Inhibitors of the Conformational Switch Involved in CD4 Binding by the *env* Glycoprotein gp120 from Human Immunodeficiency Virus Type 1 (HIV1) †

J. Reed* and V. Kinzel

Department of Pathochemistry, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg, Germany

Received March 4, 1994; Revised Manuscript Received June 27, 1994®

ABSTRACT: A 15-residue fragment within the major continuous domain of gp120 from HIV1 that can bind independently to the CD4 receptor conserves the property of behaving as a polarity-triggered conformational switch despite displaying over 50% variability between strains. As this switch behavior (the ability to flip abruptly from β-sheet to α-helix as the medium polarity is lowered past a critical point) is closely linked to CD4-binding ability, it presents a potential strain-independent target for intervention. A number of compounds have been tested for their ability to function as switch inhibitors. All those that displayed switch inhibitory activity also have been shown to act to prevent CD4 binding and/or viral infectivity. In addition, all compounds testing positive as switch inhibitors have certain chemical characteristics in common. The groundwork has thus been established for the design of strain-independent blockers of CD4 binding based on the strategy of switch inhibition.

The binding of the *env* glycoprotein gp120 of human immunodeficiency virus type 1 to the T-cell receptor CD4 constitutes the first and crucial step in the infective process for most cells and, as such, a logical point at which to prevent it. The immunological approach to this problem has been complicated by the extreme variability of gp120 among viral strains (Hahn et al., 1985; Wong-Staal et al., 1985). Even the major continuous domain of gp120 involved in CD4 binding (Laskey et al., 1987) (and the only one capable of binding as an isolated fragment) displays 50% variability despite the necessity for maintaining an invariable function. This laboratory has previously shown that a 15-residue segment of this domain of gp120 conserves the ability to adopt a particular secondary structure *despite its sequence variability* (Reed & Kinzel, 1991). More importantly, this region conserves the ability to flip abruptly and concertedly from β-sheet to α-helix as the medium polarity is decreased past a certain critical level. Recently, primary structure elements responsible for this conformational switch have been reported (Reed & Kinzel, 1993). Amino acid substitutions that disrupt the conformational switch also destroy the ability of the 15-residue peptides to bind to CD4, and it was concluded that the ability to flip from β-sheet to α-helix on moving from a more polar (serum) to a less polar (membrane receptor) environment is a characteristic necessary to the docking process that is preserved throughout the widest sequence variability of viral strains (Reed & Kinzel, 1991). It thus presents a target for strain-insensitive inhibition of gp120 binding to CD4; such an inhibitor would constitute a valuable addition to the arsenal of anti-HIV strategies.

Although disrupting the switch behavior by intrinsic alterations (i.e., amino acid substitutions) is an effective means of inhibiting CD4 binding *in vitro*, this approach is not particularly feasible for dealing with the natural course of HIV1 infection. We have therefore initiated a search for exogenous substances capable of inhibiting the conformational switch. There are two possible ways of carrying out such a search. On the one hand, computer-assisted molecular modeling could be used to identify the parameters of a molecule with—theoretically—ideal binding properties to the strain-averaged conformational surface. On the other hand, one could take an empirical approach by finding a number of exogenous inhibitors and determining what they have in common, subsequently using quantitative structure/activity relationships (QSAR) to refine the design. This paper describes results from following the second approach. We have begun by testing a series of substances known to prevent viral infection but whose mode of action was not fully explained in order to determine, first, if they are capable of inhibiting the conformational switch and, second, what chemical characteristics the proven switch inhibitors have in common. The results show that it is possible to disrupt the switch mechanism by administering exogenous compounds, that those compounds that are effective share a number of chemical properties, and that it is possible to produce nonpeptide substances with these properties that function simultaneously as switch inhibitors and inhibitors of CD4 binding. These experiments have established a basis that may lead to the design of a maximally effective, strain-independent inhibitor of gp120 binding to CD4.

MATERIALS AND METHODS

Peptides and Inhibitors. The 15-residue switch domain of gp120 from HIV1 strain LAV (LPCRIKQFINMWQEV) and the dibenzylated 12-residue CD4 fragment [TYIC(bzl)E-(bzl)VEDQKEE] were obtained from Bachem Biochemica GmbH, Heidelberg. The nonbenzylated 12-residue CD4 fragment was kindly donated by Prof. Dr. R. Braun of Bayer AG, Leverkusen. CPF dipeptides (*N*-carbomethoxycarbonylprolylphenylalanyl benzyl ester and *N*-carboethoxycarbonylprolylphenylalanyl benzyl ester) in both the D,D and L,L forms and the BM 50.0311 derivatives were the generous gift of Boehringer, Mannheim. All peptides were >95% pure as tested by HPLC and FAB, and delivered with data on peptide content determined by amino acid analysis. Peptide solutions of a given concentration were prepared by weighing adjusted appropriately on the basis of these data. CPF peptides were dissolved as a concentrated solution (2 mg/125 μL) in TFE,

† This work was supported in part by a grant from the Bundesgesundheitsamt.

* To whom correspondence should be addressed.

® Abstract published in *Advance ACS Abstracts,* August 15, 1994.

0006-2960/94/0433-10993$04.50/0

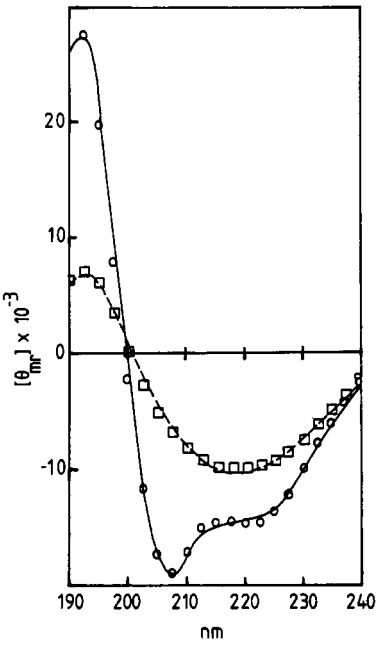

FIGURE 1: Sample CD spectra and fits. A 100 μg/mL sample of the 15-residue "switch" domain from HIV strain LAV in the presence of 10 μg/mL (350 μM) BM 50.0311 measured in 10 mM Tris·HCl, pH 7.5 (– –), and in 100% TFE (—) (□) fit to the spectrum in 10 mM Tris (55% β-sheet, 35% coil, 10% turn) and (○) fit to the spectrum in 100% TFE (50% α-helix, 40% coil, 10% turn).

and the BM 50:0311 derivatives (1 mg/50 μL) in 2 N NaOH, after which they could be brought into aqueous solution.

Circular Dichroism. Far-UV (190–240 nm) circular dichroism spectra were obtained using a Jasco J-500 automatic recording spectral polarimeter coupled to a J-DPY digital data processor. Calibration was carried out using 0.05% β-androsterone in spectral-grade dioxane as a standard. The slit width was automatically adjusted to maintain a spectral resolution of 1.0 nm throughout the wavelength range.

Samples were measured in a 1 mm dichroically neutral quartz cuvette at a sensitivity of 2.0 mdeg/cm, a time constant of 2.0 s, and a scan speed of 5.0 nm/min. Spectra presented are a 4-fold signal average with the identically measured and signal-averaged base line subtracted, converted from millidegrees to mean residue ellipticity, $[\theta_{mr}]$, for use in curve fitting and secondary structure analysis.

Curves were fitted as the linear sum of the CD spectra of model peptides in α-helix, β-sheet, extended coil, and reverse turn form (Yang et al., 1986). Model spectra for the first three structure types were an average of model peptide curves from the literature (Yang et al., 1986; Holzworth & Doty, 1965; Quadrifoglio & Urry, 1968; Greenfield & Fasman, 1969; Adler et al., 1973; Brahms & Brahms, 1980) weighted according to the frequency of the respective side chains in proteins in general. Reverse turn model spectra were taken from Crisma et al. (1984). An example of representative fits to the CD spectra at each extreme of the titration is given in Figure 1.

Switch Inhibition Test. The 15-residue LAV peptide was used as a standard for testing the ability of materials to inhibit the conformational switch. Generally, the secondary structure content of a 100 μg/mL solution of the LAV peptide at decreasing levels of polarity (titration with trifluoroethanol) was contrasted with its secondary structure content under the same range of polarity conditions in the presence of a putative inhibitor. Due to differences in the intrinsic dichroism of the inhibitor compounds used, the strategy of base line correction differed for the three classes tested.

(A) BM 50.0311 Derivatives. These substances were found to have no intrinsic dichroism in the 190–240 nm region when measured at a concentration of 360 mM in both Tris·HCl, pH 7.5, and 100% TFE (data not shown). No special correction was therefore necessary, and the two sets of curves (LAV peptide with and without inhibitor) can be directly compared.

(B) CD4 Fragments. Since tests with these involve a mixture of two peptides, it is not possible directly to isolate the secondary structure changes pertaining to the LAV 15mer alone. Accordingly, the secondary structure content of the pure CD4 peptide was calculated at the various TFE concentrations employed, and the results were used to construct a theoretical secondary structure vs TFE curve for the two peptides (LAV and CD4) combined at the ratio used in the test (2 CD4:1 LAV) were there to be no interaction between them. This was then compared to a similar plot for the physically mixed sample.

(C) CPF Dipeptides. As the substantial nonpeptide contribution to the far-UV CD spectrum in these compounds made curve fitting from simple model peptides inappropriate, a base line of the CPF dipeptide alone was subtracted from the CD spectrum of LAV + CPF at each TFE concentration used before curve fitting was carried out.

Dose–Response Relationship. The maximum slope of the β-sheet to α-helix transition as a function of medium polarity provides a quantifiable parameter through which the degree of switch inhibition can be determined. Three ways of expressing this are presented, one of which (c) is independent of the curve-fitting procedure. The maximum slope of either (a) α-helix increment, (b) β-sheet loss, or (c) the 205 nm ellipticity minimum plotted against TFE concentration for the isolated LAV peptide was defined as a standard switch or 100%. The corresponding maximum slopes in the presence of varying amounts of inhibitor were then plotted in each case as a percent of this standard.

Inhibition of LAV Peptide Binding to CD4. CD4-binding tests were carried out as described in Reed and Kinzel (1991). Briefly, HeLa T4 cells (NIH) were grown to a density of ca. 200 000 in 16 mm miniwells. The cells were chilled, washed twice with 1 mL of cold PBS, and incubated with 300 μL of 25 μM ^{14}C-labeled peptide in PBS with or without 40 μM, 400 μM, or 4 mM methyl- or ethyl-BM 50.0311 for 60 min at 4 °C. The cultures were then washed twice more with 1 mL of PBS before being lysed with 200 μL of 2% SDS in PBS. The lysate was placed in counting vials with 3 mL of Quickzint and the cell-bound radioactivity measured.

RESULTS

CPF Dipeptides. This group of peptides has been reported to be effective in preventing HIV1 infection of H9 tumor cells and human T cells (Finberg et al., 1990). Although originally chosen to mimic a critical area (Phe^{43}) on the CD4 receptor, the authors concluded that a close homology to CD4 appeared not to be connected with the activity of these compounds. Indirect evidence, however, implied that the active dipeptides bound to gp120 and remained tightly bound.

Figure 2 shows the 4 CPF derivatives tested and their effect on the behavior of the 15-residue "switch" domain within the CD4-binding region of gp120 from HIV1 strain LAV (Figure 2A). Both the D,D and L,L stereoisomers of the base form of the CPF dipeptide (*N*-carbomethoxycarbonylprolylphenylalanyl benzyl ester) were effective in eliminating the cooperativity of the β-sheet to α-helix transition in the LAV peptide occurring at 60% TFE (Figure 2B,C). In the presence of a concentration of these two derivatives at which CPF (D,D)

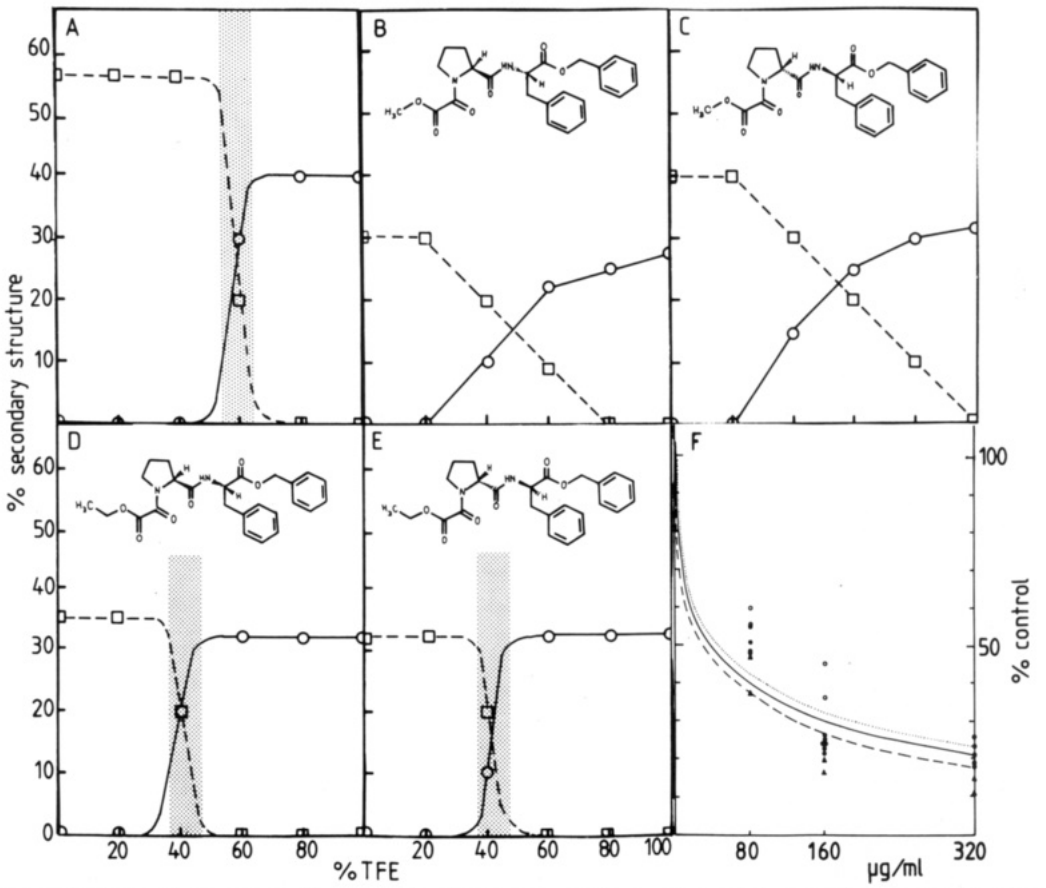

FIGURE 2: Effect of CPF dipeptides on the conformational switch domain of the *env* glycoprotein gp120 from HIV. The standard for testing was a 100 μg/mL aliquot of the 15-residue "switch" region (LPCRIKQFINMWQEV) from the CD4-binding domain of the *env* glycoprotein from HIV strain LAV, titrated from 10 mM Tris·HCl, pH 7.5, to 100% trifluoroethanol (TFE). (TFE concentrations are displayed on the *x*-axis for panels A–E.) Secondary structure estimates were carried out as described under Materials and Methods. For clarity, only α-helix (○) and β-sheet (□) contents are shown. (A) LAV peptide alone, i.e., undisturbed conformational switch. (B) LAV peptide plus 160 μg/mL *N*-carbomethoxycarbonylprolylphenylalanyl benzyl ester (L,L). (C) LAV peptide plus 160 μg/mL *N*-carbomethoxycarbonylprolylphenylalanyl benzyl ester (D,D). (D) LAV peptide plus 160 μg/mL *N*-carboethoxycarbonylprolylphenylalanyl benzyl ester (L,L). (E) LAV peptide plus 160 μg/mL *N*-carboethoxycarbonylprolylphenylalanyl benzyl ester (D,D). (F) Dose dependence of switch inhibition. Inhibitory potency was tested as above using 0, 80, 160, and 320 μg/mL *N*-carbomethoxycarbonylprolylphenylalanyl benzyl ester (D,D). The degree of switch inhibition was measured in three ways. (●) Percent of the maximum slope of the α-helix increment of LAV peptide with increasing [TFE]; (△) percent of the maximum negative slope of β-sheet loss with increasing [TFE]; (○) percent of the maximum slope of the 205 nm ellipticity minimum with increasing [TFE]. (Note that the last is a fitting-independent parameter.) Data were best fit by an exponential of the form Ae^{Bx} (—) fit to the data points (●) $r = 0.965$, (– –) fit to the data points (△) r = 0.991, and (· · ·) fit to the data points (○) $r = 0.969$.

totally prevents HIV1 infection of H9 cells and human T cells (Finberg et al., 1990), the change in secondary structure of the LAV peptide as a function of TFE concentration approaches the linear behavior seen with peptides in general, a phenomenon associated with loss of CD4-binding ability (Reed & Kinzel, 1991). The switch inhibition is a concentration-dependent phenomenon (Figure 2), with the half-maximal concentration (30–40 μg/mL) corresponding relatively closely to that required for maximal inhibition of viral infection (80–160 μg/mL) (Finberg et al., 1990).

Those CPF derivatives with anything larger than a methyl group at the *N*-terminus were found to be ineffective in preventing viral infection (Finberg et al., 1990). The D,D and L,L stereoisomers of the CPF in which an ethyl group has been substituted for the methyl group at the carbomethoxycarbonyl N-terminal moiety are both inactive as switch inhibitors (Figure 2D,E). There is still a total $\beta \rightarrow \alpha$ conversion within a sharply defined region of the polarity gradient, although the critical polarity at which it occurs is changed slightly in the presence of these compounds. This particular modification was not tested in the original study by Finberg et al.; however, in contrast to the base-form CPFs, the ethyl-substituted forms are inactive in preventing binding of sCD4 to an immobilized gp120 matrix.[1] There is thus a direct correlation in both the positive and the negative sense between the ability of the CPF dipeptides to prevent viral infection/gp120–CD4 binding and their function as "switch" inhibitors.

Benzylated CD4 Peptides. A benzylated peptide derivative comprising residues 83–94 of CD4 has been shown to prevent HIV1-induced cell fusion and infection (Nara et al., 1989; Lifson et al., 1988) through direct inhibition of viral entry. The nonderivatized peptide CD4(83–94) is inactive, and it was postulated that the function of the benzyl groups was to alter the conformation of the CD4 fragment so as to mimic the native form of this region in the intact protein. The far-UV CD spectra of CD4(83–94) and CD4(83-Cys^{86}bzl-Glu^{87}-bzl-94) under conditions of the highest (10 mM Tris·HCl, pH 7.5) and lowest (100% TFE) solvent polarities employed in the LAV switch assay are shown in Figure 3D. Derivatization causes no change in the time-averaged peptide backbone conformation at either extreme. The basis of the antisyncytial activity of the benzylated CD4 peptide alone thus cannot be due to its ability to approximate more nearly the native stereochemistry of the CD4 receptor.

[1] Dr. B. König, Boehringer Mannheim, Werk Penzberg; personal communication.

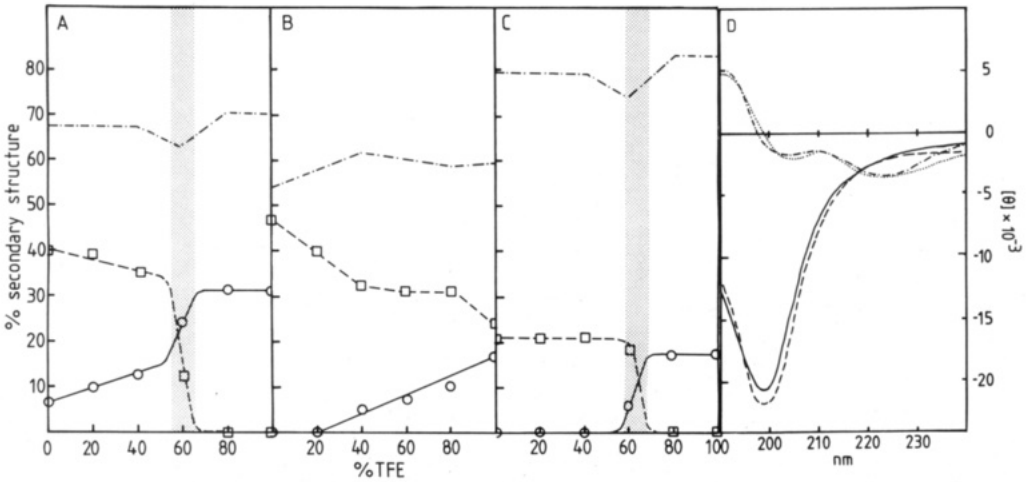

FIGURE 3: Structural comparison and effect of CD4(83–94) and CD4(83-Cys^{86}bzl-Glu^{87}bzl-94) on the conformational switch. (A) Secondary structure content as a function of polarity for a 2:1 ratio of the independent spectra of the CD4(83-Cys^{86}bzl-Glu^{87}bzl-94) peptide and the LAV peptide. (B) Secondary structure content as a function of polarity for an actual mixture of 100 μg/mL LAV peptide and 200 μg/mL (125 μM) dibenzylated CD4 peptide. (C) As in (B), but using 125 μM nonbenzylated CD4 peptide. (○) α-Helix; (□) β-sheet; (– · –) extended coil plus reverse turn. (D) Far-UV spectra of 100 μg/mL CD4(83–94) in 10 mM Tris·HCl, pH 7.5 (—), and in 100% TFE (– · –) and of 100 μg/mL CD4(83-Cys^{86}bzl-Glu^{87}bzl-94) in 10 mM Tris·HCl, pH 7.5 (– –), and in 100% TFE (· · ·).

Figure 3 shows the secondary structure content of the LAV peptide as a function of TFE concentration in the presence of 125 μM CD4(83–94) (3C) and CD4(83-Cys^{86}bzl-Glu^{87}-bzl-94) (3B). The effect of the CD4 peptides can be determined by comparing these with an algebraic sum of the independent secondary structures of the two peptides under TFE titration (3A: see Materials and Methods). CD4(83–94) has little effect, and the amount of β-sheet involved in an abrupt transition to α-helix at 60% TFE is roughly what one would expect where the LAV peptide constitutes only one-third of the protein present. In the presence of 125 μM benzylated CD4, however, the sharp $\beta \rightarrow \alpha$ transition is abolished, and β-sheet structure is preserved even at high TFE levels. A similar effect is seen with 15-residue peptides containing the switch region from gp120 of HIV1 strain ELI (data not shown), with an apparant stabilization of β-sheet structure so that full conversion to α-helix has not occurred even at >80% TFE. Here again there is a direct correlation between the ability of a substance to block gp120 binding to CD4 *in vivo* and its effectiveness as an exogenous switch inhibitor.

BM 50.0311 Derivatives. A drawback to the use of peptides or proteins as therapeutic agents is their short half-life *in vivo*. For this reason, a set of organic molecules that shared certain characteristics with the CPF peptides and dibenzyl-CD4 was tested to see whether switch inhibition could be effected by a nonproteinaceous agent. Figure 4 shows the effect of two closely related compounds on the $\beta \rightarrow \alpha$ transition of the LAV peptide. The substance in Figure 4A, with a methyl group attached to the urea moiety, totally abolished switch behavior at a concentration of 360 μM. The derivative with an ethyl group at this position (Figure 4B) had no significant effect on either the cooperativity of the $\beta \rightarrow \alpha$ transition or the critical point of solvent polarity at which it occurred. It is therefore possible to inhibit switch behavior in the CD4-binding region of gp120 by exogenous application of substances not subject to proteolytic degradation; further, this inhibition appears to be quite specific in its requirements, as apparently minor modifications (methyl → ethyl) can radically affect the inhibitory potential.

The activity of the BM 50.0311 derivatives as switch inhibitors is reflected in their behavior in the CD4-binding test. The LAV peptide binds specifically to CD4-expressing HeLa cells (Reed & Kinzel, 1991). In the presence of the methyl derivative, this binding is inhibited (Figure 5) with an apparant LD_{50} of 250 μM. The ethyl derivative, on the other hand, is very much less effective at inhibiting LAV peptide binding to CD4+ cells. (The apparant LD_{50} would be in the molar range; in practice, a nonspecific inhibition dominates as one exceeds the low millimolar level.) It has thus been possible to discover a novel substance capable of inhibiting binding to CD4 strictly on the basis of its properties as a switch inhibitor.

DISCUSSION

In searching for an exogenous inhibitor of the "switch" function of gp120, we began by examining certain known inhibitors of HIV1 infection whose basis of action was not entirely clear with the idea that these might in fact be working by preventing the $\beta \rightarrow \alpha$ switch transition. Although both the CPF dipeptides and the benzylated CD4(83–94) peptide were originally tested because of their potential as CD4 mimics, further investigation showed that this was unconnected with their function. In the case of the CPF dipeptides, the initial workers found that the various stereoisomers showed nearly identical activity, an effect incompatible with their structure being analogous to that of the native protein. Further, attempts to extend the homology by adding a Leu C-terminal to the Phe residue actually eliminated activity. In the case of the benzylated CD4(83–94), the original hypothesis—that the benzyl groups enforced a more nearly native conformation on the isolated peptide—is untenable in view of the virtually identical far-UV CD spectra of the benzylated and nonbenzylated peptides. On the other hand, both the CPF dipeptides and benzylated CD4(83–94) are successful as "switch" inhibitors. As a direct connection between elimination of the "switch" phenomenon and loss of CD4-binding ability has been established using residue substitution in the critical area (Reed & Kinzel, 1991; Cordonnier et al., 1989), it is reasonable to conclude that the actual basis of infectivity inhibition for the CPF dipeptides and dibenzylated CD4(83–94) is due to this second property.

It is possible to identify at least two chemical characteristics shared by the effective switch inhibitors that are potentially responsible for their function. The presence of a minimum of two six-membered rings—often but not always aromatic—

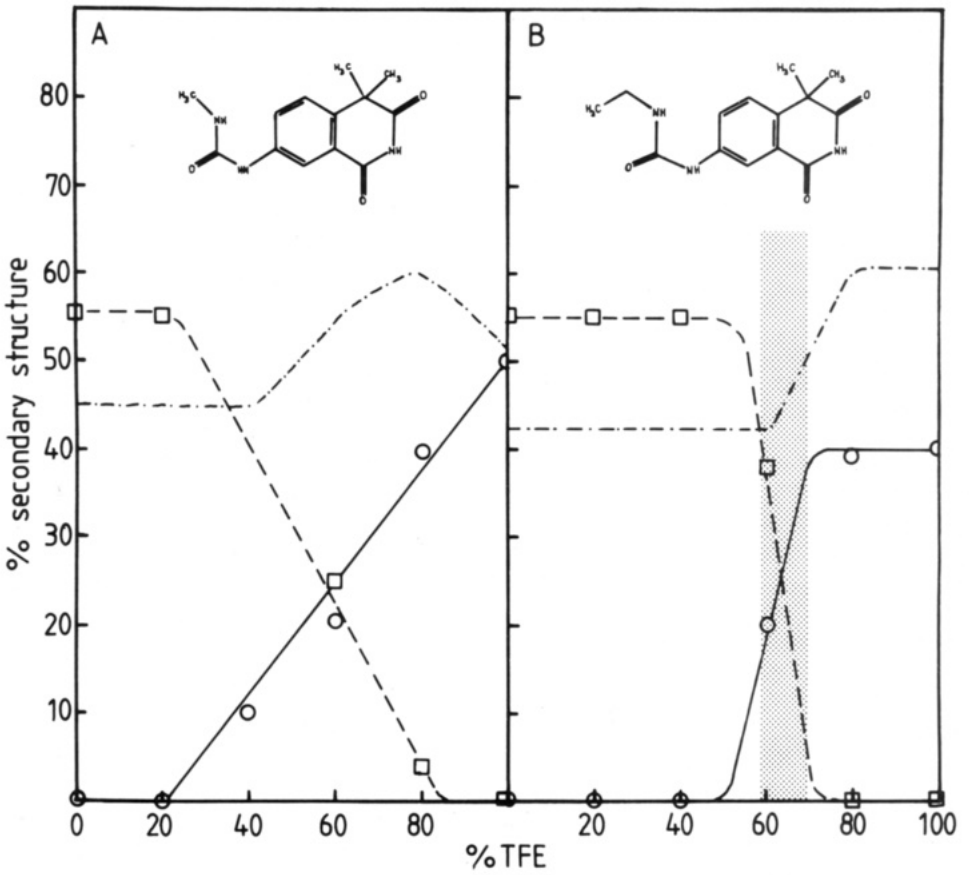

FIGURE 4: Effect of BM 50.0311 derivatives on the conformational switch. 100 μg/mL LAV peptide was TFE-titrated in the presence of 10 μg/mL (350 μM) compound. (A) methyl derivative; (B) ethyl derivative. Key as in Figure 2.

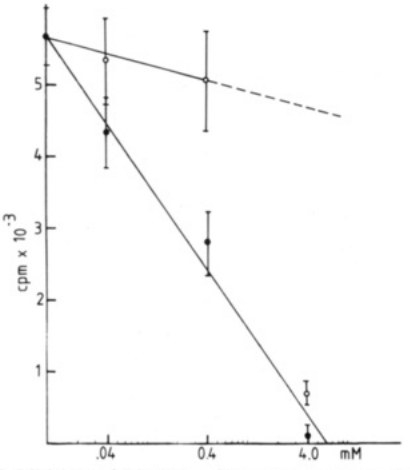

FIGURE 5: Inhibition of binding of LAV peptide to CD4 by BM 50.0311 derivatives. The amount of ^{14}C-LAV peptide binding to CD4-expressing HeLa cells was measured in the absence and in the presence of increasing amounts of BH 50.0311. Values given are the average of three independent experiments plotted on a semilogarithmic scale. (●) Values for methyl derivative. (○) Values for ethyl derivative.

within a certain distance of one another is absolutely necessary. Finberg et al. established that both the presence and the relative position of the Phe and the C-terminal benzyl ester contributed significantly to the antiviral activity of CPFs. The same pattern is seen with CD4(83–94) where the non- and the mono-benzylated forms are inactive in preventing syncytium formation (Lifson et al., 1988), and addition of a second benzyl group at a residue other than that C-terminal to the Cys^{86} bzl also produces an inactive substance. A second common factor seems to be the presence of potential negative charges in a resonance form of the carbonyl groups. This property is shared by BM 50:0311, a substance that bears out the importance of both factors since it is devoid of peptide bonds and does not in any sense mimic CD4, but does contain the critical ring and carbonyl groups.

While these two factors—six-membered rings at an appropriate distance and a certain number of carbonyl groups—may be tentatively identified as necessary to the function of an ideal gp120 switch inhibitor, it is clear that they are by no means sufficient. This is shown by the lack of activity in both the CPF dipeptides and BM 50.0311 when a relatively minor modification, the substitution of an ethyl for a methyl moiety, is made. The linkage of the methyl group is chemically rather similar in these molecules, being through a carbonyl ester in the CPFs and a ureate in the BM 50.0311. There is a suggestion that the presence and placement of the methyl group may be important to CPF activity in the work of Finberg et al., since replacement of the carbomethoxycarbonyl group with a *tert*-butoxycarbonyl abolished activity (as did changing the position of the carbomethoxycarbonyl with respect to the aromatic rings). Our data on switch inhibition by CPFs and BM 50.0311 indicate that the most conservative possible substitution, ethyl for methyl, results in a loss of activity. There seems therefore to be a fairly sensitive requirement for an aliphatic moiety no larger than a methyl group attached via a ureate or carbonyl ester linkage at a particular distance from the required ring elements. This requirement may be connected to the fact that methyl (but not ethyl) groups are capable of exerting nonbonded resonance.

As more switch inhibitors are identified, it may be possible to determine the basis of their activity. Two of the requirements so far identified are suggestive, in that a property of the switch region of gp120 is the presence of five hydrophobic

residues, all either totally or functionally conserved, at positions 404, 407, 408, 411, and 414: in fact, so placed as to form a continuous hydrophobic region when the protein adopts helical form. Two positively charged residues are conserved at positions 403 and 405, which are at opposite sides of the helical axis. The physical basis of abrupt, cooperative helical folding once the critical polarity threshold is reached is not known. It is, however, reasonable to think that a molecule with the potential for ideally placed hydrophobic and electrostatic interactions with amino acid side chains might disrupt this cooperativity. We intend to test an array of derivatives of BM 50.0311 to refine our definition of the requirements contributing to effective inhibition. A form of QSAR (quantitative structure/activity relationships) can then be used to design a rough template of the working inhibitor. This will be additionally improved using molecular modeling of inhibitor/peptide binding to optimize such parameters as, for example, the ideal separation of the aromatic rings and their positioning with respect to negative charges. Further derivatives can then be designed on this basis.

In order to be a feasible choice for eventual therapeutic use, an inhibitor of this type should have an ID_{50} at the nanomolar level. As soon as switch inhibitors within a reasonable distance of this goal are developed (ID_{50} in the low micromolar region), they will be tested for their ability to prevent viral infectivity in cell culture systems. As the switch characteristics of this region are conserved throughout the widest range of variability in the *env* glycoprotein gp120, and are a requirement for CD4 binding, the characterization and development of a maximally effective inhibitor are of more than theoretical interest.

ACKNOWLEDGMENT

We thank Dr. A. Mertens of Boehringer, Mannheim, for his generosity in furnishing many of the compounds and derivatives tested, and Dr. Thomas A. Reed for helpful discussions on the organic chemistry involved.

REFERENCES

Adler, A. J., Greenfield, N. J., & Fasman, G. D. (1973) *Methods Enzymol. 27*, 675–735.

Brahms, S., & Brahms, J. (1980) *J. Mol. Biol. 138*, 149–178.

Cordonnier, A., Montagnier, L., & Emerman, H. (1989) *Nature 340*, 571–574.

Crisma, M., Fasman, G. D., Balaram, H., & Balaram, P. (1984) *Int. J. Pept. Protein Res. 23*, 411–419.

Finberg, R. W., Diamond, D. C., Mitchell, D. B., Rosenstein, Y., Soman, G., Norman, T. C., Schreiber, S. L., & Burakoff, S. J. (1990) *Science 249*, 287–291.

Greenfield, N., & Fasman, G. D. (1969) *Biochemistry 8*, 4108–4116.

Hahn, B. H., Gonda, M. A., Shaw, G. M., Popovic, M., Hoxie, J., Gallo, R. C., & Wong-Staal, F. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4813–4817.

Holzwarth, G., & Doty, P. (1965) *J. Am. Chem. Soc. 87*, 218–228.

Lasky, L. A., Nakamura, G., Smith, D. H., Fennie, C., Shimasaki, C., Patzer, D., Berman, P., Gregory, T., & Capon, D. J. (1987) *Cell 50*, 975–985.

Lifson, J. D., Hwang, K. M., Nara, P. L., Fraser, B., Padgett, M., Dunlop, N. M., & Eiden, L. E. (1988) *Science 241*, 712–716.

Nara, P. L., Hwang, K. M., Rausch, D. M., Lifson, J. D., & Eiden, L. E. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 7139–7143.

Quadrifoglio, F., & Urry, D. W. (1968) *J. Am. Chem. Soc. 90*, 2755–2765.

Reed, J., & Kinzel, V. (1991) *Biochemistry 30*, 4521–4528.

Reed, J., & Kinzel, V. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 6761–6765.

Wong-Staal, F., Shaw, G. M., Hahn, B. H., Salahuddin, S. Z., Popovic, M., Markham, P. D., Redfield, R., & Gallo, R. C. (1985) *Science 229*, 759–762.

Yang, J. T., Wu, C.-S., & Martinez, H. M. (1986) *Methods Enzymol. 130*, 208–269.