Aromatic Side-Chain Contribution to Far-Ultraviolet Circular Dichroism of Helical Peptides and Its Effect on Measurement of Helix Propensities†

Avijit Chakrabartty, Tanja Kortemme, S. Padmanabhan, and Robert L. Baldwin*

Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Received December 31, 1992

ABSTRACT: Peptides of the sequence Ac-XKAAAAKAAAAKAAAAK-amide, where X is Tyr, Trp, or Ala, produce circular dichroism spectra that are typical of the α-helix; there are, however, significant differences between the Tyr, Trp, or Ala peptides in the magnitudes of the far-ultraviolet bands. A tyrosine or tryptophan residue is needed in each peptide in order to measure accurately the peptide concentration and the mean residue ellipticity. The N- or C-terminal position is chosen because helix fraying is greatest at each end and the Tyr or Trp residue should influence the helix content of the peptide least at these positions. Amide proton exchange measurements by proton nuclear magnetic resonance spectroscopy indicate that the Tyr, Trp, and Ala peptides possess similar amounts of H-bonded secondary structure. Comparison of the far-ultraviolet circular dichroism and absorption spectra of these peptides suggests that the differences in circular dichroism arise in each case from an induced aromatic circular dichroism band, which is positive for Tyr and negative for Trp. Insertion of one to three Gly residues between the aromatic residue and the rest of the helical sequence reduces the induced aromatic band to insignificant levels. Using this procedure of inserting Gly residues between the Tyr and the rest of the helical sequence, we remeasured the helix propensity of Gly. We find that the Ala:Gly ratio of helix propensities is 40, as opposed to our previous estimate of 100 determined using the Tyr peptide without considering the aromatic contribution of Tyr in the analysis [Chakrabartty, A., Schellman, J. A., & Baldwin, R. L. (1991) *Nature 351,* 586–588].

Recently, several laboratories have investigated the propensities of amino acid residues to form α-helices in different short peptide systems (Padmanabhan et al., 1990; Merutka et al., 1990; O'Neil & DeGrado, 1990; Lyu et al., 1990; Scholtz et al., 1991; Chakrabartty et al., 1991; Gans et al., 1991; Kemp et al., 1991; Rohl et al., 1992; Stellwagen et al., 1992). This work was undertaken to determine (a) if classical helix–coil transition theory can adequately describe helix formation in short peptides, (b) if a system can be devised to measure solely the relative helix propensities of two amino acids, (c) if there are significant differences in helix propensity among the amino acids, and (d) how much intrinsic helix propensities contribute to helix formation in peptides and proteins. As in earlier studies (Sueki et al., 1984), the helix propensity of an amino acid in an isolated helix is equated here with its *s*-value, where *s* is the propagation parameter of the Zimm–Bragg theory for α-helix formation (Zimm & Bragg, 1959). The *s*-values measured in different short peptide systems disagree numerically but bear a simple relation to each other (Chakrabartty & Baldwin, 1992), and are quite different from those measured earlier with random-sequence copolymers using the "host-guest" technique (Wojcik et al., 1990). Given that systematic differences in the *s*-value are found with various short peptide systems, it is crucial that determinations of *s*-values be as accurate as possible so that context-dependent effects (dependence on the apparent *s*-values on neighboring residues) can be distinguished from systematic experimental errors. Here, we have identified one such systematic error, namely, a previously unnoticed contribution of an aromatic side chain to the far-UV circular dichroism (CD)[1] spectra of our peptides, which results in an underestimation of the *s*-value of an amino acid substituted for alanine. This systematic error should be present in any peptide system which contains aromatic residues in helical sequences and uses far-UV CD data to estimate fractional helix contents.

Our peptide system for measurement of helix propensities employs a helical reference peptide (sequence: Ac-YKAAAA-KAAAAKAAAK-amide). We replace one or more Ala residues by a second residue, and the change in helix content is measured by CD: then, by applying helix–coil theory (Lifson & Roig, 1961), the *s*-value of the substituted residue is calculated (Chakrabartty et al., 1991). The single Tyr at the N-terminus of the peptide enables us to determine peptide concentration with high accuracy by tyrosine absorbance (error ±1%). This procedure for peptide concentration determination offers a considerable advantage over other methods such as the ninhydrin assay or quantitative amino acid analysis which produce errors of ±5% or even more (Marqusee et al., 1989; Chakrabartty & Hew, 1991). We find, however, that the Tyr side chain produces a large positive CD band in the far-UV which complicates the measurement of helix content. The magnitude of this band is much greater than expected from model compound data. Therefore, we developed a procedure for eliminating the Tyr contribution to the far-UV CD. Using this procedure, we redetermined the *s*-value of Gly, and found that it is slightly greater than our previous estimate (Chakrabartty et al., 1991).

MATERIALS AND METHODS

Peptide Synthesis. Peptides were synthesized by the solid-phase method using the Milligen 9050 peptide synthesizer.

† A.C. is the recipient of a postdoctoral fellowship from the Medical Research Council of Canada. S.P. is supported by a postdoctoral fellowship from the Arthritis Foundation. This work was supported by a grant from the National Institutes of Health (GM 31475).

* To whom correspondence should be addressed.

[1] Abbreviations: CD, circular dichroism; FAB, fast atom bombardment; FID, free induction decay; TFE, trifluoroethanol; TMSP, trimethylsilyl propionate; [θ], mean residue ellipticity.

0006-2960/93/0432-5560$04.00/0

An active ester coupling procedure, employing pentafluorophenyl esters of 9-fluorenylmethoxycarbonyl-amino acids, was used. The N-termini were acetylated with acetic anhydride, and the peptides were cleaved from the PAL-resin (Milligen) as peptide amides with a 95:5 trifluoroacetic acid/anisole mixture. The peptides were purified by C_{18} reverse-phase chromatography, and peptide identity was confirmed by FAB mass spectrometry at the Mass Spectrometry Facility, University of California, San Francisco. Peptide purity was assessed by analytical C_{18} reverse-phase chromatography using the Pharmacia FPLC system.

Circular Dichroism Measurements. CD measurements were made using an Aviv 60DS spectropolarimeter. Measurements at 222 nm were made in a 1.0-cm quartz cell in 1.0 M NaCl and 1 mM each of sodium borate, sodium citrate, and sodium phosphate, 0 °C, pH 7. Far-UV CD spectra were taken in a 0.1-cm quartz cell in 0.10 M KF/1 mM potassium phosphate, 0 °C, pH 7, and near-UV CD spectra were taken in a 1.0-cm quartz cell in 0.10 M KF/1 mM potassium phosphate, 0 °C, pH 7. CD measurements are reported as mean residue ellipticity at a given wavelength of peptide samples and as molar ellipticity for tyrosine and tryptophan model compounds; all measurements are reported in degrees centimeter squared per decimole. Trifluoroethanol (TFE) titrations were performed according to the method of Nelson and Kallenbach (1986).

Ultraviolet Absorption Measurements. UV difference spectra of peptides were taken at room temperature in a 1.0-cm quartz cell in 0.10 M KF/1 mM potassium phosphate, pH 7, using a Hewlett Packard 8452A diode array UV spectrophotometer with a 25-s time constant.

Amide Proton Exchange Rate Measurements. Amide proton exchange was measured by ^1H nuclear magnetic resonance (NMR) spectroscopy according to the method of Rohl et al. (1992). Aqueous solutions of peptides (0.5–2 mM, 0.5 mL) were adjusted to pH 2.5 with HCl and then lyophilized to dryness. At $t = 0$ min, the peptide was dissolved in 0.5–1.0 mL of ^2H_2O containing 1 M NaCl and 5 mM sodium phosphate, pH* 2.50 (the glass electrode reading at room temperature, uncorrected for isotope effects). Thereafter, multiple one-dimensional proton spectra were acquired on a General Electric GN-Omega spectrometer at a proton frequency of 500.13 MHz. Data were collected using a 5000 Hz spectral width and a 60° pulse. The FID was a sum of 64 scans collected in 4096 complex points with a 5 ms recycle delay. Spectra were processed using FELIX (Hare Research, Inc.) on a Silicon Graphics Personal Iris computer. Average proton occupancy was determined by measuring the integral of all peaks in the amide region. For the tyrosine- and tryptophan-containing peptides, the amide peak intensities were normalized to the area of a nonexchanging aromatic resonance. Average proton occupancy was determined by extrapolating the integral peak area as a function of time back to zero time and setting this value to 1. The pH* values of the samples were measured after acquisition of the NMR spectra. The small deviations in pH* from the value of 2.5 were corrected for by expanding or contracting the time axis according to the equation: time* = (time)$10^{2.5}/10^{\text{exptl pH*}}$ (Rohl et al., 1992).

Peptide Concentration Measurements. Concentrations of stock solutions of peptides were determined by UV absorbance, by ninhydrin assay (Rosen, 1957), or by NMR. The UV absorbance method involved measuring the absorbance of peptides containing tyrosine or tryptophan in 6 M guanidine hydrochloride, pH 6.5, and then calculating the molar concentration from ϵ_{275nm} = 1450 M^{-1} cm^{-1} for tyrosine peptides (Brandts & Kaplan, 1973) and ϵ_{281nm} = 5690 M^{-1} cm^{-1} for tryptophan peptides (Edelhoch, 1967). The NMR method involved collecting one-dimensional ^1H NMR spectra of standard solutions of a tyrosine peptide (whose concentration had been determined by tyrosine absorbance) and peptide samples of unknown concentration. The standards and samples were dissolved in ^2H_2O solutions containing 0.1 mM trimethylsilyl propionate (TMSP). Spectra were collected using a 6000 Hz spectral width. The FID was the sum of 128 scans collected in 16 384 complex data points with a pulse delay of 5 ms. Spectra were processed using FELIX (Hare Research, Inc.) on a Silicon Graphics Personal Iris computer. The areas under the acetylmethyl proton and TMSP peaks of the various ^1H NMR spectra were integrated, and the areas were expressed as the ratio of the acetyl peak to the TMSP peak. A standard curve of the peak area ratio versus tyrosine peptide concentration was used to determine the concentration of a sample. The standard curve equation was concn (mM) = 0.288(ratio) + 0.007; R = 0.997.

Table I

name	sequence	$-[\theta]_{222}$[a]	$-[\theta]_{222}$[b]
yak	Ac-YKAAAAKAAAAKAAAAK-amide	25100	24700
aak	Ac-AKAAAAKAAAAKAAAAK-amide		30700
wak	Ac-WKAAAAKAAAAKAAAAK-amide	31500	33000
fak	Ac-FKAAAAKAAAAKAAAAK-amide		26600
aky	Ac-KAAAAKAAAAKAAAAKY-amide	24600	
yaky	Ac-YKAAAAKAAAAKAAAAKY-amide	24600	
ya-ak20	Ac-YAKAAAAKAAAAKAAAAKAA-amide	30500	31300
ay-ak20	Ac-AAKAAAAKAAAAKAAAAKAY-amide	29600	30500
aa-ak20	Ac-AAKAAAAKAAAAKAAAAKAA-amide		34300
yy-ak20	Ac-YAKAAAAKAAAAKAAAAKAY-amide	28300	

[a] Mean residue ellipticity at 222 nm (deg cm^2/dmol). Conditions: 0 °C, pH 7, 1.0 M NaCl, 1 mM each of sodium borate, sodium citrate, and sodium phosphate. Peptide concentration was determined by UV absorbance. Values represent the mean of five measurements. [b] Peptide concentration was determined by ninhydrin assay. Other conditions are the same as in the first column.

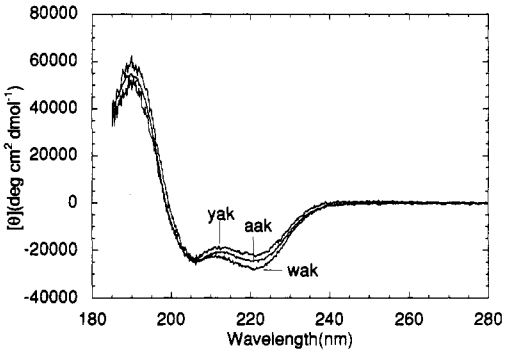

FIGURE 1: Circular dichroism spectra of yak, wak, and aak. Conditions: 0 °C, pH 7, 0.1 M KF, 1 mM potassium phosphate.

RESULTS AND DISCUSSION

Helix Contents of yak, wak, and aak Peptides. The far-UV CD spectra of three 17-residue peptides, which contain Tyr (yak), Trp (wak), or Ala (aak) as the N-terminal residue but are otherwise identical in sequence (see Table I), are displayed in Figure 1. Previously, we used the yak peptide as a reference peptide for measurement of helix propensities (Chakrabartty et al., 1991). All three spectra are typical of the α-helical secondary structure, with minima at 222 and 207 nm and a maximum near 190 nm. The intensities of the CD bands, however, differ significantly between the peptides, well outside the error limits of the measurement. In particular, the value of $[\theta]_{222}$, which is often used to estimate helical content, differs by as much as 20% (Table I). This seems to suggest that, at the N-terminus, Tyr is helix-destabilizing relative to Ala whereas Trp is helix-stabilizing.

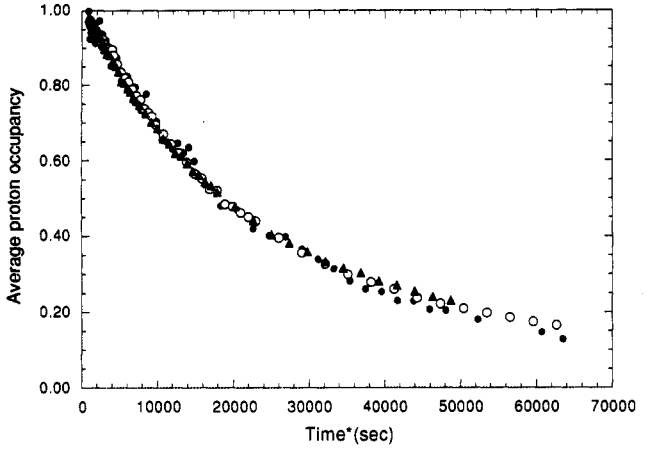

FIGURE 2: Amide proton exchange curves for yak, wak, and aak as monitored by NMR. Fractional occupancy is determined as described under Materials and Methods. Time* is an adjusted time scale which corrects for small differences in the pH* of different samples. (●) yak; (○) wak; (▲) aak.

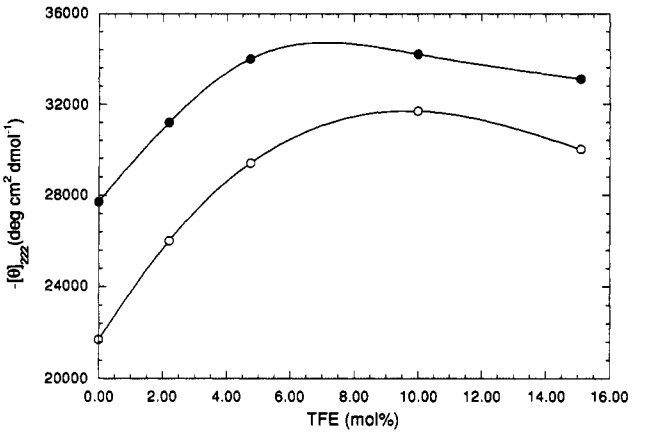

FIGURE 3: Trifluoroethanol titration of yak and aak. (●) aak; (○) yak. Conditions: 0 °C, pH 7, 0–15.25 mol % TFE.

The above interpretation is not, however, consistent with other experimental results on helical peptides, which show that substitution of a nonpolar end residue by another nonpolar residue has only a minor effect on the helicity of the peptide (Chakrabartty, unpublished data).[2] The decrease in helix content with a single Ala→Gly substitution at each Ala position of the yak peptide is greatest in the middle of the peptide and becomes small at positions close to either end (Chakrabartty et al., 1991). A similar effect was observed when Ala→Gly substitutions were made at various positions in an analog of the C-peptide of RNase A (Strehlow & Baldwin, 1989). The reduced response to substitutions at end positions occurs because the ends of isolated peptide helices are frayed. Consequently, the helix propensity of either end residue does not contribute much to the stability of the helix. The implication of these findings is that yak, wak, and aak should have minimal differences in helix content.

Helix fraying was demonstrated in different helical peptides by NMR (Osterhout et al., 1989; Pease et al., 1990; Bradley et al., 1990; Gans et al., 1991; Liff et al., 1991), and recently was described quantitatively by amide proton exchange measurements using ^{1}H NMR (Rohl et al., 1992). Measurement of the kinetics of amide proton exchange provides a sensitive assay for detecting H-bonded secondary structure (Englander & Kallenbach, 1984), and helix–coil theory has been used to relate the exchange rates observed in helical alanine-based peptides to their helix contents (Rohl et al., 1992). Therefore, we examined the rates of amide proton exchange in yak, wak, and aak. The kinetic exchange curves of yak, wak, and aak (Figure 2) are essentially superimposable, and display the complex kinetics of decay expected for isolated helices with frayed ends (Rohl et al., 1992). The identity of the three amide proton exchange curves indicates that all three peptides possess very similar amounts of H-bonded secondary structure; despite the differences in their CD spectra (Figure 1), the exchange curves indicate that these peptides possess the same helical contents.

A second experimental approach for investigating the helix contents of these peptides involves addition of the helix-inducing solvent TFE (Figure 3). Padmanabhan et al. (1990) showed that TFE can induce virtually complete helix formation in the yak peptide, and also in analogs of yak that differ modestly in helix content. At high concentrations of TFE, yak analogs with helix-destabilizing substitutions adopt helical conformations that are identical to the parent compound. If yak and aak differ in helix content, then addition of sufficient quantities of TFE should induce both peptides to form identical highly helical structures. The TFE titrations of these two peptides, however, do not converge at high TFE concentrations (Figure 3). Instead, the shapes of the two titration curves are similar, with the yak curve being displaced from the aak curve by an average value of 4500 deg cm^2/dmol. Thus, under conditions where both peptides should adopt identical helical conformations, aak and yak display differences in their circular dichroic spectra. These differences in CD spectra could, therefore, be caused by other factors such as contributions from the aromatic side chains of Tyr and Trp. These are examined in the next section.

Aromatic Contributions to the CD Spectra of yak, wak, and aak Peptides. To establish that the large differences in CD spectra between yak, wak, and aak result from contributions of the Tyr and Trp side chains, we compared the CD spectra of these peptides with their absorption spectra. Since CD depends on light absorption, aromatic CD bands should be correlated with their absorption bands. The far-UV side-chain absorption spectra of yak and wak were obtained by subtracting the aak spectrum from that of yak and wak, respectively. The side-chain absorption spectra of these two peptides were compared with corresponding CD difference spectra obtained by a similar subtraction of the aak CD spectrum (Figure 4). Whereas the yak – aak difference CD spectrum displays a broad positive CD band at 221 nm of magnitude 5000 deg. cm^2/dmol, a broad negative CD band at 220 nm of magnitude 3000 deg cm^2/dmol is seen with the wak – aak difference CD spectrum. The side-chain absorption spectra of yak and wak display large absorption bands at 225 and 221 nm, respectively. Since the difference CD bands follow the corresponding side-chain absorption bands, it is likely that the different CD spectra of these three peptides result from aromatic CD contributions.

We also examined the CD spectra of these peptides in the near-UV region. A comparison of the near-UV CD bands of yak and wak with those of acetyltyrosyl ethyl ester and acetyltryptophanyl ethyl ester, respectively, reveals some small differences (Figure 5). The phenolic CD band is diminished in the peptide, whereas the indole CD band is increased in the peptide. These bands are, however, very small in magnitude, and the small differences that we observe may be within the error limits of the measurements.

It is well-known that aromatic side-chain contributions to the far-UV CD spectra of peptides and proteins can be sizeable (Woody, 1978). The induced CD of the phenolic chromophore

[2] This statement applies when the α-amino group is blocked, for example, by acetylation.

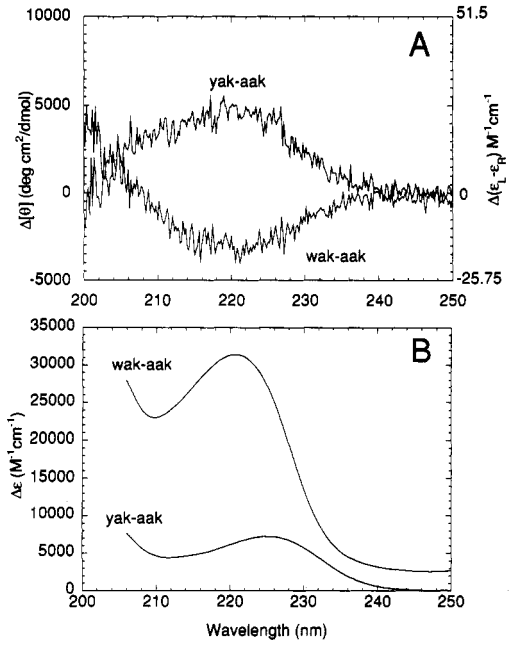

FIGURE 4: (A) Circular dichroism difference spectra. The far-UV CD spectrum of aak was subtracted from those of yak and wak. The difference in mean residue ellipticity is reported on the left vertical axis, and the right vertical axis reports the difference in molar ellipticity in terms of the difference in absorption coefficients of left and right circularly polarized light. (B) Far-UV absorption difference spectra. The far-UV absorption spectrum of aak was subtracted from those of yak and wak.

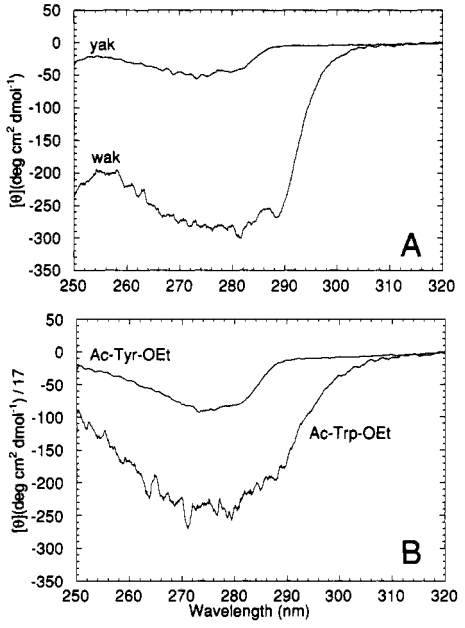

FIGURE 5: (A) Near-UV circular dichroism spectra of yak and wak. Conditions: same as Figure 1. CD is reported in units of mean residue ellipticity. (B) Near-UV circular dichroism spectra of acetyltyrosyl ethyl ester and acetyltryptophanyl ethyl ester. Conditions are the same as in (A). To facilitate direct comparisons of the CD spectra of panels A and B, data for the Tyr and Trp model compounds are reported in units of molar ellipticity divided by 17.

in the far-UV has been attributed to the L_a transition, and induction of CD is thought to arise from several mechanisms which may operate simultaneously (Hooker & Schellman, 1970). These mechanisms involve different types of coupling interactions between the L_a transition and peptide transitions as well as interactions between the phenolic chromophore and the electronic environment provided by the rest of the molecule [see Bayley (1980)]. Conformational averaging causes CD bands produced by flexible chromophores to be very small. For example, the molar ellipticity of the Tyr CD band at 225 nm in cyclic and linear peptides of sequence $(Gly)_3$-L-Tyr-$(Gly)_3$ is 6000 deg cm²/dmol (Ziegler & Bush, 1971). The diketopiperazine of sequence (*c*(L-Ala-L-Tyr), on the other hand, produces a CD band of opposite sign at 221 nm with a molar ellipticity of around −15 000 deg cm²/dmol; the greater magnitude and opposite sign of this band are a result of the conformational restriction imposed by the all-cis conformation of the diketopiperazine backbone (Snow et al., 1977). The magnitude of the Tyr CD band in yak can be obtained from the yak − aak difference spectrum (Figure 4), and it is approximately 5000 deg cm²/dmol on a per residue basis. This value translates to a molar ellipticity for the phenolic chromophore of 85 000 deg cm²/dmol. The large magnitude of this CD band suggests that the Tyr is constrained in some manner. One possibility is that the Tyr residue is fixed in the helix part time or, alternatively, the hydrophobic phenolic side chain may interact with the methyl side chains of the Ala residues (J. A. Schellman, personal communication). It should be noted that far-UV aromatic CD bands in proteins can be as large as 85 000 deg cm²/dmol, but on average, they are around 6500 deg cm²/dmol (Woody, 1978).

The Tyr band of the yak − aak difference CD spectrum cannot entirely be attributed to the L_a transition because there might be coupling between the L_a and $n\rightarrow\pi^*$ transitions in yak which will not be present in aak. A common method for distinguishing phenolic CD contributions from peptide contributions is to measure CD under conditions where the phenolic group is ionized (Snow et al., 1977; Woody, 1985); we cannot use this method, however, because the high pH required for ionization of the Tyr residue will also cause significant pH-induced conformational changes.

Aromatic Contributions to the CD of Other Analogous Peptides. We investigated the context dependence of the Tyr CD Band by synthesizing several analogs of the yak peptide (Table I). When the Tyr residue is placed at the C-terminus (peptide aky), the value of $[\theta]_{222}$ is the same as in yak (Table I), indicating that the induced CD of the phenolic chromophore is the same at both positions. When we examined a peptide with Tyr residues at both ends (peptide yaky), however, we obtained the unexpected result that its value of $[\theta]_{222}$ was the same as that of yak and aky. The expected doubling of the effect of Tyr on the CD of these helical peptides was not observed. We have no explanation for this effect. We did, however, observe analogous effects in a series of 20-residue peptides (Table I).

Results with a peptide having Phe at the N-terminus (peptide fak; Table I) indicate that the phenyl chromophore can also contribute to the far-UV CD spectrum of these helical peptides. Like Tyr, the sign of the phenyl CD band at 222 nm is positive, but it is smaller in magnitude. These observations are consistent with the theoretical calculations of Woody (1978).

Effect of Glycine Linkers on Aromatic Contributions to CD. The experimental data discussed above suggest that the induced CD of the phenolic chromophore results from interaction with the helix. The relationship between the magnitude of the induced CD and the helical content of the peptide is likely to be complex. Consequently, it seems improbable that a general method can be devised to remove by calculation the aromatic contributions from the far-UV CD spectra of these peptides. We reasoned, however, that since the induced CD arises from interactions with the helix, it may be reduced or eliminated by separating the Tyr residue from the helix by means of a flexible linker. We used Gly as the flexible linker, and inserted one to three Gly residues between the Tyr residue and the rest of the helical peptide sequence; then the CD spectra were compared with those of analogous peptides containing either Ala or Trp in place of

Table II

name	sequence	$-[\theta]_{222}$[a]		
ygak	Ac-YGKAAAAKAAAAKAAAAK-amide	25400	26400	
agak	Ac-AGKAAAAKAAAAKAAAAK-amide		25900	25200
wgak	Ac-WGKAAAAKAAAAKAAAAK-amide	26500		
yggak	Ac-YGGKAAAAKAAAAKAAAAK-amide	24000	23000	
aggak	Ac-AGGKAAAAKAAAAKAAAAK-amide		24700	23100
wggak	Ac-WGGKAAAAKAAAAKAAAAK-amide	23800		
ygggak	Ac-YGGGKAAAAKAAAAKAAAAK-amide	23500	21500	
agggak	Ac-AGGGKAAAAKAAAAKAAAAK-amide		22400	22800
wgggak	Ac-WGGGKAAAAKAAAAKAAAAK-amide	21600		

[a] Mean residue ellipticity at 222 nm (deg cm^2/dmol). Conditions: 0 °C, pH 7, 1.0 M NaCl, 1 mM each of sodium borate, sodium citrate, and sodium phosphate. Values represent the mean of five measurements. The first, second, and third values are derived from data where peptide concentrations are measured by UV absorbance, ninhydrin assay, and NMR, respectively.

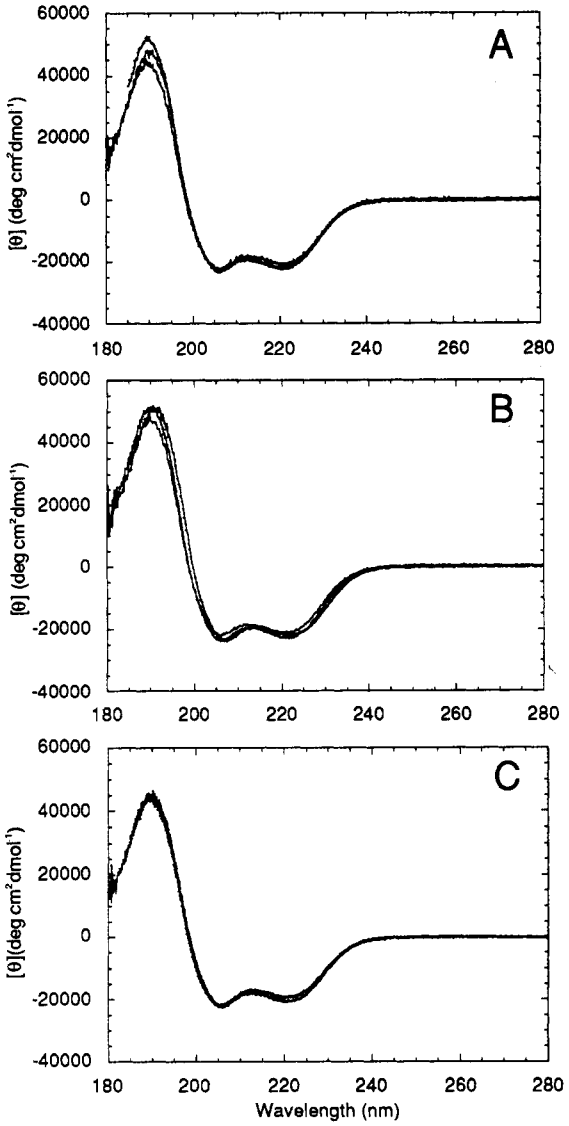

FIGURE 6: Circular dichroism spectra of (A) ygak, wgak, agak; (B) yggak, wggak, aggak; and (C) ygggak, wgggak, agggak. Conditions: same as Figure 1.

the N-terminal Tyr residue (Table II; Figure 6). Such a comparison requires that CD measurements be as accurate as possible. Since the ninhydrin method is not very accurate (error ±5%), we measured the peptide concentrations of the Ala peptides by an NMR procedure as well (see Materials and Methods). On the basis of these results, it appears that inserting one Gly residue between Tyr or Trp and the rest of the helical sequence is sufficient to reduce the induced CD to an insignificant level, within the level of error of the concentration determinations.

Table III

name	sequence	$-[\theta]_{222}$[a]
ygg-g7	Ac-YGGKAAGAKAAAAKAAAAK-amide	12900
agg-g7	Ac-AGGKAAGAKAAAAKAAAAK-amide	13700
ygg-g12	Ac-YGGKAAAAKAAGAKAAAAK-amide	8900
agg-g12	Ac-AGGKAAAAKAAGAKAAAAK-amide	9500
ygg-g17	Ac-YGGKAAAAKAAAAKAAGAK-amide	16100
agg-g17	Ac-AGGKAAAAKAAAAKAAGAK-amide	16900

[a] Mean residue ellipticity at 222 nm (deg cm^2/dmol). Conditions: 0 °C, pH 7, 1.0 M NaCl, 1 mM each of sodium borate, sodium citrate, and sodium phosphate. Values represent the mean of five measurements. Peptide concentrations are determined by UV absorbance for tyrosine peptides and by ninhydrin assay for alanine peptides. Data for the corresponding reference peptides, yggak and aggak, that lack an internal glycine residue are given in Table II.

Measurement of s(Gly) Using Peptides Containing Gly Linkers. The magnitude of the induced CD of Tyr in the yak peptide is quite large (5000 deg cm^2/dmol), and it caused a 17% underestimation of the helix content of this peptide. Since yak was used as the reference peptide in our previous evaluation of the helix propensity of Gly (Chakrabartty et al., 1991), probably the induced CD of Tyr introduced some error into this determination. To eliminate this error, the s-value of Gly was redetermined using the peptide yggak as the reference peptide. Three peptides, each with a Ala→Gly substitution in a different position, were synthesized, and then the data were analyzed with the Lifson–Roig theory (Lifson & Roig, 1961) by the procedure of Chakrabartty et al., (1991), using the same nucleation parameter as before. We also synthesized three analogous alanine peptides as controls. The sequences and data are in Table III, and the fit of the data to Lifson–Roig theory is shown in Figure 7. It should be noted that all of the Gly residues, including those in the flexible linker, were included in the fitting procedure. The parameters which best fit the helix contents of these peptides are (Lifson–Roig parameters) $\langle w \rangle = 1.64$ and $w(\mathrm{Gly}) = 0.053$; when translated to Zimm–Bragg parameters (Qian & Schellman, 1992), these give $\langle s \rangle = 1.54$ and $s(\mathrm{Gly}) = 0.05$. The parameters $\langle w \rangle$ and $\langle s \rangle$ refer to the average helix propagation parameters for all amino acid residues except Gly; the average is taken over all other residues in the peptide. Our previous estimate produced the values $\langle w \rangle = 1.66$, $w(\mathrm{Gly}) = 0.016$, $\langle s \rangle = 1.56$, and $s(\mathrm{Gly}) = 0.015$ (Chakrabartty et al., 1991), which do not produce a satisfactory fit to the data presented here (Figure 7). Other results (M. Scholtz, umpublished results; A. Chakrabartty, unpublished results) indicate that $s(\mathrm{Ala})$ and $s(\mathrm{Lys}^+)$ are close to 2.0 and 0.6, respectively, which are consistent with $\langle s \rangle = 1.54$ given here. Thus, using yggak as the reference peptide, we find the $s(\mathrm{Ala}):s(\mathrm{Gly})$ ratio is 40, as opposed to the ratio of 100 from our previous work.

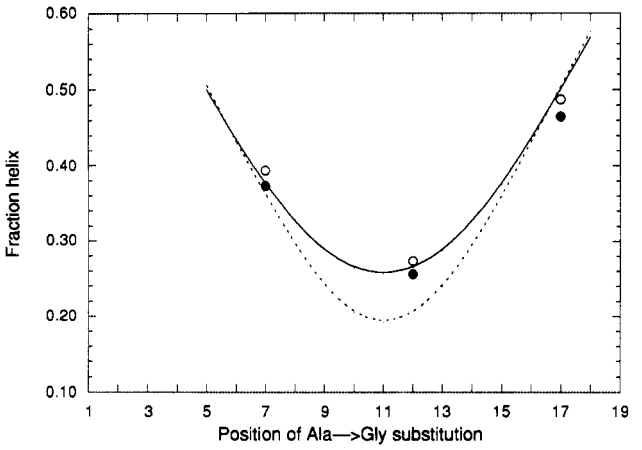

FIGURE 7: Experimental and computed fractional helix contents. The data points and curves represent experimental and computed values, respectively. Experimental fractional helix contents were determined from the $[\theta]_{222}$ measurements in Table III, using $-40000(1 - 2.5/n)$ and 0 deg cm^2/dmol as the values for 100 and 0% helix, respectively; n is the number of amino acid residues. Experimental helix contents for the two reference peptides are given in Table II. The solid curve was computed from the Lifson–Roig equation using the best-fit parameters $\langle s \rangle = 1.54$, $s(\text{Gly}) = 0.05$, and $\sigma = 0.0029$. The dashed curve was computed from the Lifson–Roig equation using the previously determined parameters of $\langle s \rangle = 1.56$, $s(\text{Gly}) = 0.015$, and $\sigma = 0.0029$ (Chakrabartty et al., 1991). (●) Tyr peptides; (○) Ala peptides (sequences and $[\theta]_{222}$ measurements are in Table III).

CONCLUSIONS

In summary, aromatic residues in helical peptides can give rise to substantial far-UV induced-CD bands, even when they are present at the frayed ends of the helix. The induced CD band is positive in the case of Tyr and Phe, and negative in the case of Trp. The broad aromatic CD bands interfere with structural interpretation of CD data and can cause significant errors in estimation of the helix content from $[\theta]_{222}$ values. The induced aromatic CD is reduced to an insignificant level, however, when the aromatic residue is separated from the helix by inserting one or more flexible Gly residues. Using this procedure, we redetermined the helix propensity of Gly, and found that the $s(\text{Ala}):s(\text{Gly})$ ratio is reduced from 100 to 40. The large induced CD that we discuss here should be present in any peptide containing an aromatic residue within a helix, and it introduces error into the measurement of helix propensities by CD.

ACKNOWLEDGMENT

We thank J. A. Schellman for helpful discussions, suggestions, and advice. We also thank S. L. Mayo for writing the binary search algorithm program for fitting CD data to helix–coil theory. We thank the Mass Spectrometry Facility, University of California, San Francisco (supported by NIH Grant RR 01614), for mass spectra of the peptides.

REFERENCES

Bayley, P. (1980) in *An Introduction to Spectroscopy for Biochemists* (Brown, S. B., Ed.) pp 148–234, Academic Press, London.

Bradley, E. K., Thomason, J. F., Cohen, F. E., Kosen, P. A., & Kuntz, I. D. (1990) *J. Mol. Biol. 215*, 607–622.

Brandts, J. F., & Kaplan, L. J. (1973) *Biochemistry 12*, 2011–2024.

Chakrabartty, A., & Hew, C. L. (1991) *Eur. J. Biochem. 202*, 1057–1063.

Chakrabartty, A., & Baldwin, R. L. (1992) in *Protein Folding: In Vivo and In Vitro* (Cleland, J., Ed.) ACS Books, New York (in press).

Chakrabartty, A., Schellman, J. A., & Baldwin, R. L. (1991) *Nature 351*, 586–588.

Edelhoch, H. (1967) *Biochemistry 6*, 1948–1954.

Englander, S. W., & Kallenbach, N. R. (1984) *Q. Rev. Biophys. 16*, 521–655.

Gans, P. J., Lyu, P. C., Manning, M. C., Woody, R. W., & Kallenbach, N. R. (1991) *Biopolymers 31*, 1605–1614.

Hooker, T. M., & Schellman, J. A. (1970) *Biopolymers 9*, 1319–1348.

Kemp, D. S., Boyd, J. G., & Muendel, C. C. (1991) *Nature 352*, 451–454.

Liff, M. I., Lyu, P. C., & Kallenbach, N. R. (1991) *J. Am. Chem. Soc. 113*, 1014–1019.

Lifson, S., & Roig, A. (1961) *J. Chem. Phys. 34*, 1963–1974.

Lyu, P. C., Liff, M. I., Marky, L. A., & Kallenbach, N. R. (1990) *Science 250*, 669–673.

Marqusee, S., Robbins, V. H., & Baldwin, R. L. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 5286–5290.

Merutka, G., Lipton, W., Shalango, W., Park, S. H., & Stellwagen, E. (1990) *Biochemistry 29*, 7511–7515.

Nelson, J. W., & Kallenbach, N. R. (1988) *Proteins: Struct., Funct., Genet. 1*, 211–217.

O'Neil, K. T., & DeGrado, W. F. (1990) *Science 250*, 646–651.

Osterhout, J. J., Baldwin, R. L., York, E. J., Stewart, J. M., Dyson, H. J., & Wright, P. E. (1989) *Biochemistry 28*, 7059–7064.

Padmanabhan, S., Marqusee, S., Ridgeway, T., Laue, T. M., & Baldwin, R. L. (1990) *Nature 344*, 268–270.

Pease, J. H. V., Storrs, R. W., & Wemmer, D. E. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 5643–5647.

Qian, H., & Schellman, J. A. (1992) *J. Phys. Chem. 96*, 3987–3994.

Rohl, C. A., Scholtz, J. M., York, E. J., Stewart, J. M., & Baldwin, R. L. (1992) *Biochemistry 31*, 1263–1269.

Rosen, H. (1957) *Arch. Biochem. Biophys. 67*, 10–15.

Scholtz, J. M., Qian, H., York, E. J., Stewart, J. M., & Baldwin, R. L. (1991) *Biopolymers 31*, 1463–1470.

Snow, J. W., Hooker, T. M., & Schellman, J. A. (1977) *Biopolymers 16*, 121–142.

Stellwagen, E., Park, S.-H., & Jain, A. (1992) *Biopolymers 32*, 1193–1200.

Strehlow, K. G., & Baldwin, R. L. (1989) *Biochemistry 28*, 2130–2133.

Sueki, M., Lee, S., Powers, S. P., Denton, J. B., Konishi, Y., & Scheraga, H. A. (1984) *Macromolecules 17*, 148–155.

Wojcik, J., Altmann, K. H., & Scheraga, H. A. (1990) *Biopolymers 30*, 121–134.

Woody, R. W. (1978) *Biopolymers 17*, 1451–1467.

Woody, R. W. (1985) in *The Peptides* (Udenfriend, S., Meienhofer, J., & Hruby, J. R., Eds.) Vol. 7, pp 15–114, Academic Press, Orlando, FL.

Ziegler, S. M., & Bush, C. A. (1971) *Biochemistry 10*, 1330–1335.

Zimm, B. H., & Bragg, J. K. (1959) *J. Chem. Phys. 31*, 526–535.