Privalov, P. L. (1979) *Adv. Protein Chem. 33*, 167–241.
Privalov, P. L. (1982) *Adv. Protein Chem. 35*, 1–104.
Privalov, P. L., & Khechinashvili, N. N. (1974) *J. Mol. Biol. 86*, 665–684.
Ragone, R., Colonna, G., Balestrieri, C., Servillo, L., & Inace, G. (1984) *Biochemistry 23*, 1871–1875.
Ramsay, G., & Freire, E. M. (1990) *Biochemistry 29*, 8677–8683.
Rhee, S. G., Chock, P. B., & Stadtman, E. R. (1989) *Adv. Enzymol. 62*, 37–92.
Schwarz, F. P. (1989) *Thermochim. Acta 147*, 71–91.
Schwarz, F. P., & Kirchhoff, W. H. (1988) *Thermochim. Acta 128*, 267–295.
Shrake, A., Fisher, M. T., McFarland, P. J., & Ginsburg, A. (1989) *Biochemistry 28*, 6281–6294.
Shriver, J. W., & Kamath, U. (1990) *Biochemistry 29*, 2556–2564.
Stadtman, E. R., & Ginsburg, A. (1974) *Enzymes (3rd Ed.) 10*, 755–807.
Stadtman, E. R., Smyrniotis, P. Z., Davis, J. N., & Wittenberger, M. E. (1979) *Anal. Biochem. 95*, 275–285.
Sturtevant, J. M. (1987) *Annu. Rev. Phys. Chem. 38*, 463–488.
Valentine, R. C., Shapiro, B. M., & Stadtman, E. R. (1968) *Biochemistry 7*, 2143–2152.
Villafranca, J. J., Ash, D. E., & Wedler, F. C. (1976) *Biochemistry 15*, 536–543.
Woolfolk, C. A., Shapiro, B. M., & Stadtman, E. R. (1966) *Arch. Biochem. Biophys. 116*, 177–192.
Yamashita, M. M., Almassy, R. J., Janson, C. A., Cascio, D., & Eisenberg, D. (1989) *J. Biol. Chem. 264*, 17681–17690.

NMR Studies of an Oligoproline-Containing Peptide Analogue That Binds Specifically to the H-2K^d Histocompatibility Molecule†

Benoit Boulat,‡,§ Lyndon Emsley,‡ Norbert Müller,∥ Giampietro Corradin,§ Janet L. Maryanski,⊥ and Geoffrey Bodenhausen*,‡

Section de Chimie, Université de Lausanne, Rue de la Barre 2, CH-1005 Lausanne, Switzerland, Institute of Biochemistry, Université de Lausanne, Chemin des Boveresses, CH-1066 Epalinges, Switzerland, Institut für Chemie, Johannes Kepler Universität, Altenbergerstrasse 69, A-4040 Linz, Austria, and Ludwig Institute for Cancer Research, Lausanne Branch, Chemin des Boveresses, CH-1066 Epalinges, Switzerland

Received May 22, 1991; Revised Manuscript Received July 18, 1991

ABSTRACT: T lymphocytes expressing variable cell surface antigen receptors recognize "processed" forms of antigen, presented on the surface of other cells by molecules of the major histocompatibility complex (MHC). Naturally processed antigenic peptides can be replaced by synthetic ones. The synthetic peptide AYPPPPPTLA (P5) is an active competitor to the antigenic peptide HLA A24 170–182 (sequence RYLENGKETLQRA) that is recognized by A24 specific T cells in association with the H-2K^d class I MHC molecule. In P5 the five prolines were designed to play the role of a rigid spacer between the residue Y and the T–L unit, so as to mimic the role of Y^{171}, T^{178}, and L^{179} in the HLA A24 antigenic peptide, since these residues have proven to be the most important with respect to the binding of the HLA A24 peptide with the H-2K^d MHC molecule. Nuclear magnetic resonance studies allow us to demonstrate that in aqueous solution P5 adopts at least three long-lived conformations that can be classified with respect to the Y^2–P^3–P^4 amide bonds as trans–trans, cis–trans, and cis–cis. Among these, the trans–trans form is present in 67% of the molecules while the two others share the remaining 33%.

Specific immune responses are mediated by T and B lymphocytes that express clonally variable antigen receptors on cell surfaces. Unlike B lymphocytes that can directly bind antigens in the native conformation, T lymphocytes apparently recognize only "processed" forms of antigens, probably peptides, presented on the surface of other cells by polymorphic molecules encoded by the major histocompability complex (MHC) (Townsend & Bodmer, 1989). Two distinct classes of MHC molecules that differ in structure, biosynthesis, and tissue distribution are known to present antigens to T lymphocytes. In general, antigenic peptides derived from endogenous proteins are presented by class I MHC molecules, whereas those from exogenous proteins are presented by class II (Germain, 1986). Both kinds of antigens can be replaced experimentally by synthetic peptides, provided they are presented by cells expressing the appropriate MHC molecules (Townsend & Bodmer, 1989).

Direct binding studies with antigenic peptides and purified MHC molecules (Buus et al., 1987) support the concept that T cell antigen recognition can be accounted for, at least in part, by the specificity of peptide interaction with MHC molecules. Thus the way in which the peptide binds to the MHC molecule is essential for T cell recognition. In an X-ray crystallographic analysis of MHC class I molecules, a groove containing the most polymorphic residues was identified as the putative antigen-binding site (Bjokman et al., 1987). Class II molecules are presumed to contain a similar site (Brown et al., 1988). The analysis of cocrystals composed of MHC molecules and

† This research was supported in part by the Swiss National Science Foundation, by the Commission pour l'Encouragement de la Recherche Scientifique, and by Spectrospin, Fällanden.
* To whom correspondence should be addressed.
‡ Section de Chimie, Université de Lausanne.
§ Institute of Biochemistry, Université de Lausanne.
∥ Johannes Kepler Universität.
⊥ Ludwig Institute for Cancer Research.

0006-2960/91/0430-9429$02.50/0

defined antigenic peptides has not yet been reported nor has the structure of bound peptides been determined. Moreover, most antigenic peptides have no defined structure in aqueous solution.

The antigenic peptide A24 170-182 ($RY^{171}LENGKET^{178}L^{179}QRA$) that is recognized by A24-specific T cells in association with the H-$2K^d$ (K^d) class I MHC molecule can compete with other unrelated antigenic peptides that are also recognized by K^d-restricted T cells (Maryanski et al., 1988; Pala et al., 1988). By comparing amino acid substituted peptides as competitors, the residue Tyr^{171} and the pair of residues Thr^{178}/Leu^{179} were identified as possible contact residues for the K^d molecule (Maryanski et al., 1990). In an attempt to understand the conformation that peptides assume as they bind to MHC molecules, we have designed polyproline-containing peptide analogues (Maryanski et al., 1990) where the polyproline unit is designed to act as a rigid spacer between the putative contact residues. By this approach, a highly active competitor analogue (AYP_5TLA) of A24 was synthesized in which Tyr was separated from the Thr/Leu pair by a pentaproline spacer. Ala residues were included at the N- and C-terminal ends of the analogue to prevent the charged ends of the peptide from coinciding with the nominal contact residues. Polyproline was chosen as a spacer due to its limited conformational possibilities compared to other homo-oligo amino acid sequences (Chao & Bersohn, 1978; Okabayashi & Isemura, 1968; Sato, 1984), since polyproline is normally thought to occupy a largely all-trans conformation. Similar peptides with Pro_4 or Pro_6 spacers (i.e., shorter or longer distances between the "active" sites) were at least 10-fold less active (Maryanski et al., 1990).

In the present study, we examine the conformation of the pentaproline analogue AYP_5TLA in solution using information obtained by nuclear magnetic resonance (NMR) experiments. Our results demonstrate that the four amide bonds *within* the polyproline spacer assume an all-trans conformation in at least 75% of the molecules. Nevertheless, we present evidence that the AYP_5TLA peptide can adopt at least *three* relatively long-lived conformations with cis/trans isomerization about the Tyr^2–Pro^3 *and* the Pro^3–Pro^4 amide bonds.

Materials and Methods

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, and the exact composition was determined by amino acid analysis.

NMR. NMR samples were prepared from lyophilized peptides. The samples weighed 10 and 50 mg and were dissolved in the appropriate solvent, i.e., 90% H_2O/10% D_2O or 99.9% D_2O or 99.9% DMSO-d_6, and made up to between 0.5 and 0.65 mL. The pH (or pD) was adjusted with NaOH (NaOD) or HCl (DCl) solutions. Unless otherwise stated, the samples were adjusted to a pH of 6.5. We routinely add an internal standard of DSS (dimethylsilyl-1-propanesulfonic acid) with all chemical shifts being referenced to the methyl resonance at 0 ppm.

NMR spectra were obtained on Bruker AM-400 and MSL-300 spectrometers equipped with digital phase shifting hardware. We recorded 1D and 2D spectra of both ^1H and ^{13}C. The 2D experiments were used to assign the spectra, in particular double-quantum-filtered correlation spectra (DQF-COSY) (Piantini et al., 1982; Shaka et al., 1983; Rance et al., 1983), soft-COSY (Brüschweiler et al., 1987; Cavanagh et al., 1987; Emsley & Bodenhausen, 1991a,b), total correlation spectroscopy (TOCSY) (Braunschweiler & Ernst, 1983; Bax & Davis, 1985a), and heteronuclear ^{13}C–^1H spectra (Ernst et al., 1987). For complete assignment of the NH protons, we recorded DQF-COSY and TOCSY spectra in H_2O solution with suppression of the water resonance achieved by coherent presaturation (Zuiderweg et al., 1986); for all other purposes we used spectra recorded in D_2O or DMSO-d_6 solution so as to avoid the need for suppression of the water resonance. We recorded one-dimensional carbon-13 spectra at natural abundance using the INEPT technique (Morris & Freeman, 1979; Aue et al., 1980). In order to determine the secondary structure, we used both the NOESY (Jeener et al., 1979; Neuhaus & Williamson, 1989) and ROESY (Bothner-By et al., 1984; Davis, 1987) techniques. In both the ROESY and TOCSY experiments, the spin-lock was achieved by using a decoupler phase coherent with the transmitter carrier. For ROESY experiments we tried both CW and pulsed spin-lock (Bax & Davis, 1985b; Kessler et al., 1987), while for TOCSY we used 16 cycles of an MLEV-17 sequence (Bax & Davis, 1985a) having a total duration of 70 ms. Soft-COSY experiments were implemented with self-refocusing 270° Gaussian pulses (Emsley et al., 1989, 1991a,b) using an external Bruker selective excitation unit. Processing of the spectra was done on ASPECT and X32 computers equipped with array processors using standard Bruker software; the parameters used are given in the figure captions.

Circular Dichroism. Ultraviolet circular dichroism (CD) spectra were recorded for AYP_nTLA, where n = 2, 4, 5, or 6 at 295 K, in H_2O, on an Jobin Yvon circular dichroism spectrophotometer using 1-cm cells at pH 6.5.

Results and Discussion

Due to its supposed structural rigidity, the PPPPP sequence greatly simplifies the biochemical problem of designing analogues, but from an NMR point of view the determination of the structure by analysis of each proline spin system per se is very difficult, due to extensive degeneracies. Nevertheless, we shall see that we have been able to obtain quite instructive information about the conformation of the putative contact sites.

The A24 analogues were chosen on the hypothesis, based on previous CD and NMR studies (Chao & Bersohn, 1978; Okabayashi & Isemura, 1968; Sato et al., 1984), that for polyproline P_n segments with n greater than five there is a tendency for the structure to assume a conformation in which the amide bonds are all-trans. To test this hypothesis, we first performed CD experiments in the wavelength range of 200–260 nm on the series of compounds of the form AYP_nTLA (n = 2, 4, 5, and 6). The results indicate that as n increases the successive spectra approach the spectrum of polyproline II chains (all-trans). In accordance with previous ideas, this result can be interpreted as an indication that the percentage of trans proline amide bonds increases as n increases. However, from CD spectra we cannot determine the conformation of individual peptide bonds, as CD gives only qualitative results based on the form of the observed spectrum as compared to that of a known reference [in this case the reference compound is polyproline II, the crystal structure of which is known (Sasisekharan, 1959)]. Thus we cannot distinguish, if for example 80% of the P_5 peptide is thought to be trans, whether each amide bond is 80% trans and 20% cis or whether four bonds are 100% trans and one is 100% cis. For this type of detailed information, we must take recourse to NMR.

In 1D NMR spectra of AYP_5TLA we immediately observe at least two distinct species in the sample that could not be further separated by HPLC. More specifically, in the 1D

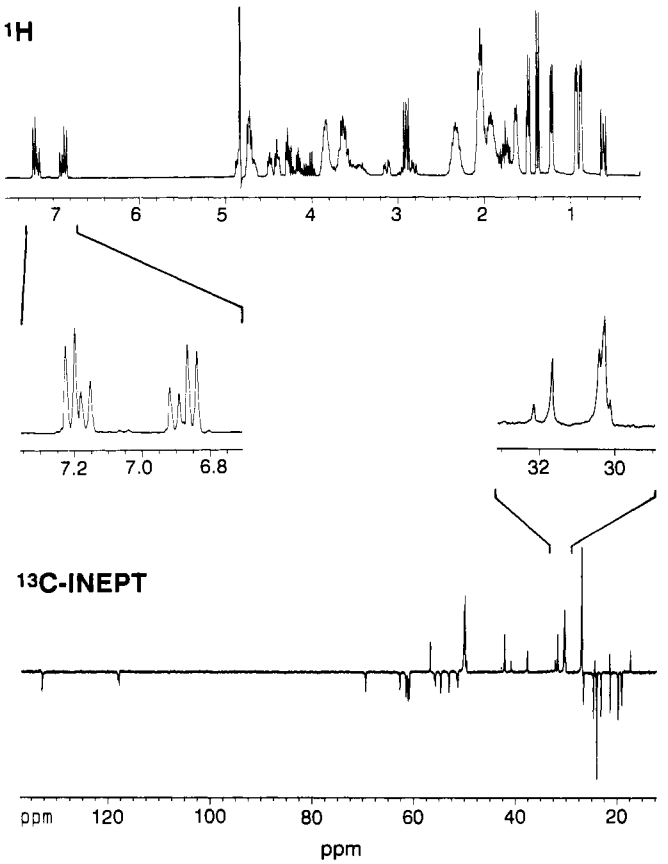

FIGURE 1: ^1H- and ^{13}C-INEPT spectra of AYP_5TLA, the latter showing negative signals for CH and CH_3 groups. The most striking features are the two sets of aromatic resonances in the ^1H spectrum (see inset) and the splitting of the proline β and γ signals into two groups in the ^{13}C spectra (β is shown in the inset). The two ^{13}C peaks at around 32 ppm probably correspond to the "cis–cis" and "cis–trans" conformations, while the peaks at 30.5 ppm correspond to the "trans–trans" conformations of the peptide.

proton spectrum there are clearly two sets of aromatic tyrosine spin systems with populations of 33% of 67% (see Figure 1). Moreover, in the ^{13}C-INEPT spectrum (Figure 1) not only do we observe two sets of aromatic resonances but the proline C^β and C^γ signals are also split into two groups in a manner that is known to be indicative of proline amide bond in cis/trans equilibrium (Grathwohl & Wüthrich, 1976; Chao & Bersohn, 1978; Dyson et al., 1988).

In order to rule out the possibility of a mixture of two noninterconverting structures, pD-jump experiments were conducted. We found that in D_2O the percentage of the minor isomer changed from 32% to 37.5% within 48 h when varying the pD from 1.5 to 9.1, as monitored by the intensities of the aromatic resonances. Further evidence for a slow isomer equilibrium has been drawn from the ratio of the aromatic proton signals in DMSO. Immediately after the lyophilized peptide was dissolved, the percentage of the minor isomer was 29% while 24 h later the value had reached a steady state at 18.7%. Changing the temperature between 298 and 323 K did not exhibit any effect on this cis/trans ratio. Since no evidence for exchange was seen in NOESY spectra, we can conclude that the exchange between the two isomers takes place on a very slow time scale.

For the identification of the spin systems, we used DQF-COSY and TOCSY in H_2O and DQF-COSY, soft-COSY, TOCSY, and ^1H–^{13}C correlation spectroscopy in D_2O. The assignments, based on multiplicity and chemical shift criteria (Wüthrich, 1987), are summarized in Tables I and II. As

Table I: Assignments of ^1H Signals in AYP_5TLA at 303 K in H_2O/D_2O Solution

residue	spin	^1H (ppm)		
		trans[a]	cis[a]	
Ala[1]	NH			
	CH^α	4.05	4.10	
	CH_3^β	1.50	1.52	
Tyr[2]	NH	7.62	8.77	
	CH^α	4.87	4.38	
	CH_2^β	2.83, 3.13	2.90, 2.95	
	CH_2^γ, CH_2^δ	6.87, 7.20	6.92, 7.12	
		trans–trans[b]	cis–trans[b]	cis–cis[b]
Pro[3]	CH^α	4.70	3.65	3.65
	CH_2^β	1.90, 2.35	1.85, 2.33	1.85, 2.33
	CH_2^γ	2.00	2.05	1.74
	CH_2^δ	3.65, 3.86	3.45, 3.52	3.40, 3.46
Pro[4]–Pro[6][c]	CH^α	4.70		
	CH_2^β	1.90, 2.35		
	CH_2^γ	2.00		
	CH_2^δ	3.65, 3.86		
Pro[7]	CH^α	4.50		
	CH_2^β	1.90, 2.27		
	CH_2^γ	2.00		
	CH_2^δ	3.65, 3.86		
Thr[8]	NH	8.17		
	CH^α	4.32		
	CH^β	4.17		
	CH_3^γ	1.22		
Leu[9]	NH	8.51		
	CH^α	4.40		
	CH_2^β	1.64		
	CH^γ	1.64		
	CH_3^δ, CH_3^δ	0.83, 0.94		
Ala[10]	NH	8.06		
	CH^α	4.17		
	CH_2^β	1.35		

[a] Refers to the conformation of the Tyr[2]–Pro[3] amide bond. [b] Refers to the conformation of the Tyr[2]–Pro[3] and the Pro[3]–Pro[4] amide bonds, respectively. [c] Due to overlap, individual assignments for Pro[4]–Pro[6] are not possible.

Table II: Assignments of ^{13}C Signals in AYP_5TLA at 303 K in H_2O/D_2O Solution

residue	C^α	C^β	C^γ	C^δ, $C^{\delta'}$
Ala[1]	51.5	20.0		
Tyr[2] (trans)[a]	56.0	b	118.1, 135.2	
Tyr[2] (cis)[a]	56.1	b	118.4, 135.1	
Pro[3] (trans–trans)[c]	61.1–61.3[d]	30.5–32.4[d]	27.2	50.0
Pro[3] (cis–trans)[c]	61.2	30.5–32.4[d]	27.2	50.0
Pro[3] (cis–cis)[c]	61.2	30.5–32.4[d]	27.2	50.0
Pro[4]–Pro[6]	61.1–61.3[d]	30.5–32.4[d]	27.2	50.0
Pro[7]	61.7	30.5–32.4[d]	27.2	50.0
Thr[8]	62.0	69.7	20.0	
Leu[9]	54.9	42.3	26.8	23.4, 24.9
Ala[10]	54.0	19.4		

[a] Refers to the conformation of the Tyr[2]–Pro[3] amide bond. [b] Not found. [c] Refers to the conformations of the Tyr[2]–Pro[3] and Pro[3]–Pro[4] amide bonds, respectively. [d] Due to overlap, individual assignments are not possible.

expected, besides the two distinct aromatic tyrosine AA'BB' spin systems, we also find *two* sets of aliphatic tyrosine H^α–H^{β_1}–H^{β_2} spin systems. In addition we also see *three* Ala H^α–H^β cross peaks, which are attributable to the change in the Ala[1] resonance due to the cis/trans isomerization of the Tyr[2]–Pro[3] bond, giving rise to two H^α–H^β cross peaks for Ala[1] and one for Ala[10]. This hypothesis is confirmed by the fact that the intensities of the two multiplets in the 1D spectrum have the same ratio to one another as those of the aromatic tyrosine resonances. The only unusual assignment is that of the H^α of Pro[3], which is shifted to a distinctly lower frequency

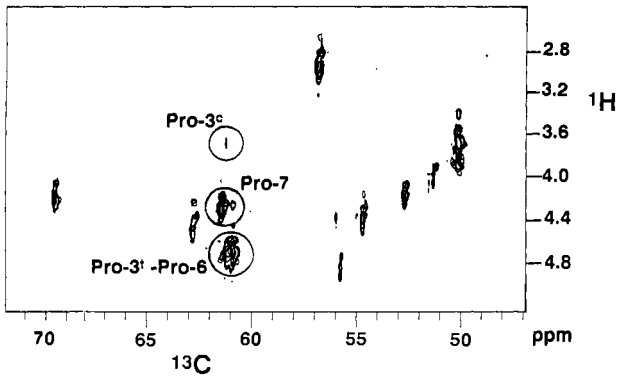

FIGURE 2: Portion of the ^1H–^{13}C correlation spectrum of AYP_5TLA showing the Pro H^α–C^α correlations between 55 and 65 ppm. The spectral width in the ω_1 dimension was 9 ppm, while in the ω_2 dimension it was 150 ppm; 4096 points were recorded in t_2 and 128 scans for each of the 1024 t_1 increments. The data were zero-filled to 2K × 4K and weighted by a Lorentz-to-Gauss function before Fourier transformation.

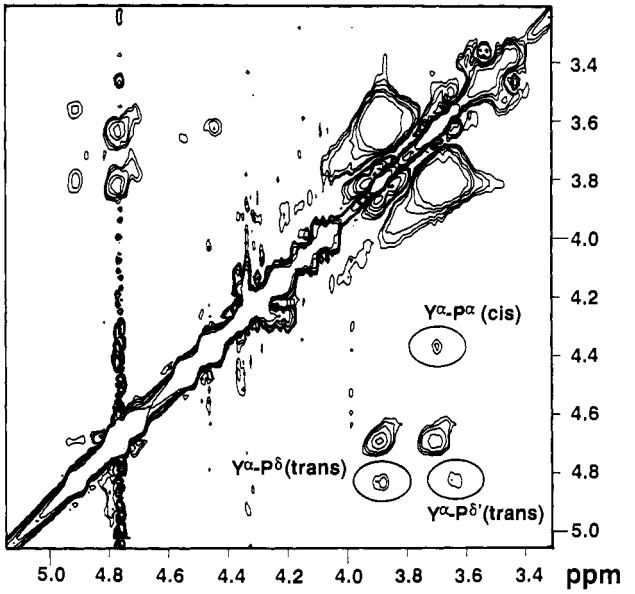

FIGURE 3: Region of the ROESY spectrum of AYP_5TLA containing the interresidue cross peaks between Tyr H^α and Pro H^α and H^δ resonances. A radio frequency field of 4 kHz was used to spin lock the magnetization for 150 ms. The spectral width in both dimensions was approximately 9 ppm; 4096 complex points were recorded in t_2 and 64 scans for each of the 512 t_1 increments. The data were zero filled to 1K × 4K and weighted by a Lorentz-to-Gauss function before Fourier transformation.

when the Tyr^2–Pro^3 bond is cis than the H^α of the other prolines. In fact, it is overlapping with the H^δ resonances of the other prolines, but ^1H–^{13}C correlation spectroscopy clearly indicates its existence (Figure 2). The H^δ resonance of Pro^3 is also shifted to lower frequency. These observations are in accordance with previous studies on smaller Tyr–Pro containing peptides (Dyson et al., 1988). As a result of severe overlap, the other proline spin systems cannot be completely disentangled. However, apart from a group of poorly resolved Pro H^α peaks belonging to Pro^4–Pro^6, we clearly see one other Pro H^α that must belong to Pro^7 (see below).

Not only does the assignment of such a highly degenerate peptide pose problems, but we expect that, due to the size of the peptide (MW = 1023), the correlation time of its motion in solution will be such that $\omega_0\tau_c \approx 1.1$, so that it will have a critical correlation time (Neuhaus & Williamson, 1989). In this regime opposite contributions to dipolar cross-relaxation will cancel so that, for example, a NOESY spectrum will not show any correlations, even for protons that are close in space. This makes the problem of secondary structure determination particularly challenging. Indeed NOESY spectra did not show *any* interresidue correlations (nor did they show any evidence of isomer exchange). Fortunately, it is possible to modify slightly the contributions to relaxation by performing so-called "rotating frame" measurements (ROESY) (Bothner-By et al., 1984; Davis, 1987) where the magnetization is locked near the tranverse plane of the rotating frame. Performing such an experiment modifies the contributions to the dipolar cross-relaxation rates so that we expect to see some weak correlations in spite of the critical correlation time. Thus ROESY experiments were used to obtain secondary structure information. No correlations could be found for either of the two alanine residues, or for the threonine, or for the leucine, reflecting the high mobility expected at the ends of the peptide chain. As shown in Figure 3, in the major trans isomer the H^α of the tyrosine has two cross peaks to the H^δ and $H^{\delta'}$ resonances of Pro^4 (and not to those of Pro^3), while in the minor cis isomer the Tyr H^α only shows one correlation to the H^α of Pro^3. The only other interresidue contacts observed in this spectrum are two sets of very intense cross peaks between the poorly resolved proline resonances at 4.70 for H^α and 3.65 and 3.86 for the two H^δ resonances.

The ROESY data can be interpreted in terms of conformation by comparing the data with contacts expected on the basis of molecular models. Using this approach, we can arrive at some fairly detailed conclusions about the structure of the peptide. First, contacts between proline^i H^α and proline^{i+1} H^δ and $H^{\delta'}$ can only occur in the presence of a trans proline–proline amide bond, supporting the CD spectroscopic results by indicating that the large majority of the proline–proline amide bonds are in trans. Secondly, cross peaks between Tyr^2 H^α and the two H^δ resonances of Pro^4 indicate a trans Tyr^2–Pro^3 amide bond in the major isomer, while the contact between Tyr^2 H^α and Pro^3 H^α is only possible when this bond is in cis, which is therefore the case for the second isomer. Thus, from the ROESY data we conclude that the bulk of the peptide chain is assuming a trans conformation but that the Tyr^2–Pro^3 bond is in a cis/trans equilibrium.

In addition, as we do not observe any ROESY cross peaks involving the proline H^α resonating at 4.50 ppm, while prolines H^α resonating at 4.70 show contacts to H^δ and $H^{\delta'}$, we conclude that the signal at 4.50 ppm corresponds to Pro^7 at the end of the oligo-proline spacer.

These conclusions can be further supplemented by the COSY and TOCSY data, which contain evidence that the Pro^3–Pro^4 bond can undergo cis/trans isomerism *provided* the Tyr^2–Pro^3 bond is in cis. We draw this conclusion from the DQF-COSY spectrum in D_2O where we see that when the Tyr^2–Pro^3 bond is in cis, the H^δ protons of Pro^3, which are shifted to a lower frequency than normal, are duplicated and show *two* cross peaks to the H^γ region. These two cross peaks appear at different frequencies in both dimensions, precluding the possibility that they are due to two inequivalent H^γ of Pro^3. We interpret this duplication of the Pro^3 H^δ and H^γ resonances in terms of a cis/trans isomerization of the Pro^3–Pro^4 amide bond within the oligoproline spacer. Unfortunately, due to overlap we can only clearly observe these peaks in 2D spectra, and the integrals are not yet reliable enough to calculate the ratio of this isomerization. To be able to decide which peak corresponds to cis and which one to trans, we performed the same type of experiments on molecules built with a smaller proline spacer (AYP_2TLA) or larger ones (AYP_6TLA, AYP_8TLA). Bearing in mind the results for polyproline oligomers which suggest that the cis conformation is more stable

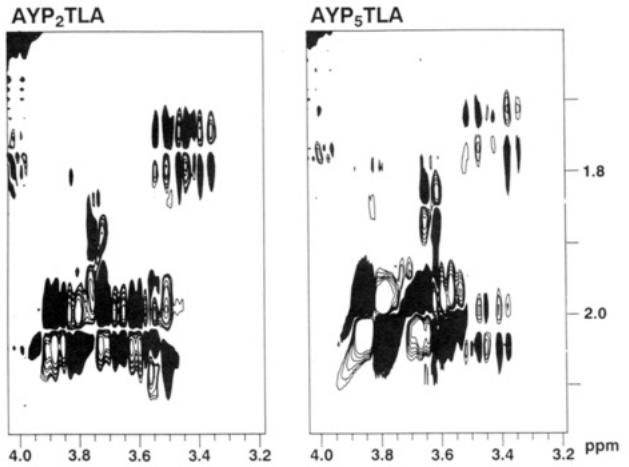

FIGURE 4: Identical regions of the DQF-COSY spectra of AYP_2TLA and AYP_5TLA. As expected, in the P_2 compound we see only one H^{δ}–H^{γ} cross peak centered at 1.74 in ω_1 and 3.45 ppm in ω_2, which we assume to correspond to the cis isomer. In the P_5 compound *two* H^{δ}–H^{γ} cross peaks appear centered at 1.74 and 2.05 ppm in ω_1 and 3.44 and 3.46 ppm in ω_2, reflecting the appearance of cis and trans isomerization around the Pro^3–Pro^4 amide bond when the Tyr^2–Pro^3 bond is cis. Overlap effects prevent us from obtaining reliable integrals. Similar spectra to P_5 are observed for P_6 and P_8 analogues.

Table III: Conformations of the Tyr^2–Pro^3 and the Pro^3–Pro^4 Amide Bonds in AYP^5TLA

Tyr^2–Pro^3	Pro^3–Pro^4	proportion
cis	cis	18% ± 10%[a]
cis	trans	15% ± 10%[a]
trans	trans	67%
trans	cis	not obs

[a] Estimated from COSY/TOCSY spectra, see the text for details.

in the dimer than in higher oligomers and that for chains with more than five prolines an all-trans conformation is assumed, we should be able to assign the peaks. Indeed, in AYP_2TLA only one H^{δ}–H^{γ} cross peak remains, clearly giving the assignment of the cis conformation (see Figure 4). Rather surprisingly, however, for the hexaproline- and the octaproline-containing analogues, both the cis and trans cross peaks are still present. It seems that the Tyr^2–Pro^3 bond isomerization has a "knock on" effect by favoring the isomerization of the Pro^3–Pro^4 bond, *irrespective of the length of the oligoproline chain.*

Conclusions

Due to their "floppiness", we have not been able to say anything specific about the conformation of the Ala^1, Thr^8, Leu^9, and Ala^{10} residues on either end of the peptide chain. However, we are able to observe three distinct conformations for the central Tyr^2–Pro^7 section of the chain, shown in Figure 5. These conformations differ only in cis/trans isomerisms around the Tyr^2–Pro^3 and the Pro^3–Pro^4 amide bonds, summarized in Table III. All amide bonds from Pro^4 to Pro^7 are always trans. Classifying the conformation of the molecule in solution according to the trans or cis nature of the Tyr^2–Pro^3 and Pro^3–Pro^4 amide bonds, around 67% of the molecules are in the "trans–trans" form. Judging from the intensities of the cross peaks between Pro^3 H^{γ} and H^{δ} in the 2QF-COSY and TOCSY spectra, the "cis–cis" conformation seems to have a slightly larger population than the "cis–trans" conformation. We conclude that the four amide bonds within the pentaproline spacer adopt an all-trans conformation in between 75% and 80% of the total.

Contrary to previous indications for polyproline chains, according to which the structure tends to be increasingly

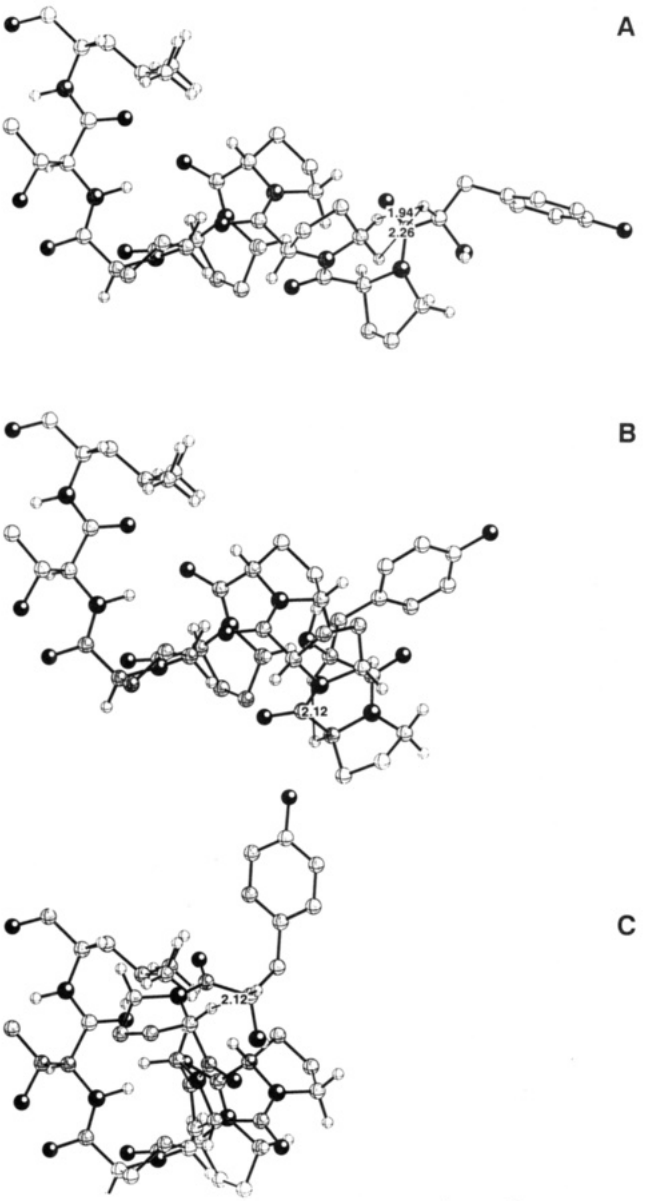

FIGURE 5: Canonical models of YP_5TL. The Pro^4 to Pro^7 amide bonds are all-trans. The Tyr^2–Pro^3 and Pro^3–Pro^4 bonds are trans–trans in panel A, cis–trans in panel B, and cis–cis in panel C. The models were made with the program BALL AND STICK (Müller & Falk, 1991). We have omitted the terminal Ala residues at both ends, and we have maintained the same (arbitrary) conformation of the Y, T, and L residues in each structure.

all-trans as n increases, our results clearly indicate that for YP_n chains the Tyr^2–Pro^3 *and* the Pro^3–Pro^4 amide bonds are always in cis/trans equilibrium, whatever the length of the chain. This seems to involve some sort of cooperative mechanism in which a cis conformation of the Tyr^2–Pro^3 amide bond in turn destabilizes the trans conformation of the adjacent Pro^3–Pro^4 amide bond. More detailed work on the mechanism of this isomerization is in progress.

As we see from Figure 5, the interresidue distances between the tyrosine, threonine, and leucine units change dramatically depending on the conformation of the Tyr^2–Pro^3 and Pro^3–Pro^4 peptide bonds. Consequently, if the preferred conformation for binding to MHC is indeed all-trans, as was the original hypothesis that motivated the construction of the AYP_5TLA peptide, we can conclude that the other two conformations should be much less active. If this is the case, it is possible that other analogues with truly rigid spacers may be even more effective for binding than AYP_5TLA. This conclusion rests

on the fact that we have seen that interconversion between isomers is slow in solution. It is quite possible, however, that during binding to the MHC molecule conversion to an all-trans conformation is facilitated.

Indeed, all our studies refer to solution conformation, while in fact we would be most interested to learn about the bound form of the peptide. This work has progressed on the assumption that the bound and the free conformations do not significantly differ. Indeed, molecular modeling studies have been made that involved inserting the AYP_5TLA peptide "by hand" into a model of the H-2K^d molecule on the basis of the X-ray derived structure of the HLA-A2 class I molecule (Maryanski et al., 1991). They found that an all-trans conformation is compatible with binding, but of course nothing can be said about the uniqueness of this particular binding scheme. Further work will concentrate on trying to determine the bound structure of the peptide using NMR. Such a project faces several problems, not the least of which are, from the NMR point of view, the molecular weight of the MHC molecule and, from the biochemical point of view, the availability of "clean" MHC.

ACKNOWLEDGMENTS

We are indebted to R. Flögel for assistance with the molecular modeling.

REFERENCES

Atherthon, E., Logan, C. J., & Sheppard, R. C. (1981) *J. Chem. Soc., Perkin Trans. 1*, 538.
Aue, W. P., Burum, D., & Ernst, R. R. (1980) *J. Magn. Reson. 38*, 375.
Bax, A. (1983) *J. Magn. Reson. 53*, 517.
Bax, A., & Davis, D. G. (1985a) *J. Magn. Reson. 63*, 207.
Bax, A., & Davis, D. G. (1985b) *J. Magn. Reson. 65*, 355.
Bjorkman, P. J., Saper, M. A., Saraoui, B., Bennett, W. S., Strominger, J. L., & Wiley, D. C. (1987) *Nature 329*, 506 and 512.
Brown, J. H., Jardetsky, T., Saper, M. A., Samraoui, B., Bjorkman, P. J., & Wiley, D. C. (1988) *Nature 337*, 845.
Bothner-By, A. A., Stephens, R. L., Lee, J. M., Warren, C. D., & Jeanloz, R. W. (1984) *J. Am. Chem. Soc. 106*, 811.
Brüschweiler, L., & Ernst, R. R. (1983) *J. Magn. Reson. 53*, 521.
Braunschweiler, R., Madsen, J. C., Griesinger, C., Sørensen, O. W., & Ernst, R. R. (1987) *J. Magn. Reson. 73*, 380.
Buus, D., Sette, A., Colon, D. M., Miles, C., & Grey, H. M. (1987) *Science 235*, 1353.
Cavanagh, J., Waltho, J. P., & Keeler, J. (1987) *J. Magn. Reson. 74*, 386.
Chao, Y. Y. H., & Bersohn, R. (1978) *Biopolymers 17*, 2761.
Davis, D. G. (1987) *J. Am. Chem. Soc. 109*, 3471.
Dyson, H. J., Rance, M., Houghten, R. A., Lerner, R. A., & Wright, P. E. (1988) *J. Mol. Biol. 201*, 161.
Emsley, L., & Bodenhausen, G. (1989) *J. Magn. Reson. 82*, 211.
Emsley, L., & Bodenhausen, G. (1991a) *Proceedings of the NATO Advanced Research Workshop, Computational Aspects of the Study of Biological Macromolecules by NMR Spectroscopy*, Il Ciocco, Italy.
Emsley, L., & Bodenhausen, G. (1991b) *J. Am. Chem. Soc. 113*, 3309.
Ernst, R. R., Bodenhausen, G., & Wokaun, A. (1987) *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford.
Germain, R. N. (1986) *Nature 322*, 687.
Grathwohl, C., & Wüthrich, K. (1976) *Biopolymers 15*, 2025.
Jeener, J., Meier, B. H., Bachmann, P., & Ernst, R. R. (1979) *J. Chem. Phys. 71*, 4546.
Kessler, H., Griesinger, C., Kerssebaum, R., Wagner, K., & Ernst, R. R. (1987) *J. Am. Chem. Soc. 109*, 607.
Maryanski, J. L., Pala, P., Cerottini, J. C., & Corradin, G. P. (1988) *J. Exp. Med. 167*, 1391.
Maryanski, J. L., Verdini, A. S., Weber, P. C., Salemme, F. R., & Corradin, G. P. (1990) *Cell 60*, 63.
Maryanski, J. L., Romero, P., Van Pel, A., Boon, T., Salemme, F. R., Cerottini, J. C., & Corradin, G. P. (1991) *Int. Immunol.* (in press).
Morris, G. A., & Freeman, R. (1979) *J. Am. Chem. Soc. 101*, 760.
Merrifield, R. J. (1986) *Science 232*, 341.
Müller, N., & Falk, A. (1991) BALL AND STICK 3.0, Cherwell Scientific Publishing, Oxford.
Neuhaus, D., & Williamson, M. (1989) *The NOE in Structural and Conformational Analysis*, VCH, New York.
Okabayashi, K., & Isemura, T. (1968) *Biopolymers 6*, 323.
Pala, P., Bodmer, H. C., Pemberton, R. M., Cerottini, J. C., Maryanski, J. L., & Askonas, B. A. (1988) *J. Immunol. 141*, 2289.
Piantini, U., Sørensen, O. W., & Ernst, R. R. (1982) *J. Am. Chem. Soc. 104*, 6800.
Rance, M., Sørensen, O. W., Bodenhausen, G., Wagner, G., Ernst, R. R., & Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun. 117*, 479.
Sato (1984) *Bull. Chem. Soc. Jpn. 57*, 1679.
Sasisekharan, V. (1959) *Acta Crystallogr. 12*, 897.
Shaka, A. J., & Freeman, R. (1983) *J. Magn. Reson. 51*, 169.
Townsend, A., & Bodmer, H. (1989) *Annu. Rev. Immunol. 7*, 601.
Wüthrich, K. (1987) *NMR of Proteins and Nucleic Acids*, Wiley, New York.
Zuiderweg, E. R. P., Hallenga, K., & Olejniczak, E. T. (1986) *J. Magn. Reson. 70*, 336.