Immunogenic Peptides Corresponding to the Dominant Antigenic Region Alanine-597 to Cysteine-619 in the Transmembrane Protein of Simian Immunodeficiency Virus Have a Propensity To Fold in Aqueous Solution†

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ABSTRACT: Two synthetic peptides corresponding to the N- and C-terminal halves of a 23 amino acid sequence representing an immunodominant domain of the simian immunodeficiency virus of macaque origin (SIV_{mac}) were examined for conformational preferences in aqueous solution by proton nuclear magnetic resonance methods. The two constituent peptides, termed A12-7 (Ala^{597}-Ile-Glu-Lys-Tyr-Leu-Glu-Asp-Gln-Ala-Gln^{607}) and A12-9 (Leu^{608}-Asn-Ala-Trp-Gly-Cys-Ala-Phe-Arg-Gln-Val-Ser^{619}), were found to contain a considerable conformational preference for states in which the backbone ϕ and ψ angles populate the α region of the Ramachandran plot. Further, for peptide A12-9, the types and intensities of the nuclear Overhauser effect (NOE) connectivities between protons in the polypeptide backbone suggest that these states appear to include helical turns. The temperature dependence of the amide proton chemical shifts indicates that some degree of intramolecular hydrogen bonding occurs in these peptides. These results are consistent with a model in which immunogenic peptides which induce antibodies reactive with the intact protein from which the peptide sequence was derived contain conformational preferences in water solution for states other than the extended-chain forms typically found in "random coil" peptides.

Certain peptide antigens have a remarkable capacity to represent immunodominant linear antigenic sites in the cognate proteins (Lerner, 1984). In order to explain this phenomenon, it has been proposed that the amino acid sequence of these peptides strongly favors the formation of particular secondary structures (Dyson et al., 1988a). These conformations are often detectable in proton nuclear magnetic resonance (NMR)[1] experiments (Dyson et al., 1985, 1988b, 1990; Williamson et al., 1986; Waltho et al., 1989). It has also been suggested that the presence of detectable conformational preferences for secondary structural elements in short peptides in water solution may provide a clue to the mechanism of initiation of protein folding (Wright et al., 1988).

Synthetic peptides have been used extensively to probe antigenic and immunogenic sites in human immunodeficiency virus proteins [reviewed in Norrby (1990)]. These studies have revealed the occurrence of a uniquely antigenic region in the N-terminal end of the transmembranous protein. This region was originally identified in human immunodeficiency virus type 1 (HIV-1) (Wang et al., 1986), and it was soon confirmed by several groups (Smith et al., 1987; Gnann et al., 1987; Chiodi et al., 1987; Närvänen et al., 1988) that infected individuals categorically produced antibodies against this site. A homologous highly immunogenic site was also identified in the closely related HIV-2 and simian immunodeficiency virus of rhesus macaque origin (SIV_{mac}) (Norrby et al., 1987; Gnann et al., 1987; Johnson et al., 1988). However, the amino acid sequence in the latter two strains of the virus was markedly different from the corresponding sequence in HIV-1. Using peptides representing this site, it therefore became possible for the first time to design serological assays that could separately identify antibodies against HIV-1 and HIV-2/SIV_{mac}. Immunochemical analysis showed that in reactions with post-human infection sera the critical amino acid residues in the SIV_{mac} antigen were Trp^{611}–Gln^{617} and in the HIV-1 antigen Gly^{592}–Leu^{602} (Norrby et al., 1989a). A minor fraction of HIV-1 antibodies also reacted with a second site, Lys^{588} and Asp^{589}. Characterization of the immunogenicity of free peptides using murine monoclonal antibodies and rabbit hyperimmune sera revealed the presence of five distinct antigenic sites (Norrby et al., 1989b). The most carboxy terminal of these sites overlapped with the immunodominant site, whereas the most amino-terminal site was identified with cross-reacting antipeptide antibodies but was inaccessible in the intact protein. In these different studies (Norrby et al., 1987, 1989a,b), the antigenic sites were represented by peptides containing 23 amino acid residues. Two shorter fragment peptides, representing both the highly immunogenic C-terminal region (12 residues) and also the cross-reactive N-terminal antigenic region (11 residues), were synthesized, with sequences corresponding to those found in the SIV_{mac} protein. This paper describes conformational studies of these peptides using ^1H NMR spectroscopy.

Materials and Methods

Peptide Synthesis. Peptides were produced by the method of simultaneous peptide synthesis (Houghten, 1985). The

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[1] Abbreviations: NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; hplc, high-performance liquid chromatography; COSY, two-dimensional correlated spectroscopy; NOESY, two-dimensional nuclear Overhauser effect spectroscopy; $d_{\alpha N}(i,j)$, $d_{NN}(i,j)$, etc., intramolecular distance between protons C$^\alpha$H and NH, NH and NH, etc. on residues i and j; $^3J_{HN\alpha}$, NH–C$^\alpha$H coupling constant; ppm, parts per million; TSS, (trimethylsilyl)propanesulfonic acid.

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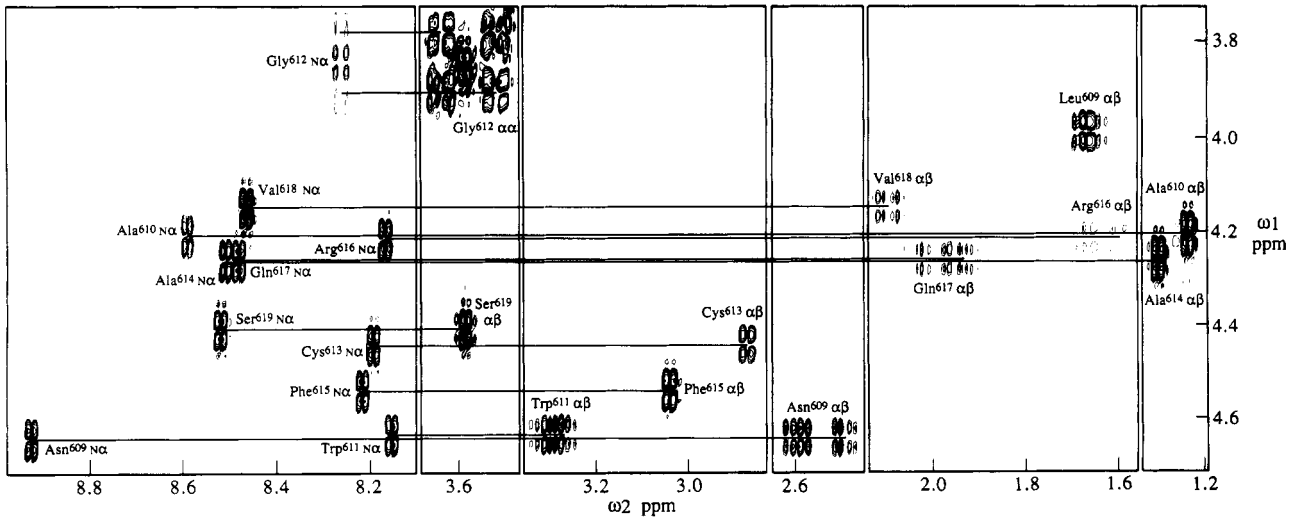

FIGURE 1: Portion of a 500-MHz phase-sensitive 2QF-COSY spectrum of peptide A12-9 at 278 K, pH 4.41, showing the cross-peaks between the NH and C$^{\alpha}$H resonances.

peptides were purified using a Waters AutoPrep 500 hplc on Vydac C-18 columns, and purity and composition were checked by analytical hplc and amino acid analysis. Two peptides were synthesized, AIEKYLEDQAQ (A12-7) and LNAWGCAFRQVS (A12-9), representing two halves of the Ala^{597} to Cys^{619} 23-mer SIV$_{mac}$ peptide (Norrby et al., 1989a,b). The entire 23 amino acid sequence was synthesized and used for immunological studies, but proved insufficiently soluble for NMR studies. The degree of purity of peptide A12-7 exceeded 90%, and that of peptide A12-9 was greater than 96% on analytical hplc. Both peptides were highly soluble in aqueous solution. The C-terminal cysteine (Cys^{619}), which does not appear to be part of the immunodominant region (Norrby et al., 1989a), was replaced by serine to eliminate the possibility of formation of an intramolecular disulfide bond. The formation of intermolecular disulfides, resulting in dimeric peptides, was avoided by sealing the freshly prepared peptide solutions under argon gas. The resonance line widths in the peptide NMR spectra indicate that the peptides are monomeric under these conditions, and no dependence of line width or chemical shift upon peptide concentration was observed.

Peptide ELISA. The peptide ELISA technique used to characterize the immunological reactions of these peptides and the SIV$_{mac}$-specific murine monoclonal antibodies and rabbit hyperimmune serum have been described previously in detail (Norrby et al., 1989a,b). Human post-HIV infection sera were collected from confirmed HIV seropositive healthy individuals. Their antibody response was type-specified by peptide ELISA (Norrby et al., 1987).

Preparation of Peptide Samples for NMR Spectroscopy. Samples were prepared as described previously (Dyson et al., 1988b,c). Peptide concentration was commonly 10 mM, in 90% ^1H_2O/10% ^2H_2O. The pH was adjusted with 0.1 M NaOH or HCl solutions, to a value in each case close to 4.5.

^1H NMR Spectroscopy. Phase-sensitive ^1H NMR spectra were acquired using Bruker AM500 and MSL300 spectrometers. The probe temperature was calibrated by the method of VanGeet (1969), using methanol. Spectra were referenced to internal dioxane (3.75 ppm downfield of TSS).

The resonances of all protons in the peptides were assigned using double quantum filtered phase-sensitive two-dimensional correlated spectroscopy (2QF-COSY) (Rance et al., 1983) and phase-sensitive two-dimensional nuclear Overhauser effect spectroscopy (NOESY) (Jeener et al., 1979). NOESY spectra (mixing time 300–400 ms) were acquired using a short Hahn echo period to improve the quality of the base line (Rance & Byrd, 1983). The H_2O peak was suppressed by gated irradiation. Spectra were routinely acquired with 2048 complex points and 64 scans for each free induction decay. Spectral widths were commonly 9 ppm in both dimensions; between 300 and 700 t_1 values were recorded for each two-dimensional spectrum.

Spectra were Fourier-transformed in both dimensions using phase-shifted sine bell window functions; the final matrix contained 2048 real points in both dimensions. Data processing was carried out on a Convex C240 computer, using the program FTNMR (Hare Research). Linear base line correction (Dyson et al., 1988b) and t_1 ridge suppression (Otting et al., 1986) were employed to enhance the quality of the NOESY spectra. A linear prediction algorithm (Tirendi & Martin, 1989) was used to enhance the quality of the spectrum close to the diagonal. A small first-order phase adjustment was usually made in ω_1.

Temperature coefficients of the amide proton resonances of each peptide were calculated from the gradient of the plot of chemical shift versus temperature, using the method of least-squares. Chemical shifts were obtained for both peptides using a series of 2QF COSY spectra at different temperatures.

RESULTS

Reaction of Purified Peptides with Site-Specific Antibodies. A rabbit hyperimmune serum against the parental SIV$_{mac}$ peptide Ala^{597}–Cys^{619} reacted strongly with each of the separately synthesized halves of the peptide (Table I). Monoclonal antibodies against five different sites of the parental peptide (Norrby et al., 1989b) confirmed that peptide A12-7 reacted with antibodies 2, 33, and 4, which involve Ala^{597}–Glu^{605} as contact residues, whereas peptide A12-9 instead reacted with antibodies 29 and 19, which react with residues Asn^{609}–Gln^{617}. Monoclonal antibody 22, which requires the presence of Cys^{619}, did not react with peptide 12-9, consistent with the substitution of Cys^{619} with serine in this peptide. Peptide A12-9, like the parent peptide, reacted with a human post-HIV-2, but not a post-HIV-1, serum, whereas peptide A12-7 showed no activity with either serum, as expected (Norrby et al., 1989b).

^1H NMR Resonance Assignments. The spectra of both of the peptides A12-7 and A12-9 were readily assigned using standard sequential assignment methods. Resonance assignments are given in Table II. Portions of the 2QF-COSY

Table I: ELISA Reactivity of Highly Purified Peptides A12-7 and A12-9 and a Control Parental Peptide, RC11-1, with Murine Monoclonal Antibodies and a Rabbit Hyperimmune Serum against the Control Peptide (Norrby et al., 1989b) and Human HIV Postinfection Sera

		ELISA extinction value in the test with murine monoclonal antibody no.						rabbit hyperimmune	human postinfection sera	
peptide	amino acid sequence	2	33	4	29	19	22	serum[a]	HIV-1	HIV-2
A12-7	Ala^{597}–Gln^{607}	0.713	1.222	1.282	0.326			1.089	0.282	0.319
A12-9	Leu^{608}–Ser^{619} [b]			0.176	1.247	0.524	0.106	1.137	0.304	1.161
RC11-1	Ala^{597}–Cys^{619}	1.725	>2.0	1.945	1.789	1.981	1.699	>2.0	0.211	1.932

[a] Dilution of reagents was 1:200 for ascites fluid containing murine monoclonal antibodies; 1:100 for rabbit hyperimmune serum, and 1:50 for human sera. [b] In peptide A12-9, Cys^{619} was exchanged for a Ser to reduce possibilities for inter- and intramolecular disulfide bond formation. [c] Not tested.

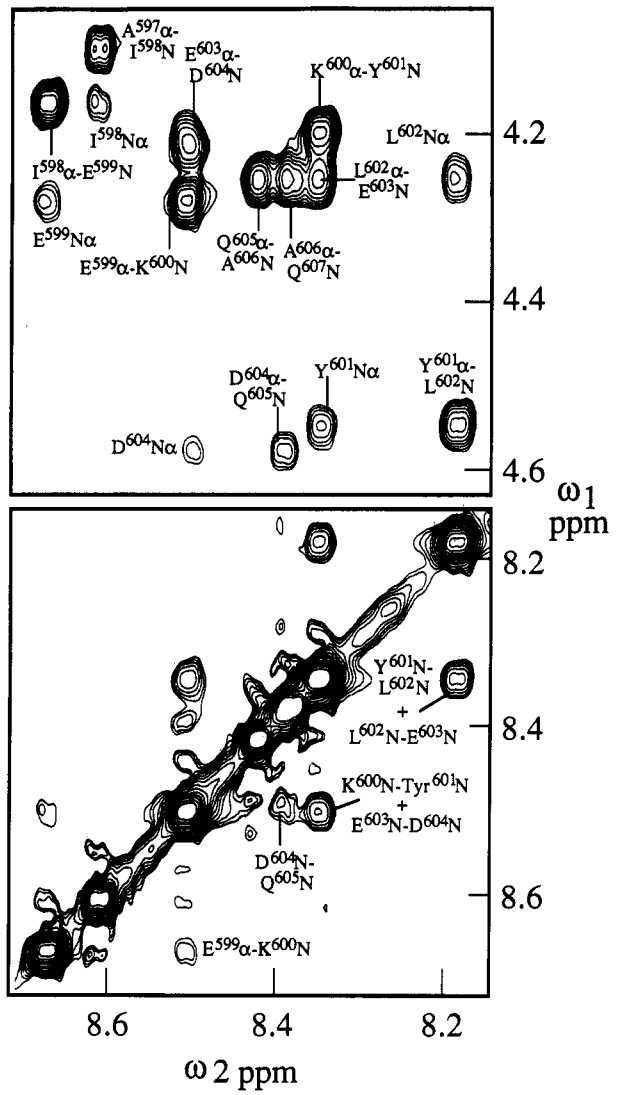

FIGURE 2: Portions of a 500-MHz phase-sensitive NOESY spectrum of peptide A12-7 at 278 K, pH 4.35. (Bottom) Amide diagonal region, showing NOE cross-peaks between amide protons of adjacent residues. (Top) NH–$C^{\alpha}H$ region.

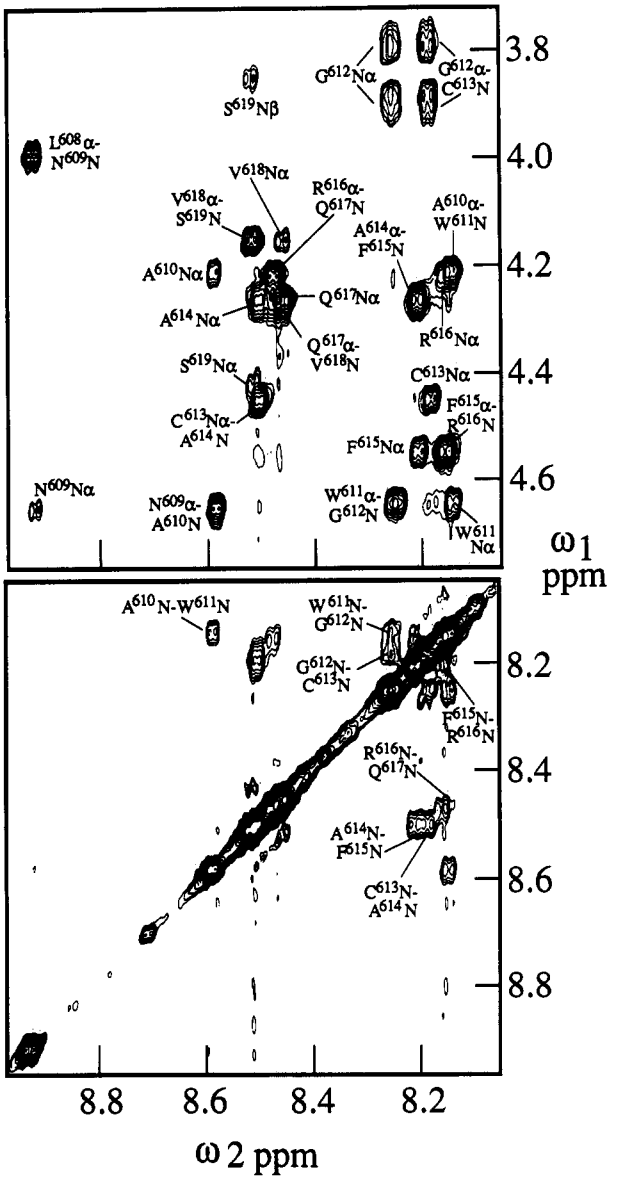

FIGURE 3: Portions of a 500-MHz phase-sensitive NOESY spectrum of peptide A12-9 at 278 K, pH 4.41. (Bottom) Amide diagonal region; (top) NH–$C^{\alpha}H$ region.

spectrum of A12-9 are shown in Figure 1 to illustrate the quality of the NMR spectra.

NMR Evidence for Structured Conformations in Solution. The observation of proton–proton nuclear Overhauser enhancements (NOEs) between certain protons in the peptide provides evidence of conformational preferences for structured forms [reviewed in Wright et al. (1988) and Dyson and Wright (1991)]. The extended-chain or β conformation of the backbone is most common for short peptides in aqueous solution; this is indicated by strong NOE connectivities between the $C^{\alpha}H$ of a given amino acid residue and the NH of the following residue, termed $d_{\alpha N}(i,i+1)$ NOEs, which have been observed in all peptides so far examined. For many peptides, this is effectively the only region of conformational space sampled: no other backbone–backbone interresidue NOEs are observed. In some peptides, however, $d_{NN}(i,i+1)$ NOEs are observed, indicating that the backbone significantly populates the α region of ϕ,ψ space. By itself, this is not sufficient evidence for folded or structured conformations. Other evidence which can be used to infer such conformations includes medium-range NOE connectivities, such as $d_{\alpha N}(i,i+2)$, $d_{\alpha N}(i,i+3)$, and $d_{\alpha\beta}(i,i+3)$ NOEs, temperature coefficients, and

Table II: Resonance Assignments

residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	$C^{\epsilon}H$	$C^{\zeta}H$
Peptide A12-7 (278 K, pH 4.35) chemical shift (ppm)							
Ala^{597}		4.10	1.52				
Ile^{598}	8.61	4.18	1.84	1.50, 1.20	0.89	0.92 ($C^{\gamma}H_3$)	
Glu^{599}	8.68	4.29	1.95, 1.88	2.34			
Lys^{600}	8.53	4.21	1.72	1.36	1.67	2.97	
Tyr^{601}	8.37	4.56	3.07, 2.98		7.10	6.80	
Leu^{602}	8.21	4.27	1.60, 1.51	1.44	0.84, 0.89		
Glu^{603}	8.36	4.22	1.97	2.35			
Asp^{604}	8.53	4.59	2.79				
Gln^{605}	8.41	4.25	1.99, 2.13	2.37			
Ala^{606}	8.44	4.27	1.42				
Gln^{607}	8.41	4.25	1.99, 2.13	2.37			
Peptide A12-9 (278 K, pH 4.41) chemical shift (ppm)							
Leu^{608}		3.98	1.67	1.61	0.91, 0.93		
Asn^{609}	8.92	4.64	2.60, 2.50		7.65, 7.02 ($N^{\delta}H$)		
Ala^{610}	8.59	4.21	1.25				
Trp^{611}	8.15	4.63	3.33, 3.27		C2, 7.26; C4, 7.61; C5, 7.16; C6, 7.23; C7, 7.48; NH, 10.21		
Gly^{612}	8.26	3.89, 3.79					
Cys^{613}	8.19	4.44	2.88				
Ala^{614}	8.51	4.26	1.31				
Phe^{615}	8.21	4.56	3.05		7.20	7.33	7.27
Arg^{616}	8.17	4.21	1.63	1.47	3.10	7.17	
Gln^{617}	8.48	4.26	1.96, 2.04	2.34	7.66, 6.99 ($N^{\epsilon}H$)		
Val^{618}	8.46	4.15	2.10	0.94			
Ser^{619}	8.52	4.41	3.85	terminal NH, 7.73, 7.26			

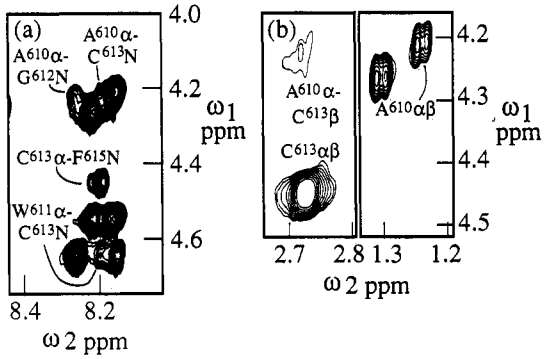

FIGURE 4: Portions of the spectrum in Figure 3 contoured to show the lower intensity medium-range NOE cross-peaks. (a) $d_{\alpha N}(i,i+2)$ and $d_{\alpha N}(i,i+3)$. (b) $d_{\alpha\beta}(i,i+3)$.

Table III: Amide Proton Chemical Shift Temperature Coefficients

Peptide A12-7 residue	Peptide A12-7 $-\Delta\delta/\Delta T \times 10^3$ (ppm/K)	Peptide A12-9 residue	Peptide A12-9 $-\Delta\delta/\Delta T \times 10^3$ (ppm/K)
Ile^{598}	6.0	Asn^{609}	6.8
Glu^{599}	8.1	Ala^{610}	10.8
Lys^{600}	9.2	Trp^{611}	5.9
Tyr^{601}	9.1	Gly^{612}	4.9
Leu^{602}	6.9	Cys^{613}	5.6
Glu^{603}	6.8	Ala^{614}	8.1
Asp^{604}	7.6	Phe^{615}	8.6
Gln^{605}	6.1	Arg^{616}	5.9
Ala^{606}	6.9	Gln^{617}	7.2
Gln^{607}	7.7	Val^{618}	9.6
		Ser^{619}	8.8

$^3J_{HN\alpha}$ coupling constants and other forms of spectroscopy such as circular dichroism (CD) spectroscopy.

Portions of the NOESY spectra of the A12-7 and A12-9 peptides are shown in Figures 2 and 3. A number of strong sequential $d_{NN}(i,i+1)$ NOE cross-peaks are visible in both spectra. In addition, the spectrum of A12-9 contains medium-range NOEs, indicating the presence of conformers containing helix or nascent helix (Figure 4). These results are summarized in Figure 5. It is noticeable that the amide proton resonances of peptide A12-7 are rather severely overlapped in the C-terminal region, which may cause medium-range and $d_{NN}(i,i+1)$ NOEs to be obscured by overlap with other, with stronger cross-peaks, or by the diagonal. For example, the $d_{N\alpha}(i,i)$ cross-peaks for Q^{605}, A^{606}, and Q^{607} are marked as obscured by overlap in Figure 5. These cross-peaks are obscured by the presence of the strong $d_{\alpha N}(i,i+1)$, which we assume is present, consistent with the behavior of the remainder of peptide A12-7 as well as most other short peptides. These sequential connectivities are not ambiguous: the cross-peak at 4.59, 8.41 ppm ($D^{604}\alpha$-$Q^{605}N$) establishes the Q^{605} NH chemical shift, which corresponds to one of the two coincident COSY cross-peaks at 4.25, 8.41 ppm. The two NOESY cross-peaks with this approximate $C^{\alpha}H$ chemical shift are not associated with the L^{602} $C^{\alpha}H$ at 8.44 and 8.41 ppm. The former cross-peak must be between the Q^{605} $C^{\alpha}H$ and the A^{606} NH (both established in the COSY spectrum), overlapped with the A_{606} Nα if it is present. Finally, the cross-peak at 4.25, 8.41 ppm in the NOESY spectrum must represent the $A^{606}\alpha$-$Q^{607}N$ connectivity, which obscures the $d_{N\alpha}(i,i)$ connectivities of Q^{605} and Q^{607}, if they are present.

The presence of intramolecular hydrogen bonding of the peptide amide protons can be detected by a lessening of the dependence of the amide proton chemical shift on temperature. If the proton is protected from solvent, for example, by a hydrogen-bonded conformation present in a significant proportion of the conformational ensemble, then the temperature coefficient of the amide proton chemical shift is lowered. The extent to which the temperature coefficient is reduced from normal solvent-exposed values has been used as a measure of the population of hydrogen-bonded (hence, structured) forms (Dyson et al., 1988c). The temperature coefficients calculated for the two peptides A12-7 and A12-9 are shown in Table III.

Circular Dichroism Measurements. The CD spectra of the two peptides (not shown) are typical of peptides with little or no ordered secondary structure. It has been observed in several

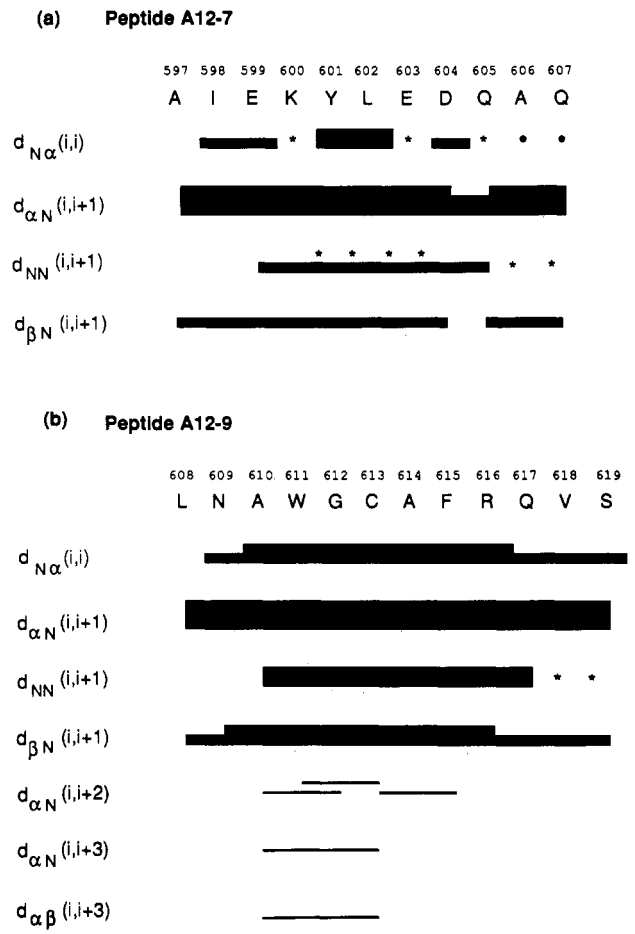

FIGURE 5: Schematic diagram showing the intensities of the NOE connectivities between various backbone protons in the sequences of (a) peptide A12-7 and (b) peptide A12-9. Asterisks indicate that a cross-peak was not observable or distinguishable due to resonance overlap.

peptide systems that the absence of a strong negative ellipticity at 222 nm in the CD spectrum may accompany NMR behavior which unequivocally indicates the presence of helical or nascent helical conformations (Dyson et al., 1988b; Waltho et al., 1989; J. P. Waltho, H. J. Dyson, and P. E. Wright, unpublished observations). This discrepancy has been interpreted in light of calculations (Manning et al., 1977) which show that distortions from perfect helix, such as are likely to occur for short peptides in conformational equilibrium in solution, considerably lessen the CD signal of helical conformations.

Discussion

The sequential assignment of the proton resonances of peptides generally relies on the presence of strong $d_{\alpha N}(i,i+1)$ NOE connectivities, which connect the amino acid spin systems in a sequence-specific manner (Wüthrich et al., 1984). In itself, the observation of these NOEs gives information on the types of conformations present, indicating that at least some, and probably a majority, of the conformers of both peptides are present in extended-chain conformations. This is to be expected. The presence in addition to this of $d_{NN}(i,i+1)$ NOEs indicates that there is a significant population of conformers containing dihedral angles in the α region of conformational space, which are present in helices and turns.

Given that the proton resonances of the A12-7 peptide are considerably overlapped, it nevertheless appears that this peptide is less structured in aqueous solution than the A12-9 peptide. The ratios of the intensities of $d_{NN}(i,i+1)$ NOEs to $d_{\alpha N}(i,i+1)$ NOEs observed for this peptide are smaller than for peptide A12-9. No medium-range NOEs indicative of helical, nascent helical, or turn conformations are observed.

For peptide A12-9, we observe in addition to relatively strong sequential $d_{NN}(i,i+1)$ NOE connectivities, medium-range $d_{\alpha N}(i,i+2)$, $d_{\alpha N}(i,i+3)$, and $d_{\alpha\beta}(i,i+3)$ NOEs (Figures 4 and 5). This is strongly indicative of the presence of a helical turn conformation. In particular, the $d_{\alpha N}(i,i+3)$ and $d_{\alpha\beta}(i,i+3)$ NOEs show that a significant population of the conformers present in solution contain helix-like structures in the region between Ala^{610} and Cys^{613}. In addition, the conformational ensemble contains extended-chain conformations [as shown by the $d_{\alpha N}(i,i+1)$ NOEs present throughout the molecule] and nascent helix and/or turn conformations [shown by the $d_{\alpha N}(i,i+2)$ NOEs]. An unusual $d_{\alpha\beta}(i,i+1)$ NOE is observed between Ala^{614} and Phe^{615} (data not shown). The intensity of the cross-peak is not greatly affected by the mixing time of the NOESY experiment, indicating that it does not arise primarily from spin diffusion processes. The incidence of such NOEs in proteins and in other peptides has been noted, and their structural implications are under study (N. J. Skelton, G. P. Gippert, and H. J. Dyson, unpublished observations).

Additional evidence for structured conformations in the region between Ala^{610} and Phe^{615} is provided by the temperature dependence of the amide proton chemical shifts. Table III shows that a significant decrease in the temperature coefficient has occurred for residues Gly^{612} and Cys^{613}, indicating a significant population of conformers for which these amide protons are protected from the solvent. It is commonly accepted that this solvent protection takes the form of hydrogen bonding. Since the NMR spectrum of peptide A12-9 is concentration-independent, hydrogen bonding or other forms of solvent protection arising from intermolecular association are ruled out. Intramolecular hydrogen bonding of the amide protons of Gly^{612} and Cys^{613} to backbone carbonyl groups of Leu^{608} and Asn^{609} or the side chain of Asn^{609} is consistent with a model in which a detectable population of conformers containing stable helical turns is present in the N-terminal portion of peptide A12-9.

Two correlations of interest can be made between the structural and immunological results presented in this paper. First, for the two peptides examined, the degree of folding correlates qualitatively with the degree of antigenicity. Thus, peptide A12-7 is less antigenic and less folded, and peptide A12-9 is more antigenic and more folded. Second, the region of peptide A12-9 containing the helical turns corresponds closely with the region of critical amino acids for reaction with human postinfection sera to HIV-2 (Norrby et al., 1989a). We have previously observed that peptide immunogens which induce antibodies reactive with the cognate sequence in folded proteins tend to be structured in solution (Dyson et al., 1988a). This general phenomenon is of great interest in the production of peptide vaccines, particularly in view of the possibility of producing peptidelike immunogens that are chemically cross-linked to force the immunogenic conformation. This would increase the effective immunogen concentration by eliminating the unproductive conformational states of linear peptide immunogens.

A cross-linked peptide has been used (Oldstone et al., 1991) to generate monoclonal antipeptide antibodies to the analogous dominant antigenic region of HIV-1, which contains the sequence LGLWGCSGKLIC. A shorter peptide with the sequence CSGKLIC was found by NMR spectroscopy to contain a reverse turn conformation when an intramolecular disulfide bridge was present, but to be unstructured in the re-

duced (dithiol) form. A small number of the antibodies raised against the HIV-1 sequence were also cross-reactive with the HIV-2 sequence, NSWGCAFRQVC, which also has the potential to form an intramolecular disulfide bond. The SIV_{mac} sequence reported in the present study has a marked similarity to the HIV-2 sequence, with the important exception that a disulfide bond is explicitly excluded by the replacement of the C-terminal Cys with a Ser, in accordance with previous findings (Norrby et al., 1989a) that the amino acid dependence of human sera containing HIV antibodies in tests with the selected peptides does not include the carboxy-terminal cysteine. We have found that this sequence contains helical turns rather than the β-turn found for the disulfide-bridged HIV-1 peptide. The differences in reactivity of the monoclonal antibodies in these two studies probably reflect differences in the state of the original peptide immunogen rather than true structural differences in the viral proteins. The high probability of induced-fit interactions in the binding of peptide antigens to antibodies has yet to be fully explored in cases such as these. In addition, the antibodies in the present study were specifically selected for cross-reaction to the native protein from which the peptide sequence was derived, which may have considerable influence on the observed immunological properties.

Of the two peptides characterized in this study, A12-9 has an exceptionally high antigenicity. However, no neutralizing activity was detectable in hyperimmune sera against an HIV-2 peptide encompassing the region represented by the SIV peptide A12-9 (Björling et al., 1991). In the same study, other HIV-2 peptides were found to be effective in inducing neutralizing antibodies. This was particularly noticeable with certain peptides representing the region matching the principal neutralizing domain V3 of HIV-1 (LaRosa et al., 1990). Conformational studies of such peptides from HIV-1 (Chandrasekhar et al., 1991) and HIV-2 (N. Assa-Munt, H. J. Dyson, E. Norrby, and P. E. Wright, unpublished observations) may provide information of value as to the types of cross-linking experiments to be employed to force the immunogenic conformation.

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