Characterization of the Calmodulin Binding Domain of SIV Transmembrane Glycoprotein by NMR and CD Spectroscopy†

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ABSTRACT: Recent experimental evidence has shown that the C-terminal peptide of the HIV/SIV transmembrane glycoprotein 41 (gp41) can bind very tightly to calmodulin (CaM). These findings imply a potential mechanism for HIV/SIV cytopathogenesis, which involves the uncoupling of some critical cellular signal transduction pathways that are normally mediated by CaM. Here, we present circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy studies of a 28-residue synthetic peptide, SIV-L, corresponding to the C-terminal portion of the SIV transmembrane glycoprotein gp41. CD studies recorded in aqueous solution show a dramatic increase in the amount of α-helical structure of the SIV-L peptide upon binding to calcium−CaM. Two-dimensional NMR experiments were performed to determine the secondary structure of the peptide in 25% aqueous trifluoroethanol solution. In this α-helix inducing solvent, the observed nuclear Overhauser effects, as well as the α^1H and α^{13}C chemical shift changes, demonstrate that a continuous α-helix is formed from W3 to L28, although there is some distortion around P17. This result is in accordance with those obtained for many other CaM-binding peptides. Subsequent one-dimensional NMR titration experiments of calcium−CaM and the SIV-L peptide suggest that the peptide can bind to CaM with a 1:1 stoichiometry and that the peptide binding involves both the N- and C-lobe of CaM. However, gel mobility shift assays suggest that the peptide CaM interaction may be more complicated, as oligomeric forms of CaM and the SIV-L peptide were found. These studies provide a potential molecular basis for HIV/SIV cytopathogenesis.

Simian immunodeficiency virus (SIV)[1] is the monkey homolog of the human immunodeficiency virus (HIV). When SIV infects the mammalian cells, it causes symptoms similar to HIV, such as virus-induced cell fusion followed by killing of the cell (Garry, 1989). The viral envelope glycoprotein gp160 is responsible for many of the biological and pathogenic properties of the SIV virion (Mckeating & Willey, 1989). Glycoprotein gp160 is synthesized as a single polypeptide chain; it is subsequently cleaved into two parts: a surface glycoprotein gp120 and a transmembrane (TM) glycoprotein gp41, which interact with each other in a noncovalent manner. Glycoprotein gp120 is critical to HIV/SIV attachment to the host cell CD4 receptor, while gp41 plays an important role in the fusion process and the following cytopathogenesis (Mckeating & Willey, 1989).

Recently, it has been shown that a synthetic peptide from the C-terminal portion of HIV or SIV gp41 binds to the calcium form of calmodulin (Ca^{2+}−CaM) with nanomolar affinity and can inhibit the CaM-dependent process of phospholipid synthesis *in vitro* (Miller et al., 1993). Similar findings have been reported by Srinivas et al. (1993). The C-terminal region in gp41 is remarkably conserved in different HIV-1 isolates, as well as in HIV-2 and SIV. The common feature of this region is that it has the potential to form positively charged amphiphilic helices, which is a characteristic motif for the CaM-binding sites of CaM-binding proteins and enzymes (O'Neil & DeGrado, 1990). Such an interaction suggests a novel mechanism for the cytopathogenesis of the HIV/SIV infection, i.e., affecting CaM-mediated signal transduction pathways (Miller et al., 1993; Srinivas et al., 1993). CaM is the most important mediator protein for the secondary messenger calcium in eukaryotic cells (Means et al., 1991; Vogel, 1994; Clapham, 1995; James et al., 1995). Depending on the cell type, it regulates up to 30 different enzymes, e.g., phosphodiesterase, myosin light chain kinase (MLCK), calcineurin, and nitric oxide synthase, etc. Because CaM is involved in so many signal transduction pathways in the cell, it is likely that the interruption of CaM regulatory functions would lead to cellular disregulation and subsequent cytopathogenesis. It has also been shown that Ca^{2+} and CaM are critical to the correct functioning of the eukaryotic cell cycle and that the deletion of the CaM gene is generally lethal to the cell (Lu & Means, 1993). Thus, it seems that the inhibition of CaM's function could at least partially contribute to T-cell anergy and cell death, two obvious phenomenon that occur when the host cells are infected by the HIV/SIV virus.

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[1] Abbreviations: 1D, one dimensional; 2D, two dimensional; CaM, calmodulin; Ca^{2+}−CaM, calcium−calmodulin; CD, circular dichroism spectroscopy; COSY, homonuclear correlated spectroscopy; DQF, double-quantum-filtered; HIV, human immunodeficiency virus; HMQC, heteronuclear multiple quantum coherence; HPLC, high pressure liquid chromatography; MLCK, myosin light chain kinase; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser enhancement spectroscopy; SIV, simian immunodeficiency virus; TFE, trifluoroethanol; TOCSY, total correlated spectroscopy.

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Calcium-saturated CaM has an unusual dumbbell-shaped structure with two domains connected by a seven-turn central α-helix (Babu et al., 1988). Each domain contains two characteristic helix−loop−helix calcium binding motifs (Strynadka & James, 1989). Upon increased intracellular Ca^{2+} ($10^{-7} \rightarrow 10^{-6}$ M), CaM will bind four Ca^{2+} and experience a substantial conformational change, exposing a hydrophobic patch on each domain that is the target for the substrate binding. Because of the large size and the instability of most CaM-binding proteins, it has been customary to study synthetic peptides of about 20 amino acid residues which comprise the entire CaM-binding domains of the target proteins (O'Neil & DeGrado, 1990). The CaM-binding peptides usually possess the same high affinity for CaM as the holoenzyme. Consequently peptides have been used extensively as models for the study of the interaction between CaM and CaM-binding proteins (Ikura & Bax, 1992; Ikura et al., 1992; Roth et al., 1991, 1992; Meador et al., 1992, 1993; Zhang & Vogel, 1994a,b; Zhang et al., 1993, 1994a,b; for reviews see Vogel, 1994; James et al., 1995; Vogel & Zhang, 1995).

Among the various forms of spectroscopy, NMR is the most powerful for the study of the CaM-binding peptides and their interaction with CaM, because it allows an investigation of residue-specific interactions (Vogel, 1994; Vogel & Zhang, 1995). In this case, the use of NMR is based primarily on the internuclear nuclear Overhauser effect (NOE) to assign the spectra and determine the peptide secondary structure in small proteins or peptides (Wüthrich, 1986). However, sometimes the peptide of interest has no obvious stable secondary structure in aqueous solution, making it difficult to assign the spectra and get useful information. In this situation, we have found that aqueous trifluoroethanol (TFE) mixtures may be helpful to induce the α-helical secondary structure that corresponds to the CaM-bound form (Zhang & Vogel, 1994b; Zhang et al., 1993). TFE has been used extensively to induce α-helices in studies of all kinds of peptides; see, for example, Nelson and Kallenbach (1989), and Sönnichsen et al. (1992). On the other hand, TFE can also stabilize β-hairpin structures in peptides, although only a few examples have been reported (Toniolo et al., 1979; Blanco et al., 1994). It is still not clear what mechanism underlies the induction of the secondary structure by TFE; however, it is generally agreed that the dominant factor is the propensity of a given amino acid sequence in the peptide to form a specific secondary structure.

Here, we have studied a synthetic peptide (SIV-L) from the C-terminal region of the SIV gp41 corresponding to amino acid residues 852−879 in the SIV transmembrane glycoprotein (Miller et al., 1993). We have studied the SIV-L peptide by CD and NMR spectroscopy and determined its secondary structure in 25% TFE aqueous solution by NMR. We also investigated the interaction between Ca^{2+}−CaM and the SIV-L peptide by NMR, tryptophan fluorescence, and gel mobility shift electrophoresis methods.

MATERIALS AND METHODS

Synthesis of the SIV-L Peptide and Preparation of Calmodulin. The 28-residue SIV-L peptide, DLWETLRRGGRWILAIPRRIRQGLELTL-NH_2, which corresponds to the amino acid sequence of residues 852−879 in the SIV transmembrane glycoprotein, was synthesized as described earlier (Miller et al., 1993). The peptide was judged to be of greater than 95% purity by amino acid analysis, mass spectroscopy, and HPLC, and it was used for CD, NMR, and fluorescence studies without further purification. We chose to work on this peptide rather than the corresponding domain of HIV, because it is devoid of Cys, a feature that facilitates the lengthy NMR experiments, as it eliminates potential covalent dimerization. (The amino acid sequences of the HIV-1 and SIV-L peptides, respectively, are RVIEVVQGACRAIRHIPRRIRQGLERIL and DLWETLRRGGRWILAIPRRIRQGLELTL. The SIV-L peptide used contained no substitutions compared to the original.) Mammalian CaM was expressed and purified from a synthetic gene in *Escherichia coli* (Zhang & Vogel, 1993). The solvents D_2O and deuterated TFE were obtained from MSD Isotopes (Montreal, Canada). All other chemicals were purchased from Sigma.

CD Spectroscopy. CD spectra of the peptide were recorded at ambient temperature (20 °C) using a JASCO J-500C CD spectropolarimeter using a cell path length of 0.1 cm. The concentration of the peptide was 10 μM in 5 mM citric acid buffer, pH 5.0. Several samples with different ratios (v/v) of citric acid buffer and TFE were studied. The peptide concentration was determined by UV absorption of the two tryptophan residues in the peptide ($\epsilon_{280}^{Trp} = 1.12 \times 10^4$ cm^2·mol^{-1}). CD spectra of Ca^{2+}−CaM complexed with the SIV-L peptide were measured at ambient temperature in 10 mM Tris buffer, 0.5 mM $CaCl_2$, pH 7.2. The CaM concentration was determined by UV absorption using $\epsilon_{276}^{1\%} = 1.8$.

Fluorescence Spectroscopy. Fluorescence measurements were performed on an Aminco SPF-500 spectrofluorometer at ambient temperature (20 °C) using a cell path length of 1 cm. The concentration of the SIV-L peptide or CaM was 10 μM. All these experiments were carried out in 10 mM Tris buffer, 100 mM KCl, pH 7.2. Mixtures (ratio 1:1) of the SIV-L peptide and CaM were measured in the presence of 1 mM $CaCl_2$ or 10 mM EDTA, respectively. The tryptophan residues from the peptide or peptide−CaM complexes were excited at 295 nm to minimize the contribution from tyrosine (of which there are two in CaM) to the emission spectra. Both excitation and emission band widths were 5 nm.

NMR Spectroscopy. Two peptide samples, one in 75% H_2O, 25% TFE−d_3 and the other in 75% D_2O, 25% TFE−d_3, were prepared for NMR studies. The concentration of each sample was close to 1.5 mM. The pH values for both samples were 5.0 (direct meter reading without correction for the isotope effect). All NMR spectra were acquired on a Bruker AMX500 spectrometer equipped with an X32 computer.

The sequence-specific assignment of all the proton NMR resonances was obtained from two-dimensional DQF-COSY (Rance et al., 1983), clean TOCSY (Bax & Davis, 1985; Griesinger et al., 1988), and NOESY (Bodenhausen et al., 1984) spectra which were acquired at 298 K in the phase sensitive mode using the time proportional phase increment technique (Marion & Wüthrich, 1983). For the NOESY experiment, 512 free induction decays were collected with a sweep width of 6024 Hz and a mixing time of 250 ms. In order to check that NOESY crosspeaks were caused by direct dipolar interaction rather than spin diffusion, we followed the buildup of NOE's in spectra with mixing times of 100 ms and 200 ms. For the TOCSY experiment, the mixing time used was 150 ms. A ^1H, ^{13}C HMQC spectrum (natural

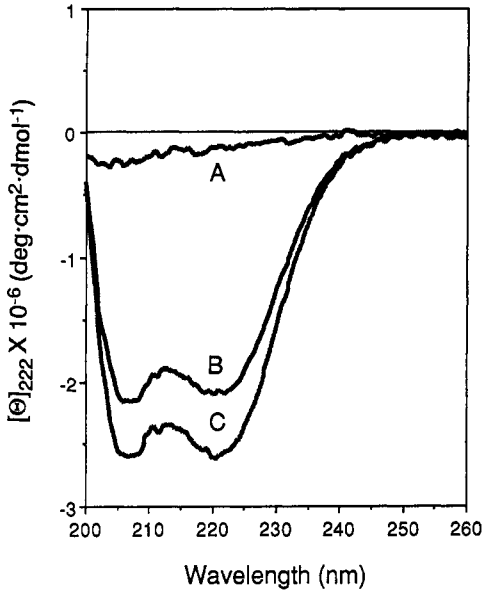

FIGURE 1: Far ultraviolet CD spectra of the SIV-L peptide (A), Ca^{2+}−CaM (B) and the SIV-L/Ca^{2+}−CaM complex (C). The SIV-L peptide and CaM concentration are 10 μM each in 10 mM Tris buffer, 0.5 mM $CaCl_2$, pH 7.2.

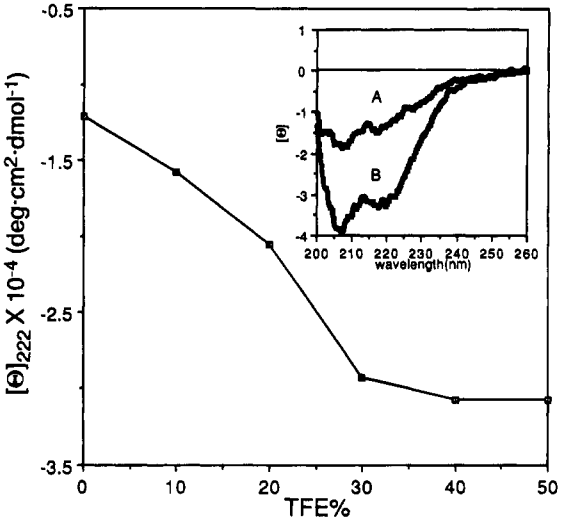

FIGURE 2: TFE titration curve of the SIV-L peptide. The mean residue ellipticity is measured at 222 nm as a function of the concentration of TFE. The inset shows the CD spectra for the SIV-L peptide in the presence of 0% (A) and 40% (B) TFE.

abundance) of the peptide in 75% D_2O, 25% TFE−d_3 was recorded using the method of Bax et al. (1983); this heteronuclear experiment was used to obtain the carbon-13 chemical shifts of the α-carbon resonances. All spectra were processed using the Bruker UXNMR software package. The data were zero filled once in the F1 dimension, and a sine-square window function was used in both dimensions before Fourier transformation.

One-dimensional titration experiments were carried out by titrating 0.85 mM Ca^{2+}−CaM, 100 mM KCl in D_2O, pH 7.0 (direct meter reading without correction for the isotope effect) with 0.85 mM SIV-L peptide in D_2O, pH 7.0; dilution effects are not considered in these experiments. Spectra were collected with a sweep width of 6024 Hz and 128 scans.

Gel Mobility Shift Assay. Nondenaturing urea−polyacrylamide gel mobility shift electrophoresis was performed following the procedure described by Erickson-Viitanen and DeGrado (1987), except that the running buffer also contained 4 M urea.

RESULTS

The SIV-L Peptide Adopts an α-Helix When Binding to Ca^{2+}−CaM. SIV-L peptide binding to CaM has been studied in 10 mM Tris buffer, pH 7.2 using CD spectroscopy (Figure 1). The peptide by itself has no obvious secondary structure in aqueous solution, because the spectrum lacks the typical minima at 208 nm and 222 nm for a stable α-helix (Johnson, 1990). Although the value of the mean residue ellipticity at 222 nm is as high as 1.2×10^4 deg·cm²·dmol^{-1}, this value is probably due to several different structures that exchange rapidly. In addition, it may contain contributions of the two tryptophan residues in the peptide sequence, since it is known that aromatic amino acid residues can make a contribution to the negative ellipticity around 220 nm (Manning & Woody, 1989; Chakrabartty et al., 1993). In addition, 2D NMR experiments also showed that the peptide has no extensive stable secondary structure in aqueous solution (see below). However, our CD studies showed (Figure 1) that addition of the SIV-L peptide to Ca^{2+}−CaM at a 1:1 ratio induced a significant amount of α-helical structure in the complex. The observed increase at 208 nm and 222 nm is a good indication of the formation of an α-helix (Johnson, 1990). Ca^{2+}−CaM generally does not gain secondary structure in the presence of a target peptide, because the peptide−CaM complex usually only bends and unravels the central helix of CaM to accommodate the peptide binding (Ikura et al., 1992; Roth et al., 1992; Meador et al., 1992,1993). We thus attribute the increased α-helical content exclusively to binding of the SIV-L peptide to CaM, as is often the case for other CaM-binding peptides (Vogel & Zhang, 1995). In contrast, no increase in the α-helical content was observed when we studied a mixture of apo−CaM and the SIV-L peptide at a 1:1 ratio (data not shown).

TFE Can Induce an α-Helix in the SIV-L Peptide. Because TFE is a well-known α-helix inducing solvent for linear peptides (Nelson & Kallenbach, 1989; Sönnichsen et al., 1992), we monitored the CD spectra of the SIV-L peptide titrated with different concentrations of TFE (Figure 2). Clearly, the α-helical content increased with the addition of TFE and reached a plateau at TFE concentrations over 40%. TFE is widely used as an α-helix inducing reagent, because it will only induce an α-helix if the amino acid sequence of the peptide has the propensity to form an α-helix. Our titration data clearly show that the SIV-L peptide has the potential to form an α-helical structure. Therefore, we decided to use a 25% TFE aqueous solution as the preferred solvent to study the SIV-L peptide using one- and two-dimensional NMR, as we have done with other CaM-binding peptides before (Zhang & Vogel, 1994b; Zhang et al., 1993). We have found that NMR spectra acquired in 25% TFE are slightly better resolved than those recorded in 40% TFE; hence, even though this is only near the titration midpoint for induced helix, the majority of the NMR work was done in a 25% TFE aqueous solution.

We first acquired a 1D proton and a 2D NOESY spectrum of the SIV-L peptide in aqueous solution and inspected the fingerprint region (αH-NH) of the spectrum. The chemical shifts of the crosspeaks are close to their typical random coil values and are degenerate which prevented us from making extensive detailed assignments of the spectrum. In addition, there were no crosspeaks in the amide−amide region (data

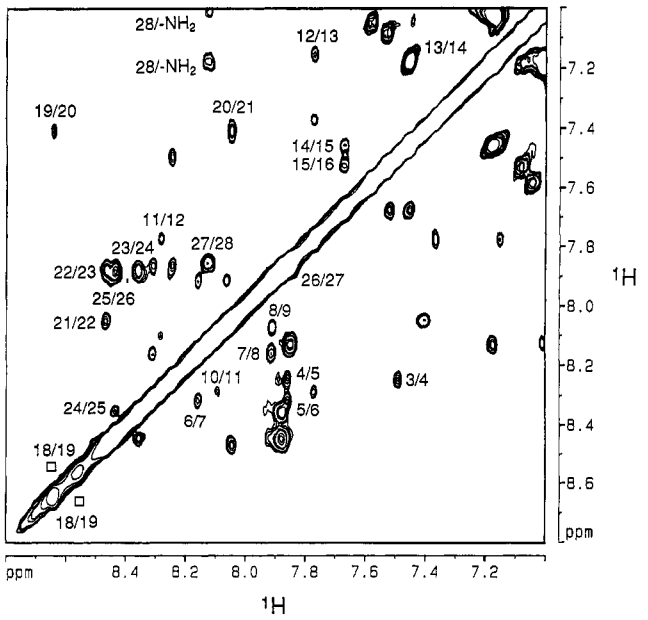

FIGURE 3: Amide−amide region of the NOESY spectrum (250 ms mixing time). The assignments of the NH−NH crosspeaks are indicated in the figure. The two boxes indicate the position of the crosspeaks for R18−R19, which are evident at lower contour levels.

not shown). All the above features indicate that the SIV-L peptide has no well-defined secondary structure under these conditions. We then acquired 1D spectra of the SIV-L peptide in 25% TFE−H_2O in the concentration range of 0.2−2 mM; no line broadening was observed in the spectra with increasing concentration (data not shown). Moreover, no crosspeaks between two peptide chains in the NOESY spectra were detected (see below). Therefore, we believe that aggregation of the peptide in 25% TFE−H_2O is negligible in these concentration ranges, and we have used a 1.5 mM concentration to acquire the NMR spectra.

The assignment of the NMR spectra of the SIV-L peptide and its secondary structure in 25% TFE−H_2O was determined using standard 2D NMR spectroscopy (Wüthrich, 1986). DQF-COSY (Rance et al., 1983) and clean TOCSY (Bax & Davis, 1985; Griesinger et al., 1988) were used to identify the spin system of each amino acid in the peptide. The crosspeaks involving the amino and α-protons of D1 were absent, probably because of fast proton exchange with solvent. We found that the 150 ms mixing time in the TOCSY experiment was quite useful for determining the spin systems of the six arginines, because the side chain guanidinium NH protons can couple back to the α-protons to give clear identification of the six α-proton positions (data not shown). Next, we acquired NOESY spectra (Bodenhausen et al., 1984) to obtain the sequential assignment of the peptide (Wüthrich, 1986). Three different mixing times have been used to obtain the NOE buildup curves (data not shown). All spectra show that the crosspeaks used for assignment and secondary structure analysis are real and that spin diffusion was negligible. Figure 3 shows the amide−amide region of the NOESY spectrum (250 ms mixing time) recorded in 25% TFE−H_2O solution. Figure 4 provides a summary of the NOE data for the amino acid residues in the peptide. The appearance of continuous short range $d_{NN(i,i+1)}$ crosspeaks and many medium range $d_{\alpha N(i,i+3)}$, $d_{\alpha\beta(i,i+3)}$ NOEs provides a strong indication for the formation of an α-helix between W3 and L28 (Wüthrich, 1986). We also measured the $^3J_{\alpha N}$ coupling constant for the peptide backbone; most values vary between 5 and 8 Hz, indicating that in 25% TFE, the α-helical structure is in exchange with extended structures. The short range d_{NN} crosspeaks increased further in intensity in 40% TFE, indicating further stabilization of the helical structure, in agreement with the CD data in Figure 2. In addition, we also observed many crosspeaks between aromatic side chains and other aliphatic side chains, consistent with α-helix formation. For example, crosspeaks could be observed between the W3 side chain protons and L6 δCH_3, the W12 side chain protons and L14 δCH_3, W12 2H, 4H, and A15 βH, all of which also support the α-helical nature of the peptide. Some irregularities were noted around the proline residue. The R18 amide proton apparently exchanges relatively fast with the solvent, so it could not be detected in the TOCSY spectra (data not shown). However, it was observed in the NOESY spectrum, where we also can see its crosspeak with P17 αH, which is helpful for the assignment of the spectrum (see supporting information). When the contour level was decreased, crosspeaks between I16 NH and P17 δH appeared. Two crosspeaks between I16 αH and P17 δH can be observed in the aliphatic region of the NOESY spectrum, while crosspeaks between I16 αH and P17 αH were absent. Thus, the proline residue is predominantly in the trans conformation (Wüthrich et al., 1984). In addition, we observed NH−NH crosspeaks between R18 and R19 when the contour level was decreased (Figure 3), as well as a crosspeak between αH of P17 and βH of I20 (Figure 4). These data support the notion that the I16-P17-R18-R19 region is still in an α-helical conformation, although some distortion exists due to the helix bending effect of the proline residue.

A table showing the complete proton assignment for all the residues in the SIV-L peptide is included in the supporting information. Carbon-13 chemical shifts for the α-carbon resonances were also provided; these were taken from the ^1H, ^{13}C natural abundance HMQC spectrum (see supporting information). Changes in the chemical shift relative to the random coil values (the chemical shift index) of the α proton and α carbon resonances can also provide a strong indication for the presence of an α-helix (Spera & Bax, 1991; Wishart et al., 1991) (Figure 4). We observed that the majority of the ^1H α-proton chemical shifts experience an upfield shift, while the ^{13}C α-carbon chemical shifts are downfield shifted; both of these trends are consistent with the typical behavior for amino acid residues involved in α-helical structure (Spera & Bax, 1991; Wishart et al., 1991). It should also be noted that the chemical shift changes indicate the presence of a distortion of the α-helix around the Pro residue. Taken together, the NOE summary and the chemical shift data (Figure 4) indicate that the SIV-L peptide adopts an α-helix from W3 to L28 in 25% TFE aqueous solution.

The Interaction of the SIV-L Peptide with Ca^{2+}−CaM Involves Both Lobes of CaM. Figure 5 shows a series of 1D NMR spectra for Ca^{2+}−CaM titrated with the SIV-L peptide up to a 1:1.1 ratio. The broad lines for the complex suggest (but do not prove) that in addition to specific interaction, some nonspecific interaction or exchange may occur. The titration could not be extended to higher concentrations of the SIV-L peptide, because at the protein and peptide concentrations used in these experiments precipitates formed at ratios higher than 1:1, indicating the formation of other oligomeric complexes. The 1D spectra show that both the N-lobe and the C-lobe of CaM are involved in the peptide binding, as aromatic resonances of

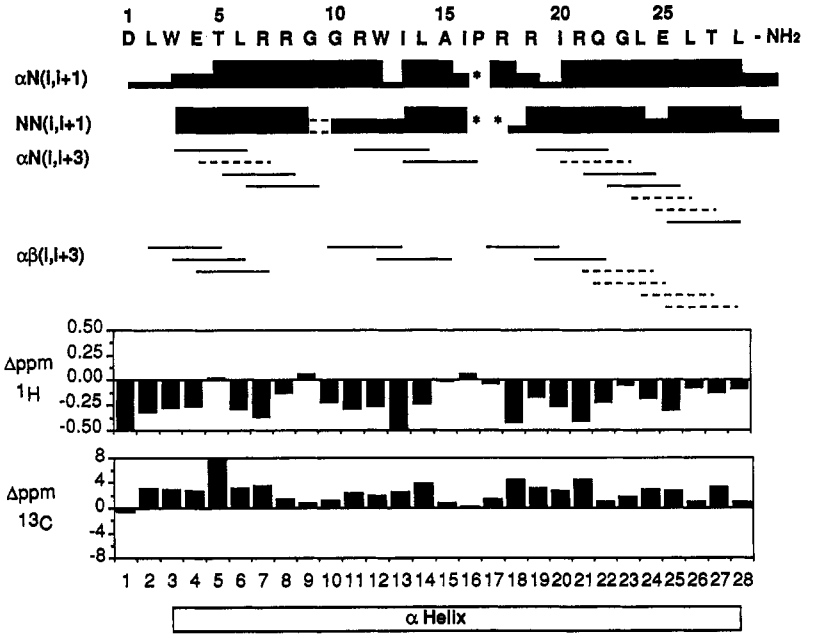

FIGURE 4: Amino acid sequence of the SIV-L peptide and summary of the short- and medium-range NOEs involving the NH, $C_\alpha H$ and $C_\beta H$ protons. Some likely NOE crosspeaks that are presumably present but could not be detected because of resonance overlap are indicated by the open box and dashed lines (i, sequence number; asterisk, not observable due to the absence of amide proton of P17). The thickness of the lines corresponds to the intensities of the measured NOEs. The differences between the chemical shifts of the αH and αC resonances of the SIV-L peptide in 25% TFE and the random coil shifts are also presented. The latter were taken from Wüthrich (1986) and Richarz and Wüthrich (1978), respectively.

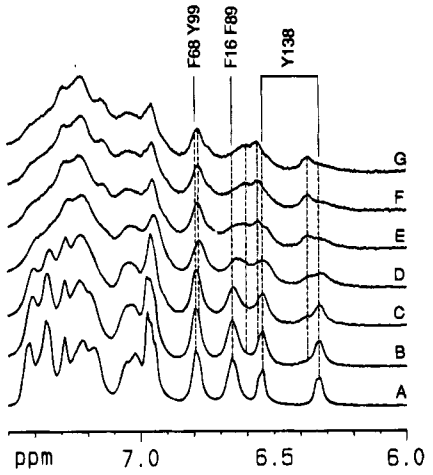

FIGURE 5: Stack plot of the aromatic regions of the ^1H NMR spectra in Ca^{2+}−CaM at different points of the titration with the SIV-L peptide at pH 7.0, 298 K. The peptide to CaM ratio from bottom to top is 0:1 (A); 0.2:1 (B); 0.4:1 (C); 0.6:1 (D); 0.8:1 (E); 1:1 (F), and 1.1:1 (G). The assignments of the resonances of several aromatic residues in Ca^{2+}−CaM are indicated in the figure. The original CaM concentration was 0.85 mM (see Materials and Methods).

both lobes of CaM change their chemical shifts upon binding the peptide. The titration experiment also confirmed that the SIV-L peptide is tightly binding to CaM because the new set of peaks increases in intensity while the original set of peaks decreases in the course of the peptide titration, showing that the two forms are in slow exchange. This result is consistent with the nanomolar binding affinity reported earlier (Miller et al., 1993). Further evidence for the binding of the SIV-L peptide to both lobes of CaM came from experiments with ^{13}C-methylmethionine-labeled CaM titrated with the SIV-L peptide to a 1:1 ratio. The nine well-resolved methionine methyl crosspeaks can provide an efficient means of monitoring changes in the two methionine-rich hydrophobic surface patches of Ca^{2+}−CaM upon peptide binding (Zhang & Vogel, 1994b; Zhang et al., 1994c; Vogel & Zhang, 1995). In the case of the SIV-L peptide, we noted that the methionine residues of both the N-lobe and C-lobe of CaM changed position upon binding the SIV-L peptide (data not shown). However, the appearance of this ^1H, ^{13}C HMQC spectrum of the complex was rather poor because of the broad lines that occurred upon peptide binding. This broadening (see also Figure 5) made it impossible to pursue isotope-filtered NMR experiments to determine the structure of this peptide bound to CaM, as has been done for the CaM-binding domain of MLCK (Roth et al., 1991; Ikura & Bax, 1992).

Further gel mobility shift experiments showed that the SIV-L peptide interaction with CaM is potentially complicated. They not only can form a 1:1 complex as the NMR and CD data indicate, but they also can form a complex between an oligomeric peptide and CaM as we observed in the urea gel experiment (Figure 6). In this experiment, we observed that the SIV-L peptide nearly saturated CaM when their ratio is 4:1. Because of the lower protein and peptide concentrations used in the urea gel experiments, the oligomeric forms could be studied, whereas they precipitated with the higher concentrations used in the NMR experiments (see above). The solubility of the oligomeric forms in the gels is not related to the presence of 4 M urea, as they were also detected on gels when it was omitted (data not shown). As suggested by a reviewer of the manuscript, these data can provide a potential explanation of the observed appearance of the ^1H NMR spectra of the complex (Figure 5), where free and bound peptide are in slow exchange, yet conformational broadening can occur due to exchange in the complex. As expected, the SIV-L peptide did not give rise to a band shift when mixed with apo−CaM; thus, the SIV-L peptide and apo−CaM do not bind to one another (data not shown).

We also acquired the fluorescence spectroscopy for the SIV-L peptide in the presence and absence of CaM. The

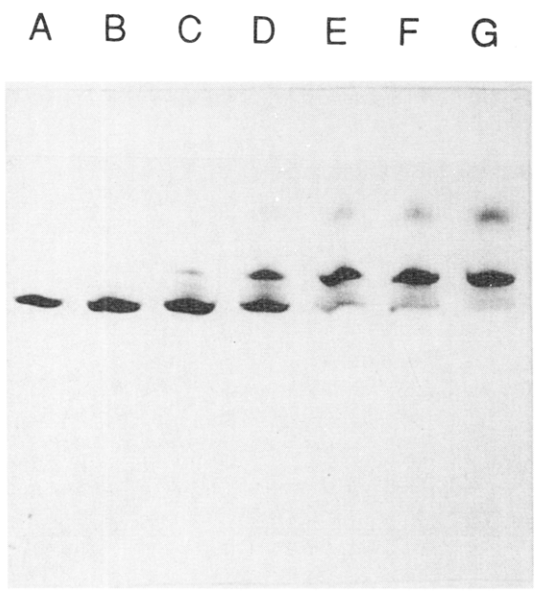

FIGURE 6: Nondenaturing urea−polyacrylamide gel electrophoresis of the SIV-L/CaM complex at different ratios in the presence of 0.1 mM $CaCl_2$ (CaM concentration 15 μM). The peptide to CaM ratio is 0:1 (lane A); 0.5:1 (lane B); 1:1 (lane C); 2:1 (lane D); 3:1 (lane E); 4:1 (lane F); and 5:1 (lane G). Control experiments were also run with peptide at the same concentrations as lane F and G, in the absence of CaM (data not shown). Under these conditions no stained bands could be detected, showing that any stained bands must contain some CaM. (The running buffer was 25 mM Tris, 192 mM glycine, 0.1 mM $CaCl_2$, and 4 M urea at pH 8.3).

emission spectra of the Trp residues in the SIV-L peptide experienced a significant blue shift, as well as an increase of the fluorescence intensity upon binding to Ca^{2+}−CaM, whereas there was little change in the presence of EDTA (apo−CaM, data not shown). These data are comparable to those reported for the CaM-binding peptides of MLCK and caldesmon, for example (Lukas et al., 1986; Zhan et al., 1991). The tryptophan residues in these peptides go to a less polar environment upon binding to Ca^{2+}−CaM. These data suggest that the SIV-L peptide binds to the same hydrophobic surfaces of CaM as the other peptides.

DISCUSSION

The envelope glycoprotein gp160, which is formed as a noncovalent complex between gp120 and gp41, is an important component of the HIV/SIV virus (Mckeating & Willey, 1989). Several synthetic peptides derived from various regions of the surface protein gp120, as well as the transmembrane protein gp41, have already been studied by spectroscopic methods. These biophysical studies have related the peptides' structure with their proposed functions; such studies have been reported, for example, for the principal neutralizing determinant of gp120 (Chandrasekhar et al., 1991), a hybrid synthetic peptide containing multiple epitopes of gp120 (de Lorimier et al., 1994) and the N-terminus of gp41 (Rafalski et al., 1990). Here we report biophysical studies of the SIV-L peptide, which is derived from the C-terminal portion of gp41 and is reported to contain the CaM-binding domain (Miller et al., 1993). Our CD data show that the SIV-L peptide binds to Ca^{2+}−CaM as an α-helix. The fluorescence and methionyl ^{13}C NMR data suggest that the SIV-L peptide binds to the two hydrophobic surface regions of Ca^{2+}−CaM. The binding is tight, consistent with the earlier reported nanomolar affinity, and the binding involves both the N- and C-lobes of CaM and is completely dependent on Ca^{2+}. The NOE summary and chemical shift data (Figure 4) indicate that the SIV-L peptide adopts an α-helix in 25% TFE aqueous solution, which probably mimics the CaM-bound state according to the CD data of the complex. Although all the spectroscopic experiments show that the SIV-L peptide can bind to Ca^{2+}−CaM in a 1:1 ratio, nondenaturing urea gel band shift electrophoresis experiments implied that the peptide may interact with Ca^{2+}−CaM in oligomeric forms.

To date, two kinds of positively charged amphiphilic α-helices have been found to bind to Ca^{2+}−CaM. One contains a 12-residue long helical peptide which separates two large hydrophobic residues that anchor the peptide on the two hydrophobic surface domains of CaM (Ikura et al., 1992; Meador et al., 1992). Another motif involves a shorter 8-residue long helical peptide between two anchoring hydrophobic residues (Meador et al., 1993; Zhang & Vogel, 1994b). Inspection of the α-helical stretch in the SIV-L peptide in 25% TFE−H_2O shows that both of the above arrangements may exist in the SIV-L peptide between W3 and L28. Therefore, at this time we cannot determine the exact mode of binding of SIV-L to CaM until we obtain data for shorter versions of the peptide.

As indicated in the Results, we observed distortions in the helix around P17 of the peptide in 25% TFE−H_2O. This is not unique to the SIV-L CaM-binding peptide. For example, nonhelical backbone conformations are also observed around V12 in the middle of the helix in the CaM-binding domain of smooth muscle MLCK (Roth et al., 1991). The deviation may allow the peptide to adjust itself to fit snugly on the two hydrophobic patches on Ca^{2+}−CaM. In globular proteins, proline residues in the center of α-helices are known to give rise to a kink of 20° or more, and these residues are often highly conserved (Barlow & Thornton, 1988), suggesting that the conserved proline residues are functionally or structurally important. An alternative explanation has been proposed (Blanco et al., 1992; Zhou et al., 1992). These authors suggest that bending is an intrinsic property of isolated natural amphiphilic helices in polar solvents rather than a consequence of packing constraints in the tertiary structure, since the bend may allow for shorter hydrogen bonds.

We also used 1D NMR titration experiments to find that the SIV-L peptide binding to CaM involves both the N- and C-domains of CaM, the binding mode observed for most other CaM-binding peptides to CaM. From the urea gel electrophoresis experiments (Figure 6), however, it appears that the SIV-L peptide can bind to CaM in different forms, such as a monomer, dimer, or tetramer. This may in fact reflect the situation when the gp41 protein binds to CaM because the envelope glycoproteins from HIV-1, HIV-2, and SIV are found as a tetramer composed of two dimers (Pinter et al., 1989; Rey et al., 1989). In fact, oligomerization is a significant form of post-translational processing important for the assembly and function of viral membrane glycoproteins (Gething et al., 1986; Rey et al., 1989; Doms et al., 1993). The subunits in the oligomers may associate with each other through noncovalent interactions and/or disulfide bonds. It is also possible that CaM binds with monomeric SIV-L because the C-terminal portion of the gp41 protein can be dissociated when the SIV/HIV virus has already infected the host cell. Finally, CaM can perhaps facilitate the oligomerization of the SIV-L peptide. In this respect it is noteworthy that oligomeric erythrocyte Ca^{2+}-ATPase has

also been reported to bind to CaM (Kosk-Kosicka & Bzdega, 1990; Kosk-Kosicka et al., 1990). Clearly, further studies are needed to elucidate these details of the interaction between CaM and the intact gp41 protein.

In conclusion, these studies provide a molecular basis for understanding the binding of the SIV-L peptide to Ca^{2+}−CaM. CD spectra show that the peptide binds in an α-helical form to Ca^{2+}−CaM. NMR experiments showed that an amphiphilic α-helical structure can be induced in the SIV-L peptide between residues W3 and L28 in aqueous TFE solution; earlier NMR studies with other CaM-binding peptides have suggested that this solvent mimics the CaM-bound environment: in the case of MLCK and caldesmon peptides, the same α-helical structures were found in TFE and in the CaM-bound form (Zhang & Vogel, 1994b; Zhang et al., 1993). Thus, it appears that the HIV/SIV peptide binds to the two hydrophobic patches of Ca^{2+}−CaM with an amphiphilic α-helix; this mode of interaction resembles that observed for several CaM activated enzymes (Vogel, 1994; James et al., 1995; Vogel & Zhang, 1995).

SUPPORTING INFORMATION AVAILABLE

A table showing the ^1H and $^{13}C_\alpha$ chemical shift values of the SIV-L peptide in H_2O/TFE mixture (75%/25%), a spectrum of the fingerprint region of the NOESY spectrum, and a natural abundance ^1H, ^{13}C HMQC spectrum of the SIV-L peptide in D_2O/TFE mixture (75%/25%) (5 pages). Ordering information is given on any current masthead page.

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