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A Conformational Switch Is Associated with Receptor Affinity in Peptides Derived from the CD4-Binding Domain of gp120 from HIV I†

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ABSTRACT: A 15-residue region within the CD4-binding domain of gp120 from HIV I was identified with use of folding algorithms as conserving the potential for forming a particular secondary structure throughout 11 sequenced HIV strains. The region chosen has a potential for forming both β-sheet and α-helix; the helical form would be amphipathic with the five hydrophobic residues all totally or functionally conserved. Five peptides were synthesized corresponding to this region in strain LAV and the strain most highly divergent from it in primary structure (Z3) plus three additional peptides with critical substitutions in the LAV sequence. The conformation of these five peptides was examined under various conditions with circular dichroism, and the results were compared with the ability of each peptide to bind to a CD4-expressing strain of HeLa cells (HeLa T4). In solution, the unmodified peptides exhibit a bistable structure, existing as β-sheet in dilute buffer and converting to α-helix under more apolar conditions. The transition is reversible and sharp, occurring at a particular point in the polar/apolar gradient with virtually no intermediate state. The ability to undergo this bistable flip is closely associated with binding ability, amino acid substitutions that eliminate binding ability also eliminating the switch, and vice versa. The transition thus may reflect conformational changes occurring in this region of gp120 as it binds to the CD4 receptor.

The high degree of variability in the envelope protein gp120 of HIV I is well-known (Hahn et al., 1985; Wong-Staal et al., 1985) and constitutes one of the strongest difficulties facing the development of an effective antiserum. The problem is increased by the fact that the majority of the predicted antigenic epitopes of gp120 coincide with regions of high sequence variability (Modrow et al., 1987). The principal neutralizing determinant has in fact been found to differ by as much as 50% among viral isolates (Myers et al., 1989). An alternative

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strategy in the control of HIV infected involves blocking binding of the virus via gp120 to the T-cell receptor protein CD4. A number of laboratories (Deen et al., 1988; Fisher et al., 1988; Hussey et al., 1988; Traunecker et al., 1988) have been successful in producing a soluble form of CD4 (sCD4) and showing that this blocks HIV infection in vitro. Use of the technique in vivo, however, is limited by the short half-life of sCD4 in the system, and even were this to be overcome, such a strategy might be complicated by the simultaneous binding of sCD4 to its natural ligand, class II MHC. The ideal approach, which would minimize interference with the normal function of the immune system, would be to block specifically and solely that portion of gp120 responsible for docking at the CD4 receptor. This ultimately requires a knowledge of the precise conformation of the gp120-binding region.

Lasky and co-workers (Lasky et al., 1987, 1988) have mapped a semiconserved region of gp120 extending from residue 397 to 440 that seems to be directly concerned with binding to CD4. The isolated 44 amino acid peptide retains the ability to interact with the receptor. The direct involvement of this area in CD4 binding has been confirmed by in vivo studies (Cordonnier et al., 1989). The region contains highly variable as well as conserved sequences, which is surprising as it must, of necessity, be functionally invariant in all infective strains.

Steric characteristics are central to binding specificity. We therefore decided to determine first whether particular conformational characteristics were being conserved in this region despite the variation in primary structure and second whether binding to CD4 was dependent on a particular structure and, if so, to characterize it. This laboratory has developed a body of experience in relating the secondary structure properties of peptides to their ability to bind to an appropriate receptor (Reed & Kinzel, 1984a,b; Reed et al., 1985, 1987, 1988, 1989). Once the exact structural requirements for initial viral binding to CD4 are understood, it may be possible to design a molecule that will interfere only with this process.

Materials and Methods

Peptides: Synthetic peptides were chosen to correspond to a 15-residue region running from Leu^{400} to Val^{414} in gp120 of the LAV strain of HIV I. The three peptides L-P-C-R-I-K-Q-F-I-N-M-W-Q-E-V (the sequence in LAV), L-P-C-R-I-K-Q-V-V-R-T-W-Q-G-V (the corresponding region in strain Z3), and L-P-C-R-I-K-Q-F-I-G-G-W-Q-G-V (a peptide in which all variable residues not forming the hydrophobic site (see Results) were substituted with Gly to disrupt secondary structure) were obtained from Cambridge Research Biochemicals, Cambridge, England. (These are referred to as the LAV, Z3, and Gly-substituted peptides in the text.) An additional three peptides identical with the first but with the N-terminal Leu labeled with ^{14}C were obtained from the same source. Specific activities were ca. 1 mCi/mmol. Two further peptides, L-P-C-R-I-K-Q-F-I-N-M-V-Q-E-V (LAV W → V) and L-P-C-R-I-K-Q-F-I-N-M-F-Q-E-V (LAV W → F), corresponding to the LAV peptide with the Trp substituted with Val and Phe, respectively, were obtained from Bachem Biochemica GmbH, Heidelberg. (These two substitutions have been shown to reduce the ability of intact gp120 to bind to the CD4 receptor (Cordonnier et al., 1989)). Peptide purity was checked with HPLC analysis and FAB and was 90% for all samples. Gel filtration with Superose 12 was used to check the aggregation state of the peptides in aqueous buffer. This showed that they remain in monomeric form at up to 10-fold the concentrations used for spectroscopic measurement (data not shown).

Circular dichroism: Circular dichroism spectra were measured with use of a Jasco J-500 automatic recording spectralpolarimeter coupled to a J-DPY digital data processor. Slit width was automatically maintained at 1.0 mm throughout the spectral range. The instrument was calibrated by use of 0.05% β-andosterone in dioxane.

CD spectra were taken from 190 to 240 nm at a sensitivity of 2.0 mdeg/cm and scanning speed of 5.0 nm/min with a 2.0-s time constant. Samples were routinely measured at a concentration of 100 μg of peptide/mL in 10 mM Tris-HCl, pH 7.5, as the aqueous buffer and in a 1.0-mm quartz cuvette at room temperature. Curves presented are the product of an at least 4-fold signal averaged sample curve with a similarly measured and signal averaged baseline subtracted. Spectra digitally recorded in millidegrees were converted to θ_{MR} (mean residue ellipticity) for curve fitting and secondary structure analysis.

Curves were fitted by use of CD spectra of model peptides in α-helix, β-sheet, extended-coil, and reverse-turn conformations (Yang et al., 1986). This method was chosen over those involving comparison with the CD spectra of known globular proteins (Provencher & Glockner, 1981; Hennessey & Johnson, 1981), as these are deliberately designed to include factors such as long-range interactions not displayed by model peptides. As the CD curves to be fitted were those of short, relatively simple peptides in solution, the use of peptide model curves was considered more appropriate.

Structure probability analysis: Analysis of the potential of primary sequences to form secondary elements was carried out with use of folding algorithms (Chou & Fasman, 1978). Potential helix-forming sequences were defined as those for which $P_\alpha > 1.05$. (P_α, P_β, and P_t are the overall probability factors for finding a given residue within α-helix, β-sheet or β-reverse turn, respectively.) Probable bend locations were defined as those for which $p(t) \geq 0.75 \times 10^{-4}$ and $P_\alpha < P_t > P_\beta$; $p(t)$ is a sequence-dependent function $p(t) = f_i f_{i+1} f_{i+2} f_{i+3}$ with the probability of a β-reverse turn beginning at residue i given as the product of the position-dependent frequencies f for each amino acid residue from position i to $i + 3$ of the tetrad.

Receptor binding assays: The degree to which the gp120-derived peptides retained the ability to bind to the CD4 receptor was examined by use of a HeLa T4 cell line supplied by the NIH AIDS Research and Reference Reagent Program (Catalog No. 154). Cells were tested for the ability to express CD4 on their surface with use of anti-CD4 mAb and FITC-conjugated rabbit anti-mouse (both the kind gift of Prof. Stefan Meuer, Division of Applied Immunology, Deutsches Krebsforschungszentrum). One hundred percent of the cultured cells tested CD4-positive by this method. The Gly-substituted and LAV W → F and LAV W → V peptides served as an internal control for binding specificity, as these retain the gross physical properties (size, number of hydrophobic side chains, and, for the latter two, charge) of the unmodified sequences.

Peptide binding was assayed in two ways: either directly, with the Leu-^{14}C-labeled analogues of the LAV, Z3, and Gly-substituted peptides, or, in the case of the unlabeled LAV W → V and LAV W → F peptides, through their ability to compete with labeled LAV peptide for binding to the receptor.

For direct bindings tests, HeLa T4 cells were grown to a cell density of ca. 200000 in 16-mm miniwells (Costar, Cambridge, MA). The cells in each well were washed twice with 1 mL of cold PBS and incubated with 300 μL of 25 μM ^{14}C-labeled peptide in PBS for 60 min at 4 °C. The cultures

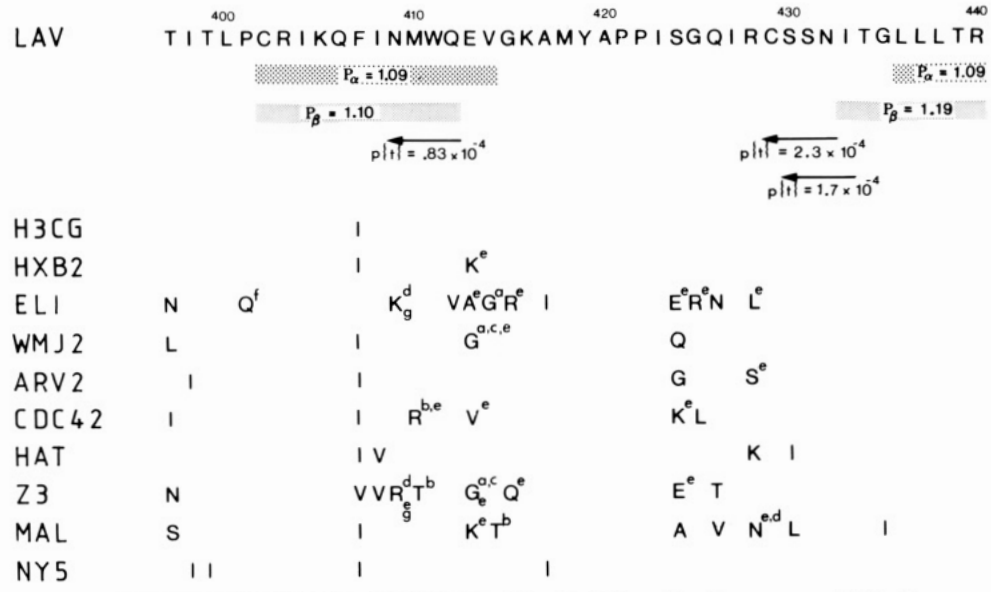

FIGURE 1: Secondary structure prediction for residues 397–440 of gp120. Predictive algorithm was applied to the sequence in HIV strain LAV (top), and the effect of amino acid substitutions occurring in other strains was compared with this. For P_α, P_β, and $p(t)$, see text. H = strong helix or β-sheet former, h = moderate helix or β-sheet former, I = weak helix or β-sheet former, b = moderate helix or β-sheet blocker, and B = strong helix or β-sheet blocker. Coarse hatching indicates areas of high α-helix probability; fine hatching indicates areas of β-sheet. β-turns are indicated by arrows. Key to footnotes: (a) shortens helix; (b) slightly weakens helix; (c) introduces possible β-bend; (d) eliminates β-bend possibility; (e) charge change; (f) extends helix; (g) strongly improves helix.

were then washed twice more with 1 mL of PBS before being lysed with 200 μL of PBS + 2% SDS. The lysate was placed in counting vials with 3 mL of Quickzint (Zinsser Analytic, Frankfurt) and the cell-bound radioactivity measured.

For competition binding tests, cells were incubated with ^{14}C-labeled LAV peptide as above in the presence of increasing concentrations of unlabeled LAV, LAV W → V, or LAV W → F. The incubated cultures were then washed, lysed, and counted as above.

Results

An initial analysis was carried out on the potential for forming secondary structure elements of gp120 within the 44 amino acid CD4-binding fragment (Lasky et al., 1987, 1988) with use of folding algorithms. In doing this, special attention was paid to the amino acid substitutions appearing in the 11 natural HIV variants examined (Figure 1), bearing in mind that as these variants are all infective, the substitutions concerned cannot interfere with binding ability. This analysis raised several suggestive points.

1. α-helical segment: The region corresponding to Cys^{402} to Val^{414} in LAV has a high helix-forming potential, (P_α = 1.09). It also has a slightly higher potential for β-sheet formation, but this is the less likely structure because the naturally occurring substitution of Lys for Asn^{409} in ELI and of Arg for Asn^{409} in Z3 serves to strengthen helix and weaken β-sheet probability. In addition, such a helix establishes an extremely prominent hydrophobic subsite comprised of five residues (Figure 2). All of these except Val^{414} are either totally conserved (Ile^{404}, Trp^{411}) or substituted only with other hydrophobic side chains (Phe^{407} → Ile, Val; Ile^{408} → Val) throughout the 11 variants studied. The probability that this would occur by chance is extremely low.[1] The prediction is thus for a functionally important helical region running from ca. Cys^{402} to Gln^{412} and possibly extending to Val^{414} in some variants.

[1] The chance of five hydrophobic residues in a given pentadecapeptide occurring in exactly this arrangement is ca. $(0.375)^5 = 0.0077$. If purely coincidental, this means one could expect to find it in this region in roughly 1 of 120 cases; we observe it in 11 out of 11.

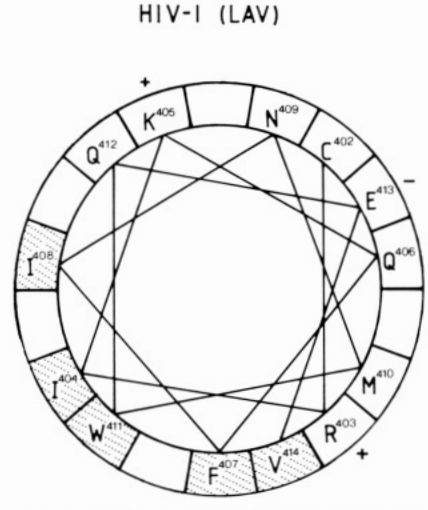

FIGURE 2: Axial projection of the gp120 sequences from Cys^{402} to Val^{414} showing alignment of hydrophobic residues and amphiphilic character of the helix.

2. β-reverse turns: Although in any single variant sequence one or more potential β-bends may occur, only one of these, corresponding to Ser^{430}–Ile^{434}, is conserved in all variants. The apparent potential for a β-bend at Pro^{421}–Pro^{422} is canceled by the extreme unfavorability of Ile^{423} in the i + 2 position; this residue is totally conserved.

3. Electrostatic interactions: With one exception, these do not appear to play an important role in binding. All charges except those on Arg^{403} and Lys^{405} in the putative helical region can vanish or even be reversed in natural variants: totally new charges are introduced in ELI, CDC 42, and Z3.

4. Conserved region Gly^{415}–Ile^{423}: What appears to be conserved here is the absence of regular secondary structure. A helix or β-sheet breaker is introduced every three or fewer residues, strongly reducing the potential for structure of this type, and the tendency to form a β-bend at Gly^{415} and Pro^{421} is undermined in each case by the occurrence of a highly unfavorable—and totally conserved—residue within the tetrad (Met^{418} and Ile^{423}, respectively). The tentative conclusion is

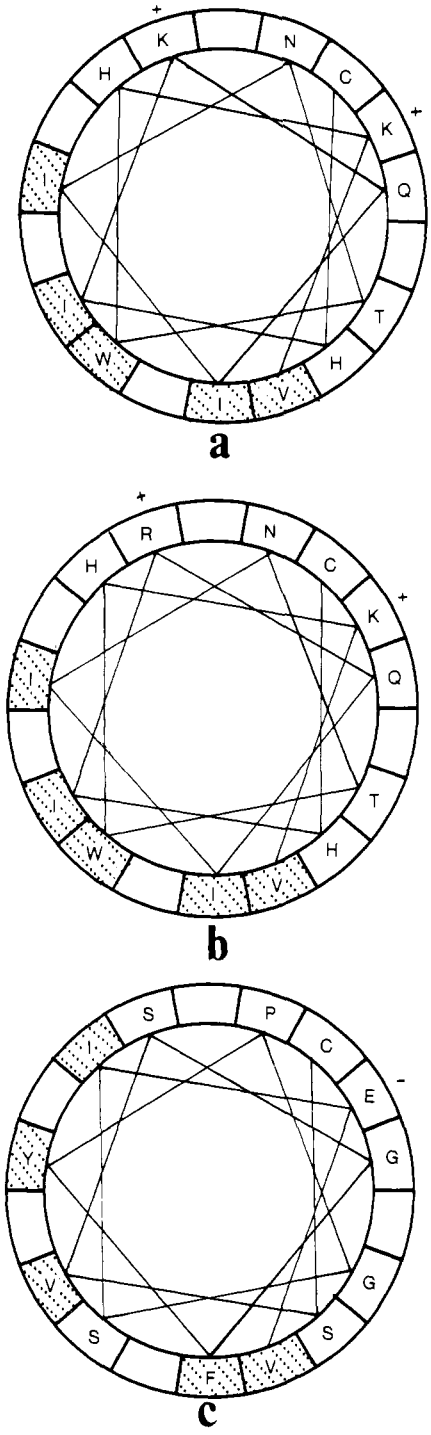

FIGURE 3: Axial projection of the sequence homologous to that in Figure 2 from two other CD4-binding viruses, HIV II (a) and SIV (b), and from the natural ligand MHC II (c). Folding algorithms indicate an α-helical potential for (a) and (b). In the case of (c), the prediction is for an α-helix at the C-terminal half modulating to a 3_{10} helix at the N-terminal portion.

that flexibility in the loop Gly^{415}–Ile^{423} is important to the function of gp120.

Two areas of potential structural importance are thus identified in the 44-residue CD4-binding fragment of gp120; a hydrophobic subsite established by a potential α-helical segment in the N-terminal region and a flexible loop containing a Pro–Pro kink flanked by conserved hydrophobic residues Tyr^{419} and Ile^{423}. With regard to the first of these, it is interesting that the sequence homologous to Cys^{402}–Val^{414} in related CD4-binding viruses also retains the potential to form an amphiphilic helix. Axial projections of the primary sequence of HIV II (Figure 3a) and SIV (Figure 3b) give a pattern very similar to that of HIV I, including the two positive charges. The corresponding area in MHC II B2 (Figure 3c) also can form an amphiphilic helix but has a negative charge. For this reason, it was decided to concentrate initially on the structural characteristics of the possible helical region and their relation to CD4 binding. To this end, five pentadecapeptides were synthesized: two corresponding to the primary sequence of this region in LAV and Z3, respectively (Z3 being the variant whose primary sequence differs most from that of LAV), plus a glycine-substituted analogue. In addition, two versions of the LAV peptide were synthesized in which the Trp was replaced once with a Val and once with a Phe. The Trp^{411} locus has been shown to be crucial for binding in the intact gp120 protein (Cordonnier et al., 1989) (see Materials and Methods).

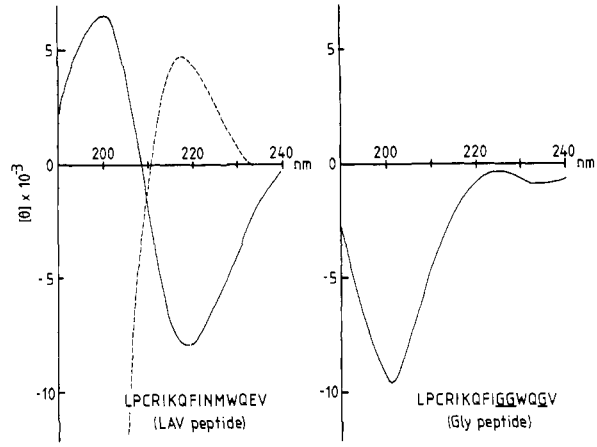

FIGURE 4: Far-UV CD spectrum of the LAV and Gly-substituted peptides. Broken line represents spectrum of model peptides in random coil conformation for comparison.

There is always the question in dealing with peptide fragments as to whether they retain in isolation the structure typically adopted in the larger molecule, in this case the 44-residue CD4-binding fragment. However, it has been found (Cease et al., 1987) that immunization with a synthetic 16-residue peptide whose sequence overlaps our putative helix area induced T-cell immunity to native gp120. Thus, we anticipated that any appropriate conformation in the gp120 fragment could exist independently in solution. Certainly, retention of the native conformation has been observed for peptide fragments even smaller than this, viz. the 13-residue N-terminal helix of bovine pancreatic ribonuclease A (Shoemaker et al., 1985) or the R-G-D-containing hexapeptide from fibrinogen (Reed et al., 1988).

Circular dichroism: Circular dichroism is a rapid and sensitive method of determining the secondary structure content of peptides and proteins. We therefore used CD to examine the conformation of the five peptides under various conditions.

The far-UV CD spectrum of the LAV peptide in buffer is shown in Figure 4. The curve clearly indicates a nonrandom configuration and in fact corresponds to 60–65% β-sheet with the remainder a mixture of random coil and reverse turn. The Z3 peptide also has a majority of its regular structure in β-sheet, although there is a small amount of α-helix as well. Thus, there does appear to be a particular secondary structure associated with this portion of the CD4-binding site on gp120 throughout the widest range of primary structure difference in the natural variants. The Gly-substituted peptide, on the other hand (Figure 4), has over 90% mixed random coil and reverse turn, showing that the Gly substitutions did in fact act

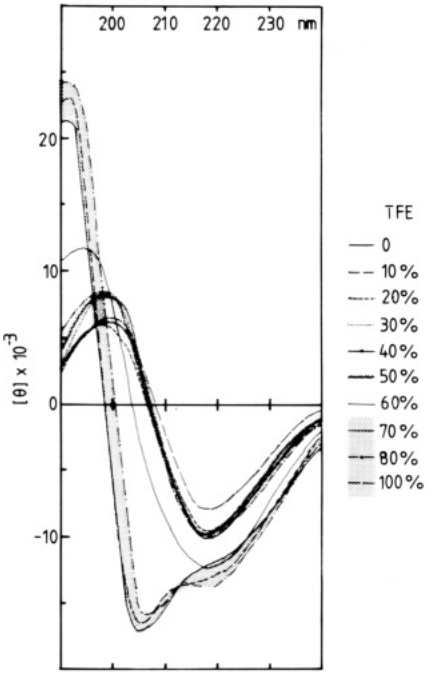

FIGURE 5: Change in far-UV CD spectrum of the LAV peptide as a function of TFE concentration. TFE concentrations from 0 to 100% were measured at 10% intervals. Coarse hatching indicates curves at concentrations where the dominant structure is α-helix; fine hatching indicates curves at concentrations where β-sheet predominates.

as planned to disrupt existing secondary structure.

It was unexpected, however, to find the major portion of the peptide in β-sheet when the correct placement of hydrophobic residues to form a continuous region in helical alignment is conserved in the binding domain through at least 11 HIV strains. As dilute buffer is not highly analogous to the presumed environment at the binding site, we checked what effect decreasing the polarity of the solvent would have on the peptide conformation.

Figure 5 shows the far-UV CD spectra of the LAV peptide under increasing concentrations of trifluoroethanol (TFE). TFE is known to favor formation of intrachain hydrogen bonds, but the gain in regular secondary structure with TFE concentration is typically gradual (see Figure 6f), a cumulative multistep process. The LAV peptide exhibits an unusual pattern of behavior. The conformation resists change up to 50% TFE, starts to alter at 60%, and by 70% TFE the change is complete with no further effect up to 100% TFE. The same phenomenon is seen in reverse if one starts at 100% TFE and decreases the concentration. This is thus a bistable, reversible system, with a sharp transition from β-sheet (state 1) to α-helical (state 2) structure triggered at a particularly hydrophobicity level (Figure 6a). The Z3 peptide also shows this behavior (Figure 6b), the change occurring at the identical TFE concentration. (The effect is not so dramatic in this peptide due to the lower initial β-sheet content and the presence of a small amount of helix already in the hydrophilic form; the end point reached in state 2, ca. 50% helix and no β-sheet, is the same as in the LAV peptide). This property is not shared by the Gly-substituted peptide (Figure 6c), which has an initial minor gain of helix at 10% TFE and then experiences no further change.

Where the conserved Trp residue in the LAV peptide has been substituted by Val (Figure 6d), there is also no abrupt transition, the W → V peptide exhibiting an almost linear gain in helicity with increasing TFE concentration, typical of the gradual gain in hydrogen-bonded structure seen generally in peptides under these conditions. The W → F substitution

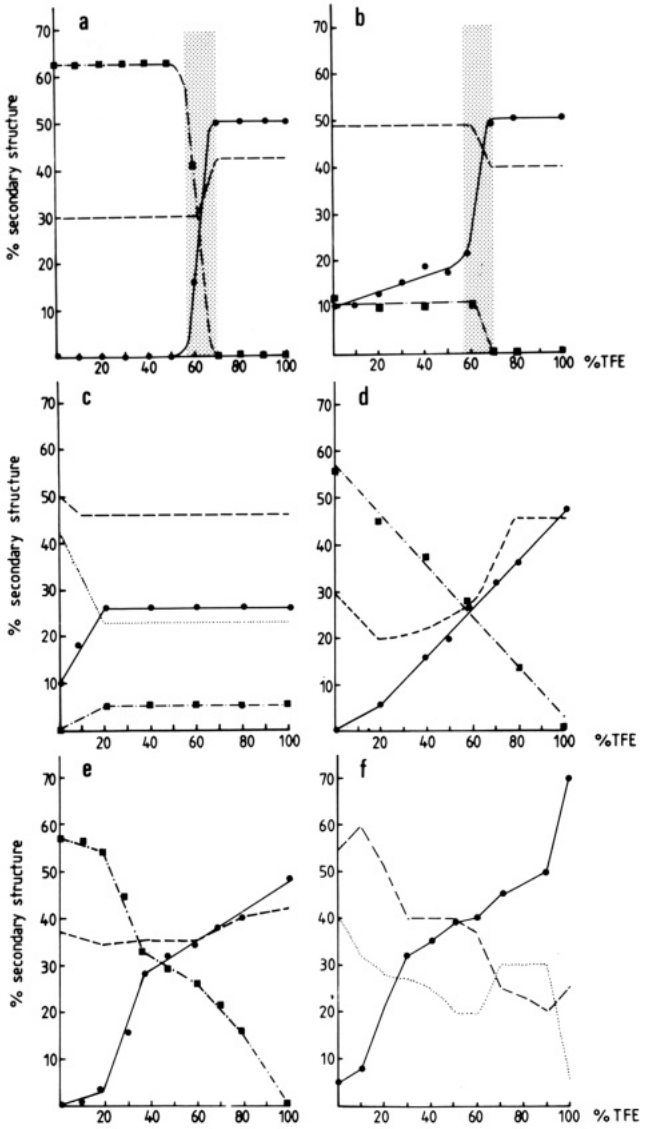

FIGURE 6: Secondary structure content of LAV peptide (a), Z3 peptide (b), Gly-substituted peptide (c), LAV W → V (d), and LAV W → F (e) as a function of TFE concentration. Panel f shows comparable curves for peptides from tubulin. The lines represent (●) α-helix, (■) β-sheet, (– –) random coil, or (···) β-reverse turn.

Table I: Binding of gp120-Derived Pentadecapeptides to HeLa T4 Cells[a]

	no peptide	[*Leu*-^{14}C]LAV	[*Leu*-^{14}C]Z3	[*Leu*-^{14}C]-Gly-sub.
cpm	21 ± 2	877 ± 105	973 ± 115	55 ± 2

[a] Figures given are total recoverable label in counts per minute.

alters the behavior of the peptide as well (Figure 6e), although not so severely; an abrupt gain in helicity occurs between 20 and 40% TFE, followed by a slow linear increase.

Receptor binding assays: The relative recovery of ^{14}C-labeled LAV, Z3, and Gly-substituted peptides from CD4-positive HeLa cells is shown in Table I. Both the LAV and Z3 pentadecapeptides retain the ability to bind to CD4 characteristic of the HIV envelope glycoprotein from which they were derived. The peptide in which Gly was substituted for all nonconserved, nonhydrophobic residues, however, binds very poorly to HeLa T4 cells.

Figure 7 shows the effect of autocompetition on binding of labeled LAV-peptide. The binding abilities of the W → V and W → F modified LAV peptides were compared through their ability to compete with labeled LAV at the lower con-

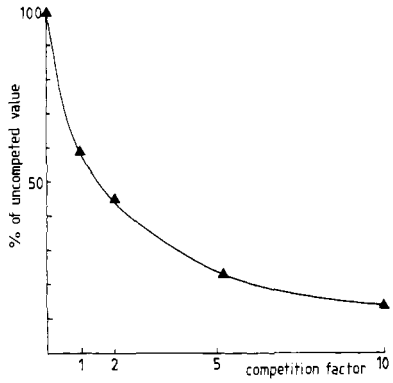

FIGURE 7: Autocompetition of labeled LAV peptide by its unlabeled analogue. HeLa T4 cells were incubated with 25 μM [*Leu*-^{14}C]LAV peptide in the presence of 0, 25, 50, 125, and 250 μM LAV peptide (competition factor = 0, 1, 2, 5, and 10). Amount of cell-bound label recoverable is expressed as percentage of the amount in the absence of competing peptide.

Table II: Allocompetition of Labeled LAV Peptide by Unlabeled W → V and W → F Derivatives[a]

	LAV W → V vs [*Leu*-^{14}C]LAV	LAV W → F vs [*Leu*-^{14}C]-LAV	LAV vs [*Leu*-^{14}C]-LAV
25 μM	90 ± 3	74 ± 10	59 ± 2.5
50 μM	86 ± 5	59 ± 10	46 ± 1.5

[a] Amount of cell-bound label recoverable is expressed as percentage (see Figure 7).

centration levels (i.e., 1 and 2 times the concentration of [^{14}C]LAV).

As shown in Table II, at up to twice the concentration of [^{14}C]LAV, a concentration at which over 50% of the labeled peptide is displaced by autocompetition, the LAV W → V peptide has no significant competitive effect, the levels of [^{14}C]LAV recovered bound to the cells being 85–90% of that in the absence of competing cold peptide. The substitution of Val for Trp in the pentadecapeptide severely reduces binding to HeLa T4 cells. Where Phe was substituted for Trp, the ability to compete with the native LAV peptide was decreased but some displacement of the labeled peptide occurred, indicating that the W → F peptide retains weak binding ability. The effect of the W → V and W → F substitutions in these peptides on CD4 binding thus reflects that observed for these point mutations in the intact gp120 protein (Cordonnier et al., 1989), further substantiating the assumption that the peptide fragment maintains the conformational characteristics of the parent molecule in this region.

Discussion

The results of the CD investigation of conformation in the gp120 peptides allow two conclusions. First, a particular secondary structure is in fact conserved in this portion of the CD4-binding region despite differences in primary structure. This argues for the functional importance of the structural elements concerned with respect to receptor binding. Second, the site further conserves the ability to exist as a conformationally bistable system with an abrupt switch between β-sheet and α-helix occurring at a particular hydrophilicity to hydrophobicity ratio. This clean-cut and precisely balanced transition as the polarity of the medium is changed is highly unusual; no other case of such behavior in a peptide comes to mind. As this ability is also conserved throughout the widest range of primary sequence variability, it may also be considered to play an important role in the binding process.

When the results of the binding studies are compared with those from CD spectroscopy, it can be seen that a close relationship exists between the ability of the peptide examined to bind to CD4-expressing cells and their ability to exhibit the abrupt transition from a predominantly β-sheet to a predominantly α-helical form at a predetermined level of hydrophobicity. The two native peptides, LAV and Z3, possess the switch characteristics in full and are capable of binding to CD4-positive HeLa cells. Under the same conditions, the Gly-substituted peptide does not show significant binding: this peptide increases its helix content at much lower TFE concentrations than LAV or Z3 and never achieves the 50% helix content typical of state 2 in the native peptides. Similarly, the W → V substitution in the LAV peptide has the effect of completely eliminating the bistable flip and this peptide competes very poorly with native LAV for binding. Finally, the LAV W → F peptide shows intermediate characteristics in both respects: an abrupt increase in helicity at relatively low TFE concentrations is followed by a linear rise to 50% helix so that its helix content at 60–70% TFE, where native peptides have completed the transition, is only 35%. The ability of the W → F LAV peptide to compete with labeled LAV for the CD4 receptor is intermediate between that of native LAV and the W → V substituted peptide.

It has been suggested (Mutter & Hersperger, 1990) that in amphiphilic peptides the driving force governing the stabilization of β-sheet structure in aqueous solution may be self-association into a bilayer arrangement shaped by hydrophobic interactions. Gel filtration of the gp120-derived peptides (see Materials and Methods) showed, however, that they are monomeric under the conditions in which β-sheet structure is adopted (i.e., in aqueous buffer), even when the concentration is raised to levels considerably higher than those used for the CD measurements. The lack of self-association is understandable when one considers that while the LAV and Z3 peptides from a hydrophobic domain when coiled in helical form, there is no regular alternation of polar and apolar residues so that amphiphilicity is not a characteristic of the β-folded arrangement. Any self-association would therefore involve the burying of polar side chains, which would tend to offset the free energy contribution of the hydrophobic interactions.

The water at interfaces in biological systems, such as that trapped in the binding site of a membrane-bound receptor, has properties very different from those of bulk water and may be quite apolar in character (Kuntz & Kaufmann, 1974; Cooke & Kuntz, 1974). The solution conditions that foster the formation of state 1 (β-sheet) and state 2 (α-helix) therefore correspond roughly to the physical environment typical of the free and bound ligand. The emulation of apolar but aqueous conditions by an organic solvent such as TFE, while it may be a close approximation in some respects, is admittedly not a perfect simulation of conditions pertaining at the ligand/receptor interface. The extremely close correspondence, however, between the conformational behavior of the gp120-derived peptides in this solvent system and their biological activity in receptor binding assays argues for the physiological relevance of the phenomenon observed.

If, in fact, these two states represent the free and bound conformations, then the marked difference in the two has important implications for immunological approaches to control of HIV infection. In particular it may help to explain why anti-idiotopic antibodies to CD4 have had little success in blocking gp120 binding in vitro even though exhibiting a high affinity for anti-CD4 (Emmerich, 1989; Rieber et al.,

1989). Antibodies directed against epitopes exhibiting the conformation on the receptor may be unable to recognize the unbound form. Such a radical structural change on binding would also have consequences for antibodies directed against this area in the free virus.

It would be intellectually satisfying if one could find in one of the areas on the CD4 DI loop recently identified (Ryu et al. 1990) as important for gp120 binding a set of physical characteristics providing a match for the LAV and Z3 peptides in helical form. Unfortunately the region 41–59, which is most heavily implicated in HIV binding, does not appear to have an affinity with either side of the helix; there is no obvious hydrophobic locus (although Phe^{43} is solvent exposed), and the predominant charge is positive. A slightly better candidate is the 77–85 region, with its cluster of mutation-sensitive negative charges, but the connection of this area with the 41–59 domain is difficult to visualize, as the authors point out. It seems increasingly likely that the gp120/CD4 binding interaction is an extremely complex multidomain process in which each domain plays a defined but interconnected role.

The function of a specifically triggered conformational switch is naturally a key question. The recent paper by Montagnier's group (Cordonnier et al., 1989) contains a speculation relevant here. They postulated that the closeness of two critical mutation sites, one for CD4 binding at Trp^{411} and that affecting viral tropism at Ile^{404}, might mean that the region was involved in transmitting a conformational change in gp120 after it binds to the receptor. The bistable conformational flip we have observed in this region is well suited to function as such a signal and appears to be induced by a change in physical conditions similar to that which could occur on binding. A further interesting point is that the two mutation sites, Ile^{404} and Trp^{411}, are adjacent when the peptide is in helical form (Figure 2).

The initial theoretical prediction of helical folding in the Cys^{402}–Val^{414} segment encouraged the development of a tentative strategy to block gp120 binding by designing a molecule having a hydrophobic site of the same dimensions as that observed in the amphiphilic helix and containing in addition two negative charges so placed as to favor electrostatic interaction with Arg^{403} and Lys^{405}. Such a molecule should be specific for the corresponding area in all HIV variants while binding poorly, if at all, to the analogous region (negatively charged) in MHC II. In addition, it would be less sensitive to conservative side-chain substitutions than an antibody directed against this epitope in any one HIV strain. The discovery that the helical form may only occur after binding means that this strategy is probably impractical as originally conceived. (Designing a blocking molecule to the β-sheet form is more difficult but still a possibility once the steric specifications are more exactly known.) On the other hand, it is interesting to consider the consequences of specifically inducing the conformational flip from β-sheet to α-helix in a physiological solvent system. If an induced fit involving both ligand and receptor occurs on gp120 binding to CD4, the state I form may be necessary for initial contact, with state 2 binding poorly. Further, if a conformational change in this sequence is the signal to the virus that it is suitably bound, induction of the change in the absence of the receptor could act to abort the infective mechanism. These possibilities are under examination.

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Registry No. L-P-C-R-I-K-Q-F-I-N-M-W-Q-E-V, 132938-31-9; L-P-C-R-I-K-Q-V-V-R-T-W-W-G-V, 132938-32-0; L-P-C-R-I-K-Q-F-I-G-G-W-W-G-V, 132938-33-1; L-P-C-R-I-K-Q-F-I-N-M-V-Q-E-V, 132938-34-2; L-P-C-R-I-K-Q-F-I-N-M-F-Q-E-V, 132938-35-3.

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Skeletal Structure of Clathrin Triskelion in Solution: Experimental and Theoretical Approaches†

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ABSTRACT: The physicochemical properties of the clathrin triskelion were determined by dynamic and static light-scattering and sedimentation analyses in Tris and triethanolamine (TEA) buffers of about pH 8, in which the clathrin triskelion has been found to be in different conformational states by electron microscopy [Heuser, J., & Kirchhausen, T. (1985) *J. Ultrastruct. Res. 92*, 1-27]. Dynamic light-scattering measurements provided diffusion coefficients ($D^0_{20,w}$) of 1.22×10^{-7} and 1.23×10^{-7} cm^2/s, and ultracentifugal analysis gave sedimentation coefficients ($s^0_{20,w}$) of 8.39 and 8.32 S in Tris and TEA buffer, respectively. The average Stokes radius of the protein was determined to be 175 Å from its diffusion and sedimentation coefficients and its molecular weight. Static light-scattering analysis provided molecular weights of 6.58×10^5 and 6.41×10^5 and radii of gyration of 311 and 301 Å in the respective buffers. These results indicate that the clathrin triskelion has a similar conformation in the two buffers. For clarification of the skeletal structure of the clathrin triskelion in solution, the physicochemical parameters were calculated by using two models in which the clathrin arms are bent at various angles in a plane, on the basis of the Bloomfield approximation and a formula derived to estimate the radius of gyration of proteins consisting of various structural units. Values for the Stokes radius, diffusion and sedimentation coefficients, and radius of gyration in the ranges of 178-170 Å, $(1.20\text{-}1.26) \times 10^{-7}$ cm^2/s, 8.26-8.66 S, and 316-266 Å, respectively, were obtained with these models with the arms bent in the range of 0-60°. Comparison of these experimental and theoretical values suggests that, in weakly alkaline solution, the clathrin triskelion adopts a pinwheel-like structure with the arms more extended than in the polyhedral lattice, where the arms are bent at an angle of 60°.

In most cells, receptor-mediated endocytosis and intracellular protein transport are known to be mediated by coated pits and coated vesicles (Goldstein et al., 1985). These pits and vesicles are coated with a polyhedral lattice of a coat protein, clathrin. The assembly unit of this clathrin coat, termed a triskelion, has a molecular weight of 6.5×10^5 and forms a pinwheel-like structure consisting of three heavy and three light chains (Keen, 1985; Pearse & Crowther, 1987; Brodsky, 1988). Recently, the amino acid sequences of the heavy (Kirchhausen et al., 1987a) and light (Jackson et al., 1987; Kirchhausen et al., 1987b) chains have been deduced from the sequences of their cDNA clones.

The three arms of the clathrin triskelion are believed usually to be bent to some extent. However, electron-microscopic observations have indicated that in Tris buffer the arms are bent clockwise, whereas in triethanolamine (TEA)[1] buffer they are bent counterclockwise and are more extended (Heuser & Kirchhausen, 1985; Kirchhausen et al., 1986). Since an evaporation step is included in the procedure for preparation of samples for electron microscopy, the images may not reflect the "true" conformation of the clathrin triskelion in solution. Therefore, we attempted to determine the exact structure of the clathrin triskelion in solution. In the present study, we examined the physicochemical properties of the clathrin triskelion at about pH 8 in Tris and TEA buffers, mainly by a light-scattering technique, compared the experimental results with theoretical estimations, and found that the clathrin triskelion forms a pinwheel-like structure with rather extended arms and that its structure is the same in the two buffers.

Experimental Procedures

Clathrin Preparation. Clathrin was purified from crude coated vesicles of bovine brain as described previously

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[1] Abbreviations: TEA, triethanolamine; DTT, dithiothreitol.

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