides that incorporate a central bend (or bulge) and extended ends. One important implication of our modeling of the A24 peptide based on the polyproline analog is that peptides with different backbone conformations can bind to the same restriction element.

Experimental Procedures

Cells

The isolation of K^d-restricted, HLA-specific CTL clones from DBA/2 ($H\text{-}2^d$) mice immunized with syngeneic P815 cells transfected with either HLA-CW3 or HLA-A24 genes is presented elsewhere (Maryanski et al., 1986a, 1986b, 1988). CTL clones are designated by the HLA gene expressed by the P815 transfectant used for immunization. The NP-specific CTL clone T5/5 (Taylor et al., 1987) was a kind gift from Dr. B. A. Askonas.

Peptide Synthesis and Purification

The F-moc, t-Bu strategy for solid-phase peptide synthesis was used as described by Merrifield (1986) and Atherthon et al. (1981). HPLC-purified peptides were >90% pure by analytical HPLC. Lyophilized peptides were dissolved in 0.7% sodium bicarbonate buffer or water and further diluted in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS).

Cytolytic Assay

P815 cells (10^6) were labeled with 150 μCi of sodium [^{51}Cr]chromate as described (Cerottini et al., 1974) for 1 hr at 37°C and washed three times. Labeled targets (2×10^3 in 50 μl volumes) were added to wells of V-bottomed microtiter plates containing 100 μl volumes of the appropriate peptide diluted in DMEM supplemented with 5% FCS and HEPES. CTLs (6×10^3 cells) were added in 50 μl volumes. After a 4 hr incubation at 37°C, the supernatants (100 μl) were harvested for counting. The percentage of lysis was calculated as: 100 × [(experimental − spontaneous release)/(total − spontaneous release)]. For competition experiments, the target cells were incubated for 15 min with the competitor peptide (100 μl volume) before addition of a suboptimal concentration of the antigenic peptide (50 μl volume). CTLs (50 μl volume) were added after a further 15 min of incubation at room temperature. The plates were then incubated for 4 hr at 37°C. The percentage of control lysis was calculated as: 100 × [(percentage of lysis with competitor − background lysis)/(percentage of lysis without competitor − background lysis)]. Background lysis represents the percentage of lysis of the target cells in the absence of peptides. The relative competitor efficiency of the peptides was calculated for the peptide concentration required to obtain 50% of control lysis.

Structural Modeling of the Peptides

Atomic coordinates for regular backbone conformations of the polyproline II helix ($\varphi = -78$, $\psi = 149$), the 3_{10} helix ($\varphi = -49$, $\psi = -26$), the α helix ($\varphi = -57$, $\psi = -47$), and a slightly twisted extended chain ($\varphi = -113$, $\psi = 112$) were generated by application of dihedral angular rotations (φ and ψ, given above) to a standard polypeptide backbone model. Both the backbone and side chain conformations of the terminal Y, T, and L residues of the sequence AYP$_5$TLA were determined by statistical analysis of a library of 48 highly refined protein structures (Bernstein et al., 1977) to determine the most probable conformations for residues preceding or following proline sequences in globular proteins. These most probable structures (Janin et al., 1978; Jones and Thirup, 1986; Ponder and Richards, 1987; Weber et al., 1989) were then fitted (Kabsch, 1978) to the central polyproline II helices to define the conformation of the peptides shown in Figure 10, top.

The A24 peptide RYLENGKETLQRA incorporates six amino acids between Y and TL residues, versus five amino acids for the AYP$_5$TLA peptide. Accordingly, we searched the structural data base as described above for six-residue conformations that would span a length approximately equal to the polyproline P$_5$ segment, and allow similar orientations of the connected Tyr, Thr, and Leu side chains to those in the AYP$_5$TLA peptides. In fact, these criteria are quite restrictive and admit only a small family of related structures that are composed of extended end segments connected by a kink, exemplified in Figure 11 as a 3_{10} helical turn, as plausible A24 peptide models.

Cα backbone coordinates from the crystal structure determination of the HLA-A2 major histocompatibility antigen (Bjorkman et al., 1987a) were obtained from the Brookhaven Protein Data Bank (Bernstein et al., 1977). Side chain positions were appended to these coordinates as described in Results to generate the model in Figure 11.

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