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Solution Conformational Preferences of Immunogenic Peptides Derived from the Principal Neutralizing Determinant of the HIV-1 Envelope Glycoprotein gp120†

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Received May 14, 1991; Revised Manuscript Received July 3, 1991

ABSTRACT: With standard one- and two-dimensional proton NMR techniques, a common structural motif has been identified in water solutions of short peptide sequences derived from the envelope glycoprotein gp120 of HIV-1. Three peptides of lengths 12, 24, and 40 residues (termed RP342, RP142, and RP70, respectively) were synthesized, each containing a central amino acid sequence common to many HIV-1 isolates. In addition, RP70 contained a disulfide bond between cysteine residues close to the ends of the molecule, forming a loop that is thought to constitute an important structural and immunological component of the intact glycoprotein. Peptides RP70 and RP142 showed evidence for the presence of a significant population of conformations containing a β-turn in the conserved sequence Gly-Pro-Gly-Arg. Strong nuclear Overhauser effect (NOE) connectivities were observed between the amide protons of the arginine and the adjacent glycine. A weak NOE connectivity was observed between the $C^{\alpha}H$ of the proline residue and the NH of the Arg [a $d_{\alpha N}(i,i+2)$ NOE connectivity], confirming the presence of a conformational preference for a turn conformation in this sequence. The remainder of the peptide showed evidence of conformational averaging: no NMR evidence for a uniquely folded structure was obtained for any of the peptides in water solution. Circular dichroism (CD) spectra indicated that no ordered helix was present in water solutions of RP70, although a CD spectrum that indicated the presence of approximately 30% helix could be induced by the addition of trifluoroethanol. Changes are observed in the NMR spectrum of RP70 in TFE/water mixtures consistent with helix formation, and the β-turn is apparently retained.

Several candidate vaccines designed to provide protection from human immunodeficiency virus type 1 (HIV-1) infection are based on the viral envelope glycoprotein gp160 and its derivatives gp120 and gp41 (Bolognesi, 1989). Although several sites on these proteins have been reported to be targets for neutralizing antibodies, it is now clear that the principal neutralizing determinant (PND)[1] is located within a disulfide-linked loop in the V3 region of gp120 (Rusche et al., 1988; Palker et al., 1988; Javaherian et al., 1989). Due to sequence variability in this region, antisera raised against the PND of one isolate generally do not neutralize other isolates (Matthews et al., 1986; Putney et al., 1986; Goudsmit et al., 1987; Rusche et al., 1988), and this type specificity has been an obstacle for vaccine development. Analysis of 245 PND sequences, however, revealed that certain amino acid residues are relatively conserved (LaRosa et al., 1990). The sequence Gly-Pro-Gly-Arg, in particular, was present in 84% of the PNDs studied, and significantly, antisera directed against the slightly longer sequence Gly-Pro-Gly-Arg-Ala-Phe were shown to neutralize multiple isolates (Javaherian et al., 1990). The

† This research was supported in part by Grant CA 27498 from the National Institutes of Health. K.C. was supported by a fellowship from the Repligen Corp.
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[1] Abbreviations: NMR, nuclear magnetic resonance; CD, circular dichroism; 2QF-COSY, double-quantum filtered two-dimensional correlated spectroscopy; NOESY, two-dimensional nuclear Overhauser effect spectroscopy; R-COSY, relayed-COSY spectroscopy; PND, principal neutralizing determinant.

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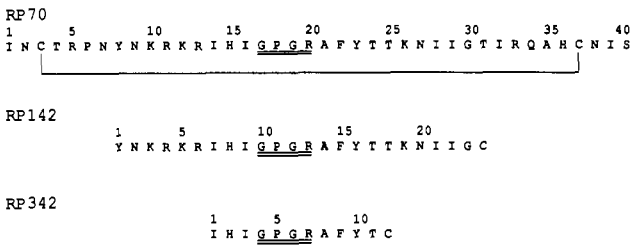

FIGURE 1: Schematic diagram showing the amino acid sequences of the three peptides from gp120, aligned to indicate their sequence identity. Sequences correspond to the MN isolate. Peptides are C-terminal amides.

conserved sequence may therefore be the most appropriate target for vaccine development. It may also play a biological role in HIV-1 infection (Hattori et al., 1989; Stephens et al., 1990).

To increase understanding of the nature of the conserved Gly-Pro-Gly-Arg sequence and the role of the disulfide-linked loop, we have examined the solution conformational preferences of three synthetic peptides, RP70, RP142, and RP342 (Figure 1), using ^{1}H NMR and CD spectroscopy. The peptides contain, respectively, 40, 24, and 12 amino acid residues corresponding to the relatively common MN isolate (LaRosa et al., 1990); all contain the conserved Gly-Pro-Gly-Arg sequence. The peptide RP70 contains two cysteine residues, which are disulfide-bridged both in the gp120 protein (Leonard et al., 1990) and in the peptide under study. The other two peptides do not contain disulfide bridges, permitting the evaluation of the requirement for the integrity of the loop itself as a structural element stabilizing conformations that bind to neutralizing antibodies.

The use of proton NMR techniques to evaluate the conformational preferences of peptides in solution is widespread. It is particularly useful in the absence of other structural data such as X-ray crystal structures. It has been found that, in several cases where peptide immunogens had been shown to elicit protein-reactive antibodies, conformational preferences for defined secondary structures in water solution were also observed for the epitope and nonepitope regions of the immunizing peptide (Dyson et al., 1985, 1988a; Williamson et al., 1986; Waltho et al., 1989; Dyson et al., 1991). Both proton NMR and CD spectroscopy have been used for this purpose (Wright et al., 1988; Dyson & Wright, 1990). In this paper we present evidence that the Gly-Pro-Gly-Arg sequence in the PND shows a preference for a reverse turn conformation in water solution that is apparently independent of the disulfide bridge in the loop. Other studies indicate that peptide immunogenicity may be dependent on the integrity of such disulfide-bridged structures (Oldstone et al., 1991).

The actual conformations of linear peptide antigens bound to antipeptide antibodies has been the subject of a considerable amount of recent crystallographic and NMR work [for example, Stanfield et al. (1990) and Tsang et al. (1990)]. The crystal structure of an antibody bound to a peptide related to those in the present solution study is in progress (I. A. Wilson, personal communication).

Materials and Methods

Sample Preparation for NMR Experiments. All of the peptides used in this study were prepared by solid-phase peptide synthesis as reported elsewhere (Profy et al., 1990) and were purified by reverse-phase HPLC. The peptides are C-terminal amides, N-terminal amines.

Samples for NMR spectroscopy were prepared in 90% ^1H_2O and 10% ^2H_2O. The pH was adjusted to a value between 4 and 4.5 by using small aliquots of NaOH and HCl solutions. No measurements were made for solutions of ^2H_2O. Dithiothreitol (DTT) was added to samples of RP342 and RP142 to ensure that no intermolecular disulfide bonds formed. Dioxan was added to each sample as a chemical shift reference.

NMR Spectroscopy. NMR experiments were performed on Bruker AM500 NMR spectrometers equipped with digital phase shifting hardware. All experiments were carried out at 5 °C. Both the pH range and temperature were chosen to minimize amide exchange (Wüthrich, 1986). Two-dimensional double-quantum filtered correlated spectroscopy (2QF-COSY) (Rance et al., 1983), nuclear Overhauser enhancement spectroscopy (NOESY) (Jeener et al., 1979), rotating frame NOESY (ROESY; Camelspin) (Bothner-By et al., 1984), double-quantum (2Q) (Braunschweiler et al., 1983; Rance et al., 1989), total correlation spectroscopy (TOCSY; HOHAHA) (Bax & Davis, 1985; Rance, 1987), and relayed COSY (R-COSY) (Eich et al., 1982) data were acquired according to standard procedures (Ernst et al., 1989). Mixing times of 300 (RP70), 400 (RP142), and 500 ms (RP342) were used in the NOESY spectra. Suppression of the H_2O peak was achieved by presaturation. Spectra were typically acquired as 512 free induction decays of 2048 complex points each with a spectral width of 10 ppm in both dimensions. Spectra were processed on a SUN work station or on CONVEX computer using software provided by Dennis Hare. Phase-shifted sine bell filter functions were used in both dimensions, and a baseline correction (Zuiderweg et al., 1985; Dyson et al., 1988c) was employed in the ω_2 dimension. The final transformed spectrum contained 2048 real points in both dimensions.

Temperature Dependence of the Amide Proton Chemical Shift. Temperature coefficients were calculated as the gradient of the linear plot of chemical shift against temperature for each of the amide protons in the three peptides. Since the amide protons of the two longer peptides were severely overlapped in one-dimensional spectra, a series of two-dimensional spectra were acquired at four temperatures (278, 288, 298, and 308 K). Amide proton chemical shifts for RP142 were obtained from the fingerprint region of a series of 2QF-COSY spectra. Data were incomplete for several of the amide protons due to overlap of their $C^\alpha H$ resonances with that of water at higher temperatures. The temperature coefficient data for RP70 were obtained by using a series of NOESY spectra, since the 2QF-COSY is insufficiently resolved to give definitive information on certain of the amide protons of greatest interest.

Spectra were acquired for RP70 in a 20% (v/v) TFEd_3/water solution, by using the signal from the methylene deuterons of the TFE to lock the spectrometer frequency.

Circular Dichroism Spectroscopy. All samples for circular dichroism (CD) were 39 μM peptide and were obtained by dilution of a stock solution of the peptide in water at pH 4.4. Trifluoroethanol (TFE) concentrations were calculated as reported earlier (Dyson et al., 1988b). CD spectra were recorded on an AVIV 61 DS spectrometer at 25 °C. Mean residue ellipticity was calculated for each sample as described by Adler et al. (1973).

Results and Discussion

Resonance Assignments for the Peptides. Assignment of the proton resonances in the NMR spectra of the three peptides was carried out according to established procedures (Wüthrich, 1986; Dyson & Wright, 1990). For each peptide, 2QF-COSY and NOESY spectra were acquired. For peptides that are largely disordered in solution, resonance overlap becomes increasingly severe as the length of the peptide increases.

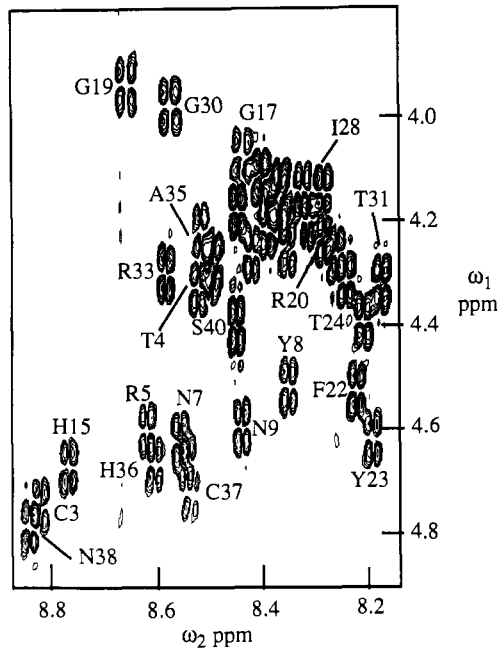

FIGURE 2: Portion of a 500-MHz phase-sensitive 2QF-COSY spectrum of RP70 in 90% ^1H_2O/10% ^2H_2O, pH 4.20, at 278 K. The region shown includes all of the cross peaks representing the scalar connectivity between amide proton and $C^\alpha H$ of each amino acid residue. Selected cross peaks are labeled.

This is illustrated in Figure 2, which shows the fingerprint ($C^\alpha H$–NH) region of a 2QF-COSY spectrum of RP70. The NH portion of the spectrum is quite well dispersed, but the $C^\alpha H$ region, especially in the vicinity of 4.2 ppm, is badly overlapped, necessitating the use of relayed NMR spectra in order for assignment of side-chain proton resonances to be completed for RP70 and for RP142. Complete assignment was achieved for all side-chain resonances, by utilizing the favorable dispersion of the amide proton resonances in R-COSY and TOCSY spectra; the assignments are shown in Table I. Many of the side-chain proton resonances of the same amino acid type are indistinguishable, especially for peptides that are unstructured in solution; these are indicated in Table I by asterisks. Sequential assignment of the proton resonances was completed by using standard techniques from the NOESY spectrum of each peptide. Portions of NOESY spectra of the three peptides, with certain of the sequential assignments marked, are shown in Figures 3–5.

Circular Dichroism Specta of RP70. The CD spectrum of RP70 in water, which, due to the presence of a disulfide-bonded loop, might be expected to show a greater preference for structured conformers, indicates the presence only of "random" structures. No helix is indicated by the spectra of RP70 in water solution at 298 (Figure 6) or 278 K (data not shown). Additional of TFE apparently mediates formation of some helix, although at maximum this amounts to only 25–30% of the molecule, (calculated from the accepted value of −36 000 to −40 000 deg cm^2 dmol^{-1} for 100% helix). The addition of TFE results in the observation of an isodichroic point (Figure 6a), indicating that the transition is probably two-state.

Largely Disordered Conformations of RP70, RP142, and RP342. It is generally recognized that the presence of an ordered conformation in solution is indicated by characteristic CD and NMR spectra (Dyson & Wright, 1990). The CD spectrum of a helix consists of a pair of minima at 208 and 222 nm and is thought to indicate the presence of at least seven residues in a series of conformations containing a true helix (Goodman, 1990). The NMR evidence for a helical conformation consists of a stretch of NOE connectivities between the amide protons of consecutive residues [$d_{NN}(i,i+1)$], supported by the presence of medium-range $d_{\alpha\beta}(i,i+3)$ and $d_{\alpha N}(i,i+3)$ connectivities. The diagnostic NMR evidence for a β-turn consists of one or more $d_{NN}(i,i+1)$ NOEs within the turn, accompanied by a reduction in the magnitude of the corresponding $d_{\alpha N}(i,i+1)$ NOEs. A $d_{\alpha N}(i,i+2)$ connectivity across the turn is a requirement for positive identification of the turn. Corroborative evidence in the form of lowered $^3J_{HN\alpha}$ coupling constants and reduced amide proton temperature coefficients may also be present. It appears that β-hairpins and other intramolecular β-structures are rarely observed in small peptides in water solution, apparently due to a propensity for intermolecular interactions, association, and insolubility (Narita et al., 1989).

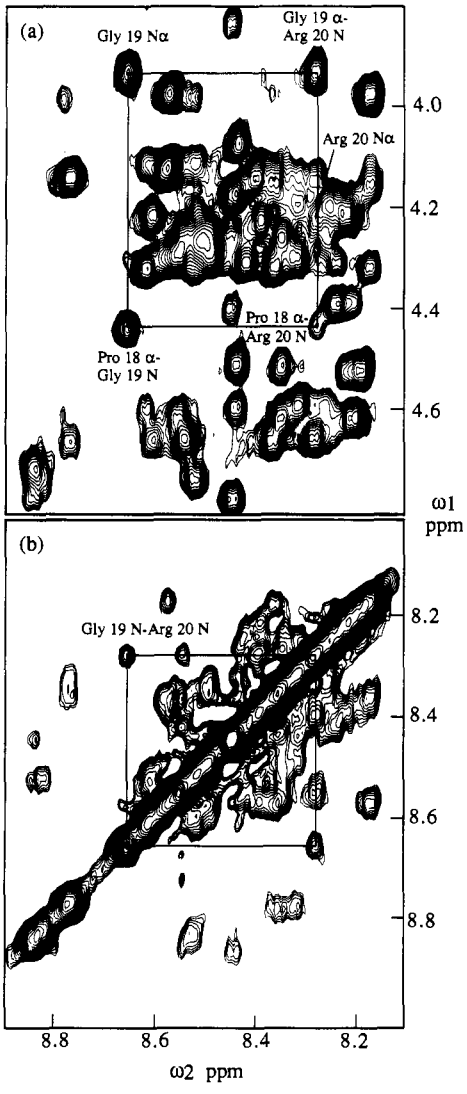

FIGURE 3: Portions of a 500-MHz phase-sensitive NOESY spectrum of RP70 in 90% ^1H_2O/10% ^2H_2O, pH 4.20, at 278 K. (a) Amide–$C^\alpha H$ region; (b) amide–amide region. Sections a and b are plotted at the same contour level.

The NOE data for each peptide indicate that the majority of the molecule in each case is disordered; that is, no single conformation (or closely related group of conformations) is present in solution. The NOE connectivities present in the spectrum of each peptide are shown schematically in Figure 7. Although a number of $d_{NN}(i,i+1)$ NOEs are seen in the NOESY spectra of the three peptides, there is no evidence for $d_{\alpha N}(i,i+3)$ NOEs (Figures 3–5) or $d_{\alpha\beta}(i,i+3)$ NOEs. At the same time, a complete set of strong consecutive $d_{\alpha N}(i,i+1)$

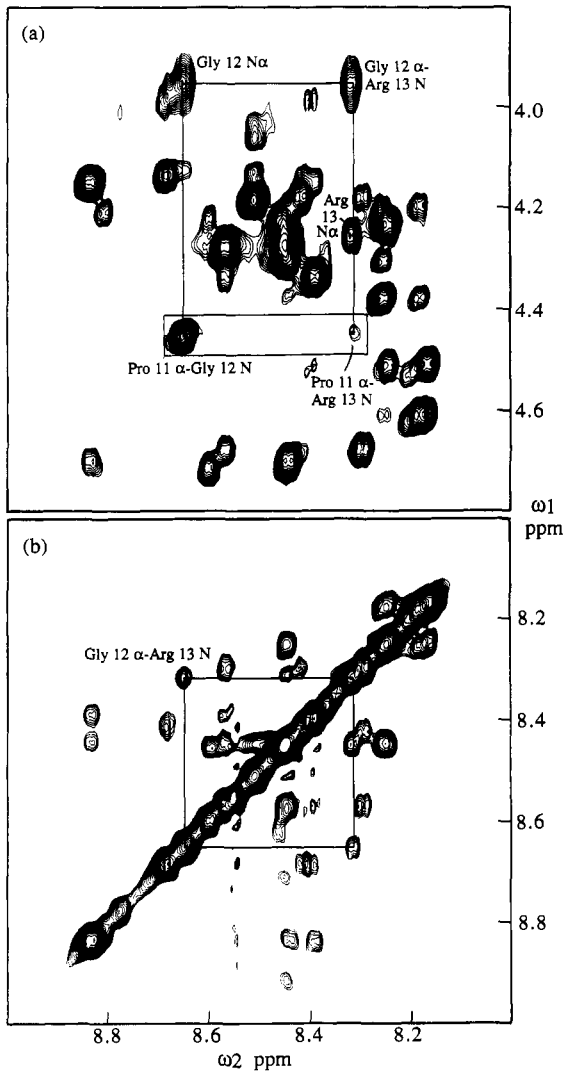

FIGURE 4: Portions of a 500MHz phase-sensitive NOESY spectrum of RP142 in 90% ^1H_2O/10% ^2H_2O, pH 4.34, at 278 K. (a) Amide–C$^\alpha$H region; (b) amide–amide region. Sections a and b are plotted at the same contour level. The inset is contoured at a lower level to show the $d_{\alpha N}(i,i+2)$ NOE connectivity between Pro 18 and Arg 20.

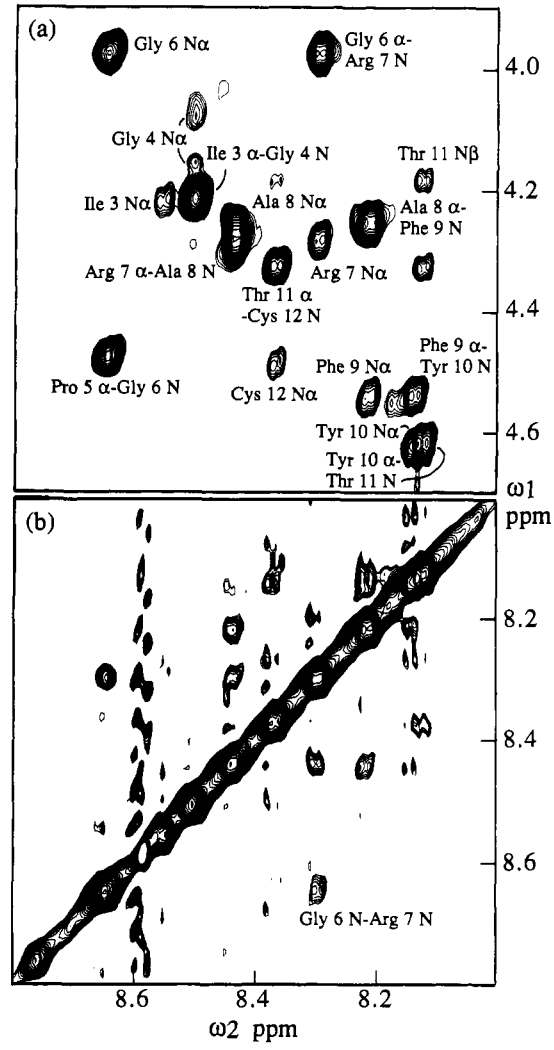

FIGURE 5: Portions of a 500-MHz phase-sensitive NOESY spectrum of RP342 in 90% ^1H_2O/10% ^2H_2O, pH 4.06, at 278 K. (a) Amide–C$^\alpha$H region; (b) amide–amide region. Sections a and b are plotted at the same contour level.

NOEs is observed throughout the peptide. These results indicate that the backbone conformational population of these peptides in water solution lies largely in the β region of (ϕ,ψ) space [strong $d_{\alpha N}(i,i+1)$ NOEs] but that there is a significant population of conformations in the α region of (ϕ,ψ) space as well [$d_{NN}(i,i+1)$ NOEs].

These results indicate that the peptides do not show a strong conformational preference for ordered helix in water solution. No evidence for a defined β-structure is seen: the characteristic CD spectrum of β-sheet (Adler et al., 1973) is absent. Despite the presence of strong $d_{\alpha N}(i,i+1)$ NOEs, the NMR spectrum shows no interstrand NOEs (for example, long-range $d_{\alpha\alpha}$ and $d_{\alpha N}$ NOEs), which rules out the presence of defined β-sheet. The presence of significant $d_{NN}(i,i+1)$, as well as $d_{\alpha N}(i,i+1)$ NOEs, is a strong indication that the peptides consist in the main of a random set of conformations in water solution.

The $d_{NN}(i,i+1)$ NOE connectivities may be indicative of nascent helix in solution (Dyson et al., 1988b), a conclusion that is supported by the appearance of a helix-specific CD signal upon addition of TFE. Further evidence for nascent helix, in the form of a number of $d_{\alpha N}(i,i+2)$ NOEs (Dyson et al., 1988b), is not apparent from the NMR spectra. The nascent helix itself consists of a population of different conformations, in which a significant proportion contain backbone conformations in that α region of (ϕ,ψ) space (Wright et al., 1988), rather than any single defined solution conformation.

Presence of a β-Turn Conformation in RP70 and RP142. While the majority of the molecule in all three peptides does not appear to contain a defined folded structure, strong evidence for one element of secondary structure in the region of the conserved Gly-Pro-Gly sequence is evidence from the NMR spectra of RP70 and RP142. The presence of a $d_{NN}(i,i+1)$ NOE between the amide protons of Gly 19 and Arg 20 of RP70 (Figure 3) is evidence for a population of conformers with backbone conformations in the α region of (ϕ,ψ) space in this region. In addition, weak NOE connectivities are observed between the C$^\delta$H of Pro 18 and the amide proton of Gly 19 (spectroscopic data not shown) and between the Pro 18 C$^\alpha$H and the Arg 20 amide proton (Figure 3). These NOEs confirm the presence of a reverse turn formed by residues –Gly 17–Pro 18–Gly 19–Arg 20– of the sequence. The observation of the $d_{\delta N}(i,i+1)$ NOE between Pro 18 and Gly 19 is an indication that at least some of the reverse turn conformations may be of type I (Wüthrich et al., 1984; Dyson et al., 1988c). The presence of a population of type II turn, in addition to the conformations containing a type I turn, cannot be ruled out: the diagnostic NOE for the type II turn is the $d_{\alpha N}(i,i+1)$ connectivity between residues 2 and 3 of the turn. This connectivity is unambiguous when the turn is present in a molecule of defined conformation such as a protein. However, in the

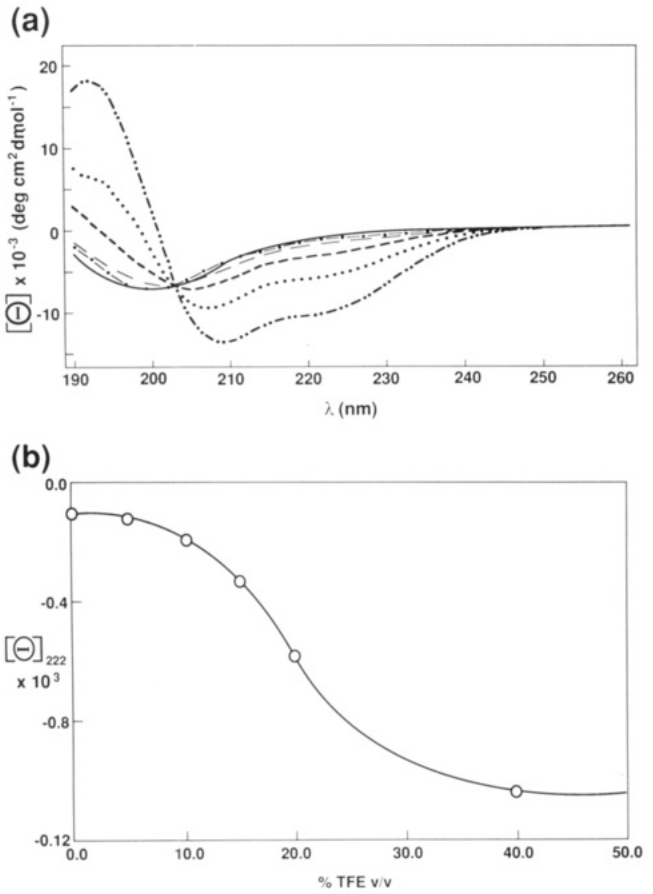

FIGURE 6: (a) CD spectrum of RP70, concentration 39 μM, in H_2O, pH 4.20, at 298 K, with increasing concentrations of TFE (% v/v). (b) Plot of molar ellipticity of RP70 at 222 nm against concentration of TFE (% v/v).

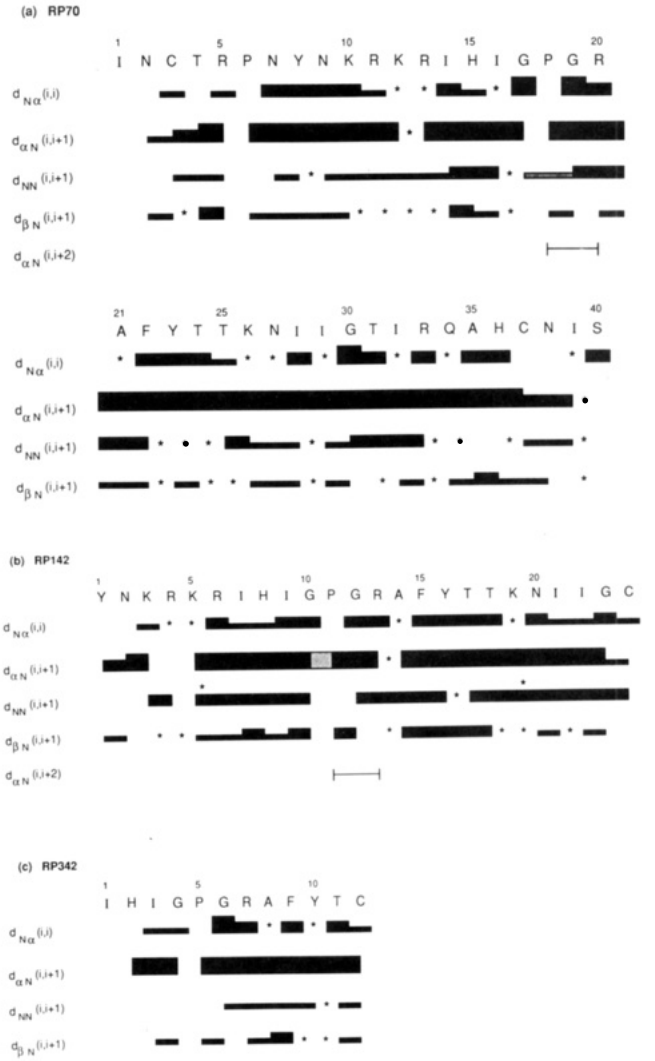

FIGURE 7: Schematic diagram showing the magnitude of various NOE connectivities observed in NOESY spectra of the three peptides: (a) RP70; (b) RP142; (c) RP342.

majority of peptides in solution, a significant population of unfolded conformers exists in dynamic equilibrium with the folded forms. The unfolded conformers give rise to a strong $d_{\alpha N}(i,i+1)$ NOE for each pair of residues in the peptide: this masks the NOE derived from the conformers containing the type II turn and gives rise to the ambiguity of interpretation referred to above.

Analysis of the NOESY spectra of RP142 (Figure 4) shows the presence of the same NOE connectivities in the Gly-Pro-Gly-Arg sequence as were observed for RP70, although the $d_{\alpha N}$(Pro,Arg) NOE cross peak is of lower relative intensity for RP142 than for RP70. This indicates that a lesser, but significant, population of reverse turn conformations is present in this region of the shorter peptide.

Absence of a β-Turn Conformation in RP342. No evidence was observed for the presence of a reverse turn in the shortest peptide, RP342 although $d_{NN}(i,i+1)$ NOE connectivities are present (Figure 5). The diagnostic NOE connectivity that establishes the presence of a turn conformation is the $d_{\alpha N}$-$(i,i+2)$ NOE across the turn, which is unambiguously observed in the spectra of RP70 and RP142 (Figures 3 and 4) but which is absent from both the NOESY spectrum (Figure 5) and the ROESY spectrum (not shown) of RP342. We conclude that the β-turn conformer is not significantly populated in this peptide. This is in contrast to observations made with the analogous peptides derived from HIV-2 (N. Assa-Munt, H. J. Dyson, P. E. Wright, and E. Norrby, unpublished observations), where the turn structure is observed in the 12 amino acid peptide as well as in the 24- and cyclic 40-residue peptides.

Temperature Dependence of Amide Proton Chemical Shift. The dependence of the amide proton chemical shift on the temperature, commonly termed the temperature coefficient, can be used as a measure of the extent of protection of the amide proton from the aqueous solvent. This is generally interpreted as an indication of intramolecular hydrogen bonding (Rose et al., 1985). For RP342, the temperature coefficients were measured for all amide protons by using one-dimensional spectra. For RP142 and RP70, the resolution of the amide proton resonances in one-dimensional spectra is insufficient for these measurements, and a series of two-dimensional spectra were used. For RP142, all of the NH–C$^{\alpha}$H cross peaks in the 2QF-COSY spectrum could be resolved at lower temperatures, but at higher temperatures certain of the cross peaks were missing due to overlap of the C$^{\alpha}$H resonance with that of H_2O. For RP70, many of the the NH–C$^{\alpha}$H cross peaks were insufficiently resolved in 2QF-COSY spectra for reliable temperature coefficients to be calculated. NOESY spectra were acquired in order to obtain information on residues of interest, particularly Arg 20.

No evidence for hydrogen bonding, expressed as a lowering of the temperature coefficient of the amide proton, is seen in the region of the turn conformation inferred from the NOE results. The temperature coefficients of Arg 7 in RP342, Arg 13 in RP142, and Arg 20 in RP70 are within normal values for completely solvent-exposed amide protons. If a hydrogen bond were present between the carbonyl group of the initial Gly of the turn and the Arg amide proton, these values would

Table I: ^{1}H Resonance Assignments

residue	chemical shift (ppm)[a] NH	$C^\alpha H$	$C^\beta H$	$C^\gamma H$	$C^\delta H$	others
			(a) RP70, pH 4.20, 278 K			
Ile 1		3.84	1.93	1.18, 1.48	0.89	0.96 ($C^\gamma H_3$)
Asn 2	8.99	4.90	2.77, 2.87			
Cys 3	8.82	4.74	2.89			
Thr 4	8.52	4.32	4.11	1.17		
Arg 5	8.62	4.60	1.72, 1.68	1.58	3.12	
Pro 6		4.30	2.13, 1.94	1.93	3.58, 3.79	
Asn 7	8.56	4.61	2.68, 2.76			
Tyr 8	8.34	4.52	3.07, 2.88		7.08	6.77 ($C^\epsilon H$)
Asn 9	8.43	4.60	2.70, 2.77			
Lys 10	8.31	4.20	1.72, 1.78	1.4	1.6*	2.95* ($C^\epsilon H_2$)
Arg 11	8.25	4.26	1.72	1.58	3.12	
Lys 12	8.35	4.25	1.70	1.4*	1.6*	2.95* ($C^\epsilon H_2$)
Arg 13	8.49	4.29	1.68, 1.72	1.6*	3.15*	
Ile 14	8.32	4.14	1.74	1.10, 1.35	0.80	0.85 ($C^\gamma H_3$)
His 15	8.77	4.67	3.10		7.18	
Ile 16	8.37	4.17	1.77	1.10, 1.40	0.80	0.86 ($C^\gamma H_3$)
Gly 17	8.43	4.08				
Pro 18		4.44	2.24, 1.96	2.00	3.62	
Gly 19	8.66	3.94				
Arg 20	8.29	4.23	1.68	1.6*	3.15*	
Ala 21	8.39	4.21	1.22			
Phe 22	8.22	4.52	2.95		7.09	7.29, 7.27 ($C^\epsilon H$, $C^\zeta H$)
Tyr 23	8.19	4.61	2.85, 3.00		7.06	6.77 ($C^\epsilon H$)
Thr 24	8.21	4.39	4.19	1.18		
Thr 25	8.24	4.31	4.21	1.20		
Lys 26	8.42	4.26	1.70	1.4*	2.6*	2.95* ($C^\epsilon H_2$)
Asn 27	8.55	4.68	2.68, 2.78			
Ile 28	8.29	4.14	1.82	1.1, 1.4*	0.83*	0.86 ($C^\gamma H_3$)
Ile 29	8.40	4.11	1.84	1.1, 1.4*	0.83*	0.90 ($C^\gamma H_3$)
Gly 30	8.57	3.98				
Thr 31	8.18	4.32	4.16	1.18		
Ile 32	8.37	4.14	1.82	1.1, 1.4*	0.83*	0.87 ($C^\gamma H_3$)
Arg 33	8.58	4.30	1.72	1.6*	3.15*	
Gln 34	8.49	4.27	1.94, 2.04	2.34		
Ala 35	8.52	4.21	1.32			
His 36	8.60	4.67	3.17		7.25	
Cys 37	8.53	4.71	2.91			
Asn 38	8.84	4.78	2.75, 2.82			
Ile 39	8.45	4.18	1.88	1.1, 1.4*	0.83*	0.90 ($C^\gamma H_3$)
Ser 40	8.45	4.40	3.83			
			(b) RP142, pH 4.34, 278 K			
Tyr 1		4.21	3.09		7.08	6.83 ($C^\epsilon H$)
Asn 2	8.82	4.72	2.68, 2.76			
Lys 3	8.61	4.23	1.73, 1.83	1.43	1.70	2.97 ($C^\epsilon H_2$)
Arg 4	8.44	4.26	1.82	1.60, 1.65	3.17	7.24 ($N^\epsilon H$)
Lys 5	8.43	4.28	1.8*	1.4*	1.6*	2.95* ($C^\epsilon H_2$)
Arg 6	8.57	4.35	1.75	1.61, 1.54	3.16	7.23 ($N^\epsilon H$)
Ile 7	8.40	4.15	1.78	1.13, 1.38	0.82	0.83 ($C^\gamma H_3$)
His 8	8.83	4.71	3.13		7.22	8.55 ($C^\epsilon H$)
Ile 9	8.44	4.19	1.79	1.12, 1.43	0.81	0.87 ($C^\gamma H_3$)
Gly 10	8.52	4.07, 4.14				
Pro 11		4.46	2.23, 1.96	1.96	3.62	
Gly 12	8.66	3.96				
Arg 13	8.32	4.26	1.68, 1.75	1.55	3.13	
Ala 14	8.42	4.22	1.24			
Phe 15	8.25	4.54	2.98, 3.09		7.09	7.27, 7.25 ($C^\epsilon H$, $C^\zeta H$)
Tyr 16	8.18	4.62	2.88, 3.02		7.06	6.77 ($C^\epsilon H$)
Thr 17	8.19	4.38	4.18	1.17		
Thr 18	8.26	4.32	4.21	1.21		
Lys 19	8.45	4.29	1.8*	1.4*	1.6*	2.95* ($C^\epsilon H_2$)
Asn 20	8.58	4.68	2.69, 2.78			
Ile 21	8.30	4.18	1.86	1.15, 1.41	0.83	0.85 ($C^\gamma H_3$)
Ile 22	8.42	4.5	1.85	1.20, 1.49	0.85	0.90 ($C^\gamma H_3$)
Gly 23	8.69	3.99				
Cys 24	8.41	4.53	2.92			
			(c) RP342, pH 4.06, 278 K			
Ile 1		3.87	1.96	1.18, 1.48	0.90	0.97 ($C^\gamma H_3$)
His 2	9.01	4.76	3.18		7.26	8.57 ($C^\epsilon H$)
Ile 3	8.57	4.20	1.81	1.15, 1.48	0.83	0.90 ($C^\gamma H_3$)
Gly 4	8.50	4.05, 4.12				
Pro 5		4.45	2.26, 1.98	2.02	3.64	
Gly 6	8.63	3.96				
Arg 7	8.28	4.26	1.73, 1.65	1.58	3.14	7.20 ($N^\epsilon H$)

Table I (Continued)

residue	chemical shift (ppm)[a] NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	others
Ala 8	8.42	4.22	1.27			
Phe 9	8.21	4.51	3.01		7.13	7.33, 7.29 ($C^{\epsilon}H$, $C^{\zeta}H$)
Tyr 10	8.13	4.60	3.00, 2.91		7.10	6.80 ($C^{\epsilon}H$)
Thr 11	8.11	4.30	4.15	1.17		
Cys 12	8.35	4.46	2.93			(C-terminal -NH) 7.35, 7.53

[a] An asterisk (*) indicates that the resonances of several residues of the same type are overlapped and cannot be distinguished.

be significantly lowered. This result is somewhat unexpected, in view of previous findings [e.g., Dyson et al. (1988c)] where the extent of hydrogen bonding within a turn conformation was found to be correlated with the population of turn conformations, as judged by the NOE results. The difference in behavior in this case may be due simply to the nature of the turn conformation in the peptide: not all turn conformations contain the 1 → 4 hydrogen bond (Rose et al., 1985).

Influence of the Disulfide Bond on Conformational Preference. In the intact gp120 protein, the sequence corresponding to the RP70 peptide is disulfide-bridged to form a loop (Leonard et al., 1990). One of the aims of the present work was to evaluate to what extent the integrity of this loop influenced the peptide conformation. We have shown that the reverse turn conformation in the squence Gly-Pro-Gly-Arg is present in RP70, which contains the disulfide loop, and in RP142, which not only contains no loop but also is considerably shorter. Since the turn conformation is apparently present in a comparable population in both peptides, we conclude that its formation does not depend on the integrity of the disulfide bond and the presence of the loop. In addition, NMR spectra of the reduced (dithiol) RP70 peptide (data not shown) are very similar to those of the disulfide-bridged form, indicating that no conformation is induced in the loop by the formation of the disulfide bond. The presence of the turn conformation is also apparently independent of the length of the peptide, above a certain threshold, which may be represented by RP342.

Comparison of Solution Conformation with Published Predictions. By use of a neural network approach, it has recently been predicted that the average PND sequence of HIV-1 should contain two antiparallel β-strands and a short C-terminal α-helix (LaRosa et al., 1990). The amino acid residues between the strands (corresponding to the conserved Gly-Pro-Gly-Arg sequence) were predicted to adopt a type II β-turn structure on the basis of homology with sequences of known turns in protein crystal structures (Wilmot & Thornton, 1988). The prediction of a reverse turn is consistent with our NMR results. However, we do not observe evidence in water solution for the ordered β-strands and α-helices predicted in the calculation (LaRosa et al., 1990), and we see no evidence for a unique folded conformation of the peptide: there are no intramolecular long-range NOEs, and the CD spectrum indicates a random structure. Another indication of folded structure in a peptide is the presence in the NMR spectrum of resonances shifted from their normal positions in the spectrum, corresponding to the side chains that pack in the interior of the folded molecule [see, for example, Lee et al. (1990)]. No such shifted resonances are observed for RP70, RP142, or RP342 in water solution, providing further evidence that the peptides are present as random populations of many different conformations.

Conformation of RP70 in TFE/Water Mixtures. The apparent formation of a degree of ordered helix in RP70 upon addition of TFE to the water solution of the peptide (Figure 6) may be an indication that a defined conformation has been induced that can be studied by NMR. Complete analysis of the NOESY spectrum of RP70 in 20% (v/v) TFE is complicated by broadness and overlap of the resonance lines and will require extensive further spectroscopy. Nevertheless, it is possible to make several observations: first, some of the resonances are shifted considerably from their positions in the absence of TFE, while others remain substantially unchanged. Second, there is evidence for the presence of the β-turn found in the water solutions of RP70 in the spectrum in 20% TFE. Third, the magnitude of certain of the $d_{NN}(i,i+1)$ NOE connectivities compared to the corresponding $d_{\alpha N}(i,i+1)$ NOEs is apparently greater in 20% TFE than in H_2O, and medium-range $(i,i+2)$ and $(i,i+3)$ NOEs can be seen in the spectrum, an indication that ordered helix may have formed. While a definitive description of the conformation of RP70 in TFE/water solutions must await a further extensive spectroscopic study, the indications from the above observations are that a helical conformation may be forming in the C-terminal region of the peptide, consistent with the predictions of LaRosa et al. (1990). No evidence for the β-strand regions predicted in that paper has been observed as yet.

Significance of the Observed Reverse Turn. The presence of a well-defined β-turn conformation in high population in the Gly-Pro-Gly-Arg sequence may be significant for the immunological role of the PND. This sequence is conserved among HIV-1 isolates (LaRosa et al., 1990) and has been shown to be the binding site for neutralizing antibodies (Javaherian et al., 1989, 1990). By contrast, the sequences surrounding the conserved tetrapeptide are highly variable. It is perhaps not surprising that no evidence of a defined structure is found for these regions in water solution. Regions of peptide immunogens that contain conformational preferences for ordered or nascent secondary structure have been implicated in the mechanism of induction of antipeptide antibodies (Dyson et al., 1988a). The present results are consistent with this hypothesis, in that the region of high population of ordered structure is also the site of antibody binding. Definitive information on this important point will shortly be forthcoming in the form of an X-ray crystal structure of a monoclonal antibody with a peptide related to those reported here (I. A. Wilson, personal communication).

ACKNOWLEDGMENTS

We thank Drs. Peter Wright, Ian Wilson, and James Boyd for valuable discussions and Lawrence Eckler for technical contributions.

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