that these rates are indeed well correlated with τ_L^{-1}.[18] However, molecular solvation experiments[19] using time-resolved fluorescence spectroscopy have shown that this process is not controlled by τ_L. Furthermore, the relaxation is not a single exponential. Robinson et al. have suggested that using bulk water relaxation times to describe hydration dynamics of an ion depends on the ion involved.[8] For a large ion, the interaction between the ion and individual water molecules is not very specific, partly because of the large distance from the solvent to the generally delocalized charge on the ion. Little molecular reorientation of the water is required when this charge changes position or intensity, and the τ_D process is not dominant. On the other hand, for small ions, such as e^-, H^+, or OH^-, water physically wraps itself around the charge, and the ionic solvation process is more likely to be determined by τ_D. This picture is fully consistent with the 1-ROH*-2-S proton dissociation data presented here.

Table III compares the rate parameters for 1-ROH*, 2-ROH*, and 1-ROH*-2-S at 25 °C. The excited-state acidities (pK_a*) determined from k_{dis} and k_{rec} are 0.4, 1.58, and 2.72 for 1-ROH*, 1-ROH*-2-S, and 2-ROH*, respectively. In comparison with 1-ROH*, 1-ROH*-2-S has an activation barrier that slows down proton dissociation. 1-ROH*-2-S also shows a somewhat smaller steric/mobility factor Ω than the other two molecules (0.24 vs ~0.5). This may be caused by the presence of the $-SO_3^-$ group, which could interfere with the dissociation/recombination path. In spite of these differences, all three of these excited-state weak acids require a water structure $(H_2O)_{4\pm1}$ to activate the proton dissociation channel, and furthermore the rate-limiting step is regulated by the Debye rotational time τ_D of water. The consistency between the experimental findings and the theoretical model indicates that specific structural and dynamic effects of water play an important role in these weak acid dissociations. Any sort of continuum solvent model must give an incomplete picture for these types of problems.

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Conclusions

The excited-state proton dissociation/recombination in the excited state of 1-ROH-2-S has been explored in aqueous solutions. The results are compared with those from 1- and 2-ROH. The presence of intramolecular hydrogen bonding in 1-ROH-2-S causes this molecule to behave somewhat differently than 1- and 2-ROH. On the other hand, all three molecules reflect a common water structure $(H_2O)_{4\pm1}$ for accepting the dissociated proton H^+, and they reflect a common rate-limiting step, which is controlled by orientational motions in water on the time scale τ_D.

Acknowledgment. Financial support at the SPQR Laboratory has been shared by the Robert A. Welch Foundation (D-0005, 59% and D-1094, 13%), the National Science Foundation (CHE8611381, 15%), and the State of Texas Advanced Research Program (1306, 13%).

Registry No. 1-ROH-2-S, 61293-41-2; 1-ROH*-2-S, 567-18-0.

Conformational Aspects of the Interaction of Bradykinin and Related Peptides with Sodium Dodecyl Sulfate

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Abstract: Conformational aspects of the interaction of SDS with bradykinin (BK) and related peptides were probed by CD, ^1H, ^{13}C, and ^{19}F NMR. The spectrum of [99% ^{13}C-2-Gly^6]bradykinin, pH 8.3, confirmed the high cis/trans ratio about the sixth peptide bond. Addition of 5.2 mM SDS broadened both the cis and trans ^{13}C resonances but only shifted the trans. Moreover, the cis/trans ratio increased substantially. Thus, the cis isomer is enhanced in the complex. ^{19}F NMR of [Gly^6,*p*-fluoro-Phe^8]bradykinin indicated that the *p*-fluoro-Phe^8 also sensed the cis and trans isomers of Pro^7. Addition of the same molar ratio of SDS:BK analogue as used for [^{13}C-2-Gly^6]BK gave a spectrum showing a similar increase in cis/trans ratio, but the cis ^{19}F NMR peak was shifted the most. The strong interactions of monomeric SDS with bradykinin and its C-terminal tetrapeptide fragment, SerProPheArg, were reflected in a generalized broadening of all ^1H signals accompanied by selective shifts and changes in coupling constants of some resonances. For the trans conformer of SerProPheArg the significantly shifted resonances were α- and β-Ser, β^t- and γ^t-Pro, and the ring protons of Phe. Previous and current CD spectra also indicated changes in conformation upon interaction with SDS. The presence of selective shifts of the NMR resonances bears upon the interpretation of the CD bands.

The aqueous solution structure of the peptide hormone bradykinin (BK, Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9) and selected analogues has been extensively investigated.[1-10] Those studies indicated that BK has, despite the three Pro residues,[6] <10% cis conformers based on the lack of multiple ^1H or ^{13}C peaks for a given resonance, and it has little, if any, peptide backbone hydrogen bonding (<20% of Ser^6-NH is H-bonded[6,10]) despite the predicted[10] high probabilities for γ-turns for the Pro^{2}Pro^{3}Gly^{4}Phe^5 and Ser^{6}Pro^{7}Phe^{8}Arg^9 sequences based on protein data. Those studies also showed little, if any, interaction between the Phe residues[1,4] and that all the side-chain atoms underwent rapid internal motions.[6] Consequently, in aqueous solutions BK appears to exist largely in a disordered state or at

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least in a diversity of states on an NMR time scale.[10]

BK elicits a variety of effects in animals. It is involved in the perception of pain and inflammation, and it is a vasodilator. The cell membrane receptors for BK have not been isolated, but recent evidence indicated that the pain receptors are localized in the substantia gelatinosa, dorsal root and in some small cells in the dorsal root and trigeminal ganglia regions of the guinea pig nervous system.[11] Little is known about the receptor-binding site, but it likely will contain anionic moieties which interact with the Arg residues and a hydrophobic portion to interact with the Pro and Phe residues. A disordered state, or a diversity of states, for the solution structure of BK, however, does not appear compatible with specific site binding to a cellular receptor. Therefore, we have investigated the structural changes of BK, BK analogues, and its C-terminal tetrapeptide accompanying interaction with sodium dodecyl sulfate at concentrations well below its critical micelle concentration. BK is somewhat unique among small peptides in that it interacts strongly with anionic detergents and lipids,[12,13] probably partly because of its net positive charge over virtually the whole pH range. The earlier CD studies[12,13] indicated substantial conformational changes in BK and its C-terminal tetrapeptide even for nonmicellar SDS solutions. This report extends those studies to a lower SDS:peptide molar ratio and uses ^1H, ^{19}F, and ^{13}C NMR as well as CD to probe the ensuing conformational changes.

Experimental Section

Materials and Methods. Bradykinin and related peptides were synthesized by the solid phase method[14] and purified by counter current distribution methods as previously described.[2,15] [99% ^{13}C-2]Glycine was from ICN-KOR Isotopes, Cambridge, MA, and *t*-BOC-*p*-fluoro-D,L-Phe was from Southwest Foundation, San Antonio, TX. The L-isomer of [Gly^6,*p*-fluoro-Phe^8]BK that was used in this study was fractionated from the D-isomer by counter current distribution.

^1H NMR spectra were obtained on a Bruker WM-400 spectrometer at ambient temperatures of a 5-mm carbon/hydrogen probe and referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in "100%" ^2H_2O. Proton coupled and decoupled ^{13}C NMR spectra were obtained with the same instrument and probe but were referenced to *p*-dioxane in ^2H_2O whose resonance was taken as 67.4 ppm downfield from that of tetramethylsilane. ^{19}F NMR spectra were acquired with a 5-mm specific ^{19}F probe and were referenced to freon ($CFCl_3$) taken as zero ppm by using trifluoroacetate in ^2H_2O, pH 8.34 (all pH values given for ^2H_2O solutions are uncorrected meter readings except where otherwise indicated), as a secondary reference (−122.15 ppm).

Peptide solutions were prepared from weighed lyophilized quantities based on analysis for peptide. The solution pH was raised to either 7.4 or 8.4 from initial values of 4.0 to 5.5 with 1 M NaO^2H in ^2H_2O. Solutions containing SDS were prepared either by mixing peptide and detergent solutions at twice the final concentration (individually pH adjusted to give the desired final value) or by directly dissolving solid SDS with peptide solutions which had been adjusted to a pH slightly above the desired final value. The levels of SDS (electrophoresis purity, Bio-Rad Laboratories, Richmond, CA) used were always well below the critical micelle concentration, and all solutions were freshly prepared for each acquisition.

The CD spectra of Ser-Pro-Phe-Arg were recorded at 27 °C on a Cary Model 60 spectropolarimeter with a Model 6001 CD attachment. The instrument had been modified by a Cary service engineer to eliminate possible artifactual signals on passing through intense absorption bands. Slits were programmed for a 1.5-nm band width at each wavelength. Reagent grade *d*-10-camphorsulfonic acid (Eastman Kodak, Rochester, NY) was used to calibrate the instrument according to the procedure of Chen and Yang.[16] Cell pathlengths of 0.02–1.0 cm were used as dictated by the solution's absorbance. Mean residue ellipticities, $[\theta]_{mrw}$ (deg cm^2 dmol^{-1}), were based on the mean residue weight of 126.4 g/mol for the peptide. Each spectrum reported was the average of two or more superimposed scans.

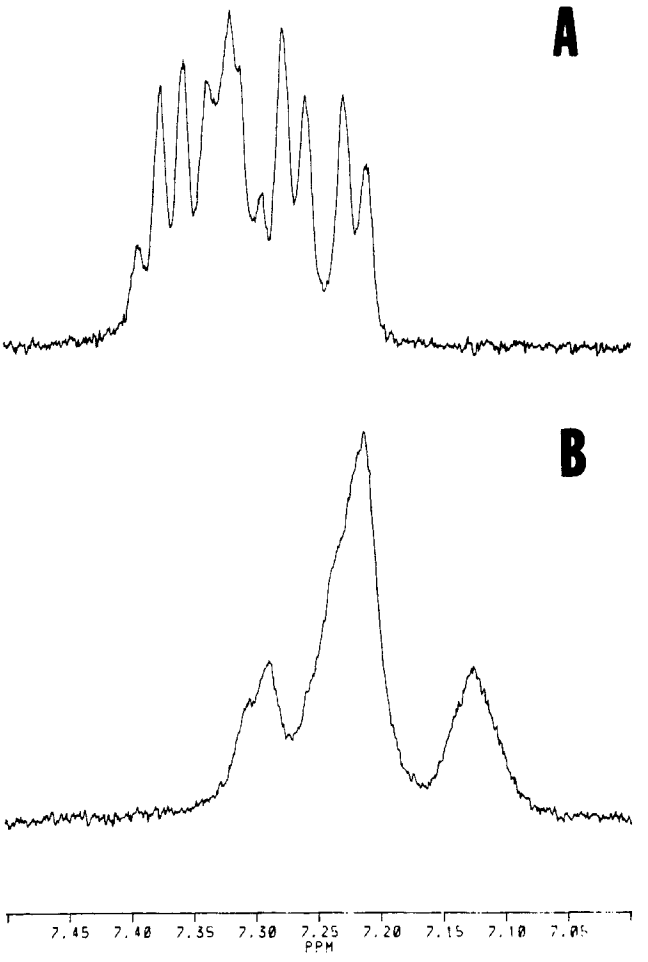

Figure 1. ^1H NMR spectrum of the aromatic region of bradykinin, 23°. A. 2.1 mM BK in ^2H_2O, pH 8.44. The spectrum was obtained from 128 60° pulses by using a 4000-Hz sweep width over 16 384 data points and solvent presaturation during a 3-s delay. It was processed with a 0.25 Hz line broadening after zero-filling to 32 768 data points. B. Same as A except that the solution was made 5.2 mM in SDS, final pH 8.34, and 880 acquisitions were used.

Results and Conclusions

Bradykinin–SDS. The readily comparable ^1H chemical shifts for 2.1 mM BK in ^2H_2O, pH 7.4, were all within 0.01 ppm, most agreed within 0.005 ppm of the shifts previously reported[10] for the same pH, and their apparent coupling constants agreed within ±0.2 Hz of those from the simulated spectrum.[10]

At a molar ratio of 7.4:1 SDS:BK all ^1H resonances were extensively shifted and broadened as indicated for the aromatic protons of Figure 1. This evidence for strong interactions between peptide and detergent was consistent with the earlier CD data[13] which suggested a cooperative interaction due to self-association of SDS-complexed peptide. The complex does not represent micelle formation since it had been shown that the critical micelle concentration of SDS, 8.2 mM, remained virtually the same in the presence or absence of BK.[13] The individual amounts of broadening due to association and exchange rate phenomena are not obtainable from these data, but both effects appear to be present. From the three broadened peaks observed for the aromatic protons of the two Phe residues (Figure 1) it is apparent that the chemical shift differences were diminished and that the rings were not as highly immobilized in the complex as they are

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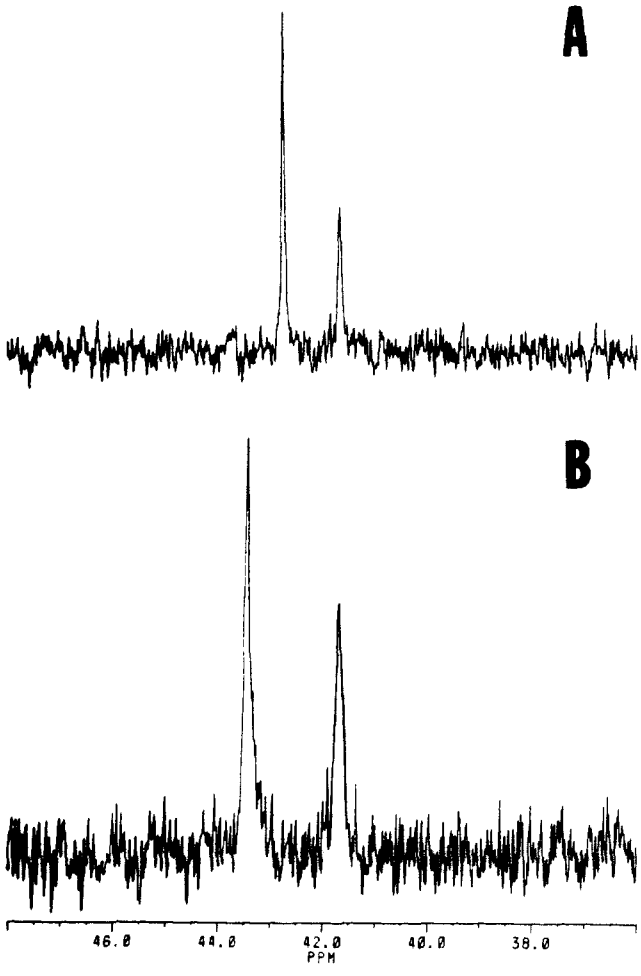

Figure 2. Proton decoupled ^{13}C NMR spectrum of [99% ^{13}C-2-Gly^6]BK, 28°. A. 0.69 mM peptide in 30% ^2H_2O, pH 8.41. The spectrum was acquired with 3600 90° pulses by using 16 384 data points over a 20 000 Hz spectral range and a 1-s relaxation delay. It was processed with a 1.5-Hz line broadening after zero-filling to 32 768 data points. B. Same as A except that the solution was made 5.2 mM in SDS, final pH 8.4, and 21 721 pulses were acquired.

Table I. Cis/Trans Ratios of [99% ^{13}C-2-Gly^6]BK and [Gly^3,*p*-fluoro-Phe]BK in the Presence and Absence of SDS

		^{13}C		^{19}F	
	pH	cis/trans	δ(cis–trans), ppm	cis/trans	δ(cis–trans), ppm
London et al.[19]	5.6	0.62	−1.14		
this study, native	8.4	0.52	−1.07	0.50	0.62
+ 5.2 mM SDS	8.4	0.72	−1.75	0.68	1.00

in some proteins.[17,18] The aliphatic peaks (not shown) were also extensively shifted and rather uniformly broadened at the same molar ratio of SDS:BK. As seen in Figure 1, the extensive broadening at this ratio precluded any observations of changes in spin–spin coupling constants. At lower ratios of SDS:BK for the same peptide concentration and at lower pHs the solutions precipitated soon after preparation.

The two ^{13}C NMR peaks for the methylene carbon of 0.69 mM [99% ^{13}C-2-Gly^6]BK (Figure 2) confirmed the ^{13}C NMR studies of London et al.[19] suggesting an unusually high cis/trans ratio for this BK analogue. That study showed that each of the three X-Pro bonds in native BK had <10% cis isomer but that when Gly was substituted for Ser^6 the cis/trans increased to ca. 0.6 at the Gly^6-Pro^7 bond. In comparison, the ^{13}C enriched analogue in 30% (v/v) $^2H_2O/H_2O$ had average ratios of 0.52, assuming

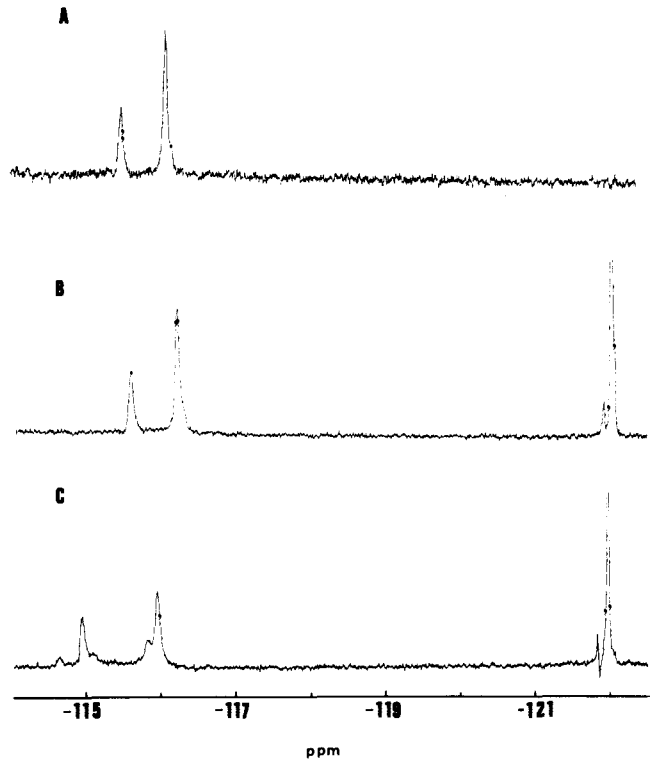

Figure 3. Proton coupled ^{19}F NMR of [Gly^6,*p*-fluoro-Phe^8]BK, 23°. A. 0.69 mM peptide acetate in "100%" ^2H_2O, pH 8.34. The spectrum was obtained with 2048 ca. 80° pulses over a 17 857 Hz spectral width by using 16 384 data points and a 1.5-s relaxation delay. It was processed with a 2.0 Hz line broadening after zero-filling to 32 768 data points. B. Same conditions as for A except for using the trifluoroacetate salt of the analogue and 30% ^2H_2O. C. Spectrum acquired identically with that in B at the same peptide concentration but with 5.2 mM SDS added. The small signal immediately downfield from the intense TFA peak is considered to be a carrier frequency artifact.

comparable relaxation times for the isomers, and chemical shift differences of −1.066 ppm for duplicate determinations which agree reasonably well with the earlier results (Table I).

The calculated Gaussian line widths at half-height, $\Delta\nu_{1/2}$, were consistently about 35% greater for the cis isomer than those for the trans form. A greater cis $\Delta\nu_{1/2}$ for [Gly^6]BK could reflect (i) its smaller proportion, (ii) several incompletely resolved forms in slow exchange with even more poorly resolved trans isomers, or (iii) a subpopulation of cis conformers with intermediate or slow interconversion rates among themselves. The latter possibility was supported by the temperature dependence studies which yielded the following data at 5, 27, and 45°: δ(cis − trans) = −1.14, −1.08, and −0.99 ppm; cis/trans intensity ratios = 0.45, 0.43, and 0.49; and cis/trans $\Delta\nu_{1/2}$ ratios = 1.32, 1.29, and 1.01, respectively. The decreasing values of δ(cis − trans) with increasing temperature indicated faster rates of isomerization, whereas the accompanying substantial decreases in the $\Delta\nu_{1/2}$ ratios, at essentially constant intensity ratios, were compatible with enhanced rates of interconversion within subpopulations of cis conformers.

At a molar ratio of 7.5:1 SDS:[Gly^6]BK the cis/trans ratio rose to 0.72 showing that SDS interacted preferentially with the cis isomer. The chemical shift difference increased from −1.066 to −1.752 ppm (Figure 2 and Table I) which was due virtually entirely to a downfield shift in the trans peak. Line widths were also affected differently when complexed with SDS. The increases in $\Delta\nu_{1/2}$ ranged from 1.7- to 2.0-fold for the cis form and 2.3- to 2.4-fold for the trans isomer. Those results further supported the argument for several incompletely resolved cis conformers of the unreacted peptide. Similar solutions of *t*-Boc[99% ^{13}C-2]Gly with and without SDS did not show a significant change in chemical shift or line width of the C2 signal indicating a lack of any generalized effect from the addition of SDS on those NMR parameters.

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Table II. ^{1}H Resonances of SerProPheArg vs Those for the Corresponding Residues of Bradykinin and Random Coils

	SerProPheArg				bradykinin		random coil residues[a]
residue	δ,[b] ppm	pH 7.4 apparent J, Hz	δ, ppm	pH 8.4 apparent J, Hz	δ, ppm	pH 7.4 J[c] Hz	δ, ppm
Ser							
α	4.100		3.886	$J_{\alpha\beta}$ = 6.03	4.677	$J_{\alpha\beta}$ = 6.38	4.50
β	3.780		3.720	$J_{\alpha\beta'}$ = 7.06	3.771	$J_{\alpha\beta'}$ = 7.43	3.88
β′	3.773	$\lvert J_{\beta\beta'}\rvert$ = 11.7	3.614	$\lvert J_{\beta\beta'}\rvert$ = 11.1	3.717	$J_{\beta\beta'}$ = −11.4	3.88
Pro							
α	4.417	$J_{\alpha\beta^c}$ = 5.07	4.411	$J_{\alpha\beta^c}$ = 4.22	4.321	$J_{\alpha\beta^c}$ = 4.3	4.44
β^t	2.16	$J_{\alpha\beta^t}$ = 8.49	2.14	$J_{\alpha\beta^t}$ = 8.52	2.151	$J_{\alpha\beta^t}$ = 8.7	2.28
β^c	1.65		1.66[d]		1.685[d]		2.02
γ^t	1.86		1.85[e]		1.90		2.03
γ^c	1.65		1.66		1.683		2.03
δ^t	3.58		3.45		3.547		3.65
δ^c	3.67		3.68		3.586		3.68
minor Pro							
α	4.532	$J_{\alpha\beta^c}$ = 4.58 $J_{\alpha\beta^t}$ = 6.49	4.532	$J_{\alpha\beta^c}$ = 2.6 $J_{\alpha\beta^t}$ = 8.7			4.44
Phe							
α	4.595	$J_{\alpha\beta(S)}$ = 6.33	4.648	$J_{\alpha\beta(S)}$ = 5.77	4.648	$J_{\alpha\beta(S)}$ = 5.61	4.66
β(S)	3.16	$J_{\alpha\beta(R)}$ = 8.96	3.18[f]	$J_{\alpha\beta(R)}$ = 9.20	3.232	$J_{\alpha\beta(R)}$ = 9.71	3.22
β(R)	2.969	$\lvert J_{\beta(S)\beta(R)}\rvert$ = 13.96	2.945	$\lvert J_{\beta(S)\beta(R)}\rvert$ = 14.10	2.935	$J_{\beta(S)\beta(R)}$ = −13.99	2.99
2, 6 H	7.241	J_{23} = 7.05 and 7.45	7.244	J_{23} = 7.29 and 7.53	7.280		7.30
4 H	7.301	J_{34} = 5.41 and 7.16	7.305	J_{34} = 5.28 and 6.91	7.31		7.34
3,5 H	7.337		7.343		7.390		7.39
Arg							
α	4.105		4.119	$J_{\alpha\beta(S)}$ = 5.13	4.150	$J_{\alpha\beta(S)}$ = 5.15	4.38
β′	1.72		1.80	$J_{\alpha\beta(R)}$ = 7.64	1.830	$J_{\alpha\beta(R)}$ = 8.11	1.89
β	1.67		1.69		1.702		1.79
γ	1.536		1.54		1.551		1.70
δ′	3.146		3.17		3.160		3.32
δ					3.180		3.32

[a] From (22). [b] All shifts are relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in "100" ^2H_2O. [c] Partial listing of simulated shifts and coupling constants at 600 MHz.[10] [d] Overlaps with γ[c]-Pro and β-Arg. [e] Partially overlaps with β′-Arg. [f] Overlaps with δ-Arg.

A ^{1}H coupled ^{19}F NMR of [Gly^6,*p*-F-Phe^8]BK acetate at pH 8.4 without SDS showed two signals of unequal intensity separated by 0.62 ppm and whose intensities indicated a cis/trans ratio of 0.50 (Figure 3A). In this case, however, the less intense peak (−115.564 ppm) was downfield from the more intense signal (−116.184 ppm). A similar spectrum obtained for a sample of the peptide in which the trifluoroacetate (TFA) counterion had not been exchanged for acetate showed three peaks; the same two as above (cis/trans ratio of 0.49) plus a third peak at −122.161 ppm which had an apparent intensity of 1.8 times that of the sum of the BK analogue peaks (Figure 3B). (That value is reasonable for 2–3 counterions considering the likely effects of differences in relaxation times for [Gly^6,*p*-fluoro-Phe^8]BK and trifluoroacetate.) ^{1}H decoupled spectra gave essentially the same cis/trans ratio (0.54) but decreased $\Delta\nu_{1/2}$ for the peptide peaks 5-fold, from approximately 25 to 5 Hz, with little change in $\Delta\nu_{1/2}$ for the TFA signal. The lack of resolvable *o*-H to *p*-F scalar coupling in *p*-F-Phe was consistent with the observations of others.[20] Such a large decrease in $\Delta\nu_{1/2}$ when proton decoupled, which was also observed with *t*-Boc-*p*-F-D-Phe, suggested extensive unresolved couplings to all the ring protons. Of current interest, however, was the fact that even a *p*-fluoro nucleus on the Phe^8 substituent can sense the cis/trans isomerization in the Gly^6-Pro^7 bond of [Gly^6]BK.

At the same pH and at a molar ratio of 7.5:1 SDS:[Gly^6,*p*-F-Phe^8]BK-TFA the major cis and trans peaks were shifted downfield by 0.61 and 0.21 ppm, respectively (Figure 3C), from those of the native peptide which increased the separation of the peaks to 1.00 ppm (Table I). In contrast, the TFA signal shifted only 0.03 ppm. *t*-Boc-*p*-F-D-Phe showed no detectable shift or change in line width with and without SDS at the same concentrations and molar ratios. The SDS-induced peptide shifts for the major cis and trans forms observable with ^{19}F NMR were in contradistinction to those for [99% ^{13}C-2-Gly^6]BK. Thus, most of the increase in the ^{19}F Δδ was due to the downfield shift of the cis form not the trans form. The cis/trans ratio of the major *p*-F-Phe peaks in SDS, however, increased to 0.68, similar to the increase seen with ^{13}C-Gly (Table I). Thus, the ^{19}F cis/trans ratio data also indicated a preferential interaction of SDS with the cis form.

The ^{19}F spectra in SDS, however, indicated multiple slowly interconverting forms of the complexes as evidenced by the partially resolved peaks on either side of the cis peak and on the downfield side of the trans signal (Figure 3C) which were not observed in the ^{13}C spectra. These "extra" peaks were better resolved in the ^{1}H decoupled, ^{19}F spectra (not shown). This probably reflected the greater chemical shift sensitivity of ^{19}F compared to ^{13}C nuclei and/or the better signal-to-noise ratio obtained in the ^{19}F spectra. The smaller peaks may have reflected multiple slowly interconverting forms of the peptide–SDS complexes due to the self-association inferred from Job plot data.[13] Since the minor peak intensities were not considered in the cis/trans ratio calculations, that value may not be as accurate as the ^{13}C data. Complexation of [Gly^6,*p*-fluoro-Phe^8]BK with monomeric SDS increased $\Delta\nu_{1/2}$ for the ^{1}H coupled and decoupled ^{19}F signals as it had for [Gly^6]BK, but the broadenings were smaller than observed for the ^{1}H or ^{13}C peaks. As expected addition of SDS produced only about a 30% increase in $\Delta\nu_{1/2}$ for the ^{1}H coupled spectrum and about 80% when ^{1}H decoupled. Only ^{1}H decoupled spectral parameters are properly indicative of molecular motions and exchange reactions.

L-Ser-L-Pro-L-Phe-L-Arg. Homonuclear COSY[21] (Figure 4) and some specific resonance decoupling experiments allowed assignment of the ^{1}H resonances of the peptide. At pH 7.4 all major resonances were assignable except for the γ-Pro protons whose off-diagonal peaks were too close to the diagonal to be observed and the β-Ser protons which showed strong coupling. Except for the N-terminal α- and β′-Ser resonances, the α hy-

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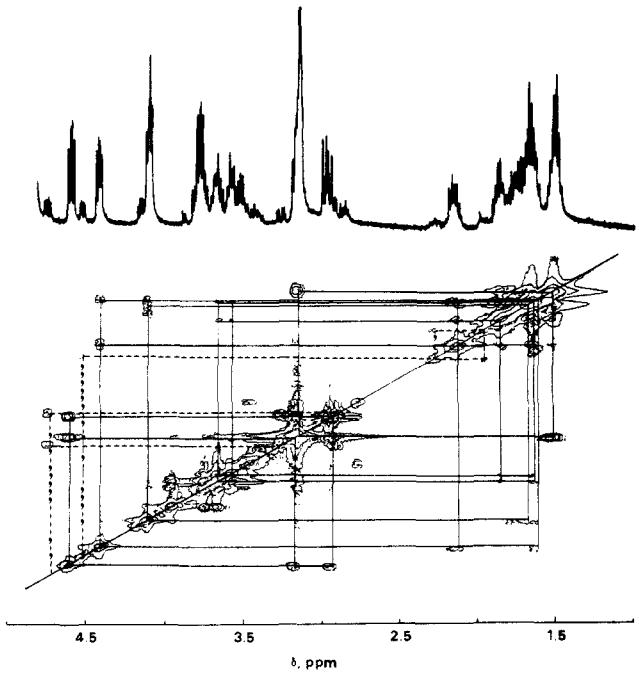

Figure 4. Symmetrized, absolute value homonuclear COSY of the aliphatic protons of SerProPheArg at 10 mg/mL, pH 7.43, in ^2H_2O, 23°. Dashed lines indicate couplings between resonances of minor forms.

drogens of Pro and Phe, and the β′-Arg, all other chemical shifts were within 0.05 ppm of those for the corresponding residues in BK[10] (Table II). Among the major Pro and Phe peaks only the β^c- and γ^c-Pro resonances were shifted (upfield) by >0.3 ppm from the predicted internal residue random coil shifts[22] suggesting a unique environment for those protons in SerProPheArg that are cis to the carbonyl oxygen of the proline residue. Interestingly, similar unusual shifts were observed for the same resonances of Pro[7] in BK[10] at pH 7.4 and at pH 8.4 (this study). One environment which would give upfield shifts for those protons is a position above the Phe ring. The appropriately smaller upfield shifts for the major β^t- and γ^t-Pro resonances (0.12 and 0.17 ppm, respectively, Table II) would also follow from such an orientation provided the proline ring was approximately parallel to that of the Phe residue. In principle ROESY experiments might be used to confirm this proposed hydrophobic interaction. However, a ROESY analysis of SerProPheArg in water[23] gave only weak to very weak cross peaks indicating rapidly interconverting forms on an NMR time scale, and no geometric analyses were reported for the peptide in this solvent.

In contrast to BK, however, the C-terminal tetrapeptide showed several minor resonances, some of which could be assigned (Figure 4). ^1H NMR amide proton studies[6] of this peptide had shown minor resonances that were interpreted as arising from a slow interconversion of cis–trans isomers of Pro in the molecule. In the aliphatic proton region (Figure 4), the assignable minor resonances of SerProPheArg represented from 12 to 18% of the total signal for a given nucleus which agreed reasonably well with the ca. 13% reported earlier and, therefore, probably reflected the same phenomenon.

Raising the pH from 7.4 to 8.4 affected the Ser chemical shifts substantially, as expected, but also shifted δ^t-Pro upfield by 0.13, α-Phe downfield by 0.053, and β′-Arg downfield by 0.08 ppm. All other observable peaks shifted by <0.02 ppm (Table II). There were, however, more generalized changes in the directly measurable apparent coupling constants (Table II). In particular, $J_{\alpha\beta^c}$ for Pro, both major and minor peaks, and the $J_{\alpha\beta}$ values for Phe decreased in the higher pH solution indicating some changes in rotomer populations (Table II).

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(23) Otter, A.; Scott, P. G.; Cann, J. R.; Vavrek, R. J.; Stewart, J. M.; Kotovych, G. *J. Biomol. Struct. Dyn.* **1988**, *6*, 609–625.

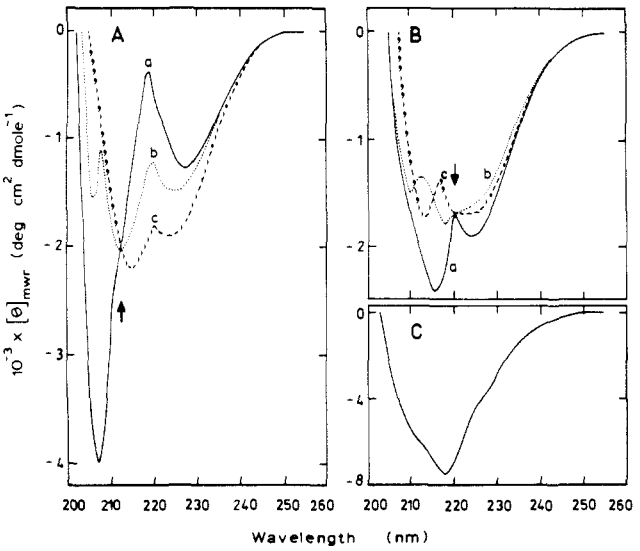

Figure 5. CD spectra of SerProPheArg in "100%" ^2H_2O. A. 0.18 mM peptide: a, pH 4.02; b, pH 7.06; and c, pH 8.44. Vertical arrow indicates the isosellipticity point. The pH 5.06 spectrum (not shown) also passed through the isosellipticity point. B. 0.4 mM peptide, pH 8.4 in the presence and absence of SDS: a, no SDS; b, 0.4 mM SDS; and c, 0.8 mM SDS. Vertical arrow indicates isosellipticity point. C. 0.4 mM peptide, pH 8.4 in the presence of 6.9 mM SDS. All pH values are meter readings.

The far UV CD spectral properties of SerProPheArg in "100%" ^2H_2O were highly pH dependent between pH 4.0 and 8.4 (Figure 5A). At pH 4.0 the spectrum was characterized by negative bands at 227.5 and 207 nm with the 207-nm band the more intense. As the pH was raised from 4.0, the amplitude of the negative band at 207 nm sharply decreased and shifted to higher wavelengths, while the amplitude of the weaker band at 227.5 increased moderately and shifted somewhat to lower wavelengths. There was an apparent isosellipticity point at 213 nm indicative of a two-state system, A = B + $^2H^+$. In that case

$$\log \frac{[\theta]_A - [\theta]}{[\theta] - [\theta]_B} = -pK + p^2H$$

where $[\theta]$ is the observed ellipticity at 228 nm, subscripts A and B designate the two states, and p^2H = pH + 0.4.[24] Assuming that the p^2H 4.42 spectrum represented pure state A and the p^2H 8.84 spectrum pure B, the ellipticities at p^2H 5.46 and 7.46 indicated a pK of 7.5 ± 0.5. Thus, the two states probably reflected the ionization of the N-terminal amino group of Ser and, therefore, correlated with the changes in ^1H chemical shifts and coupling constants between pH 7.4 and 8.4.

SerProPheArg–SDS. The interaction of SerProPheArg with monomeric SDS also was investigated by CD and ^1H NMR. To better follow the changes with increasing concentration of SDS, these data were collected at lower ratios than the 7.5:1 used with BK and its analogues. Even at low ratios the CD of SerProPheArg at pH 8.4 showed significant changes with increasing SDS (Figure 5B). For a molar ratio of 1:1 SDS:peptide the amplitude of both of the native peptide's negative CD bands (curve a) decreased, and a new negative band appeared that was superimposed on the lower wavelength feature (curve b). When the ratio was increased to 2:1, there were further changes in the spectrum which now showed two bands: a broad one centered at 224 nm and a sharper one at 213 nm (curve c). An apparent isosellipticity point at 220.5 nm indicated a two-state interaction with respect to this spectroscopic probe. At a higher SDS:peptide ratio (Figure 5C), the CD spectrum of the tetrapeptide changed extensively, and the apparent isosellipticity point was lost, perhaps indicating self-association of the complexes. Overall the CD changes occasioned by SDS are indicative of peptide conformational changes.

(24) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188–190.

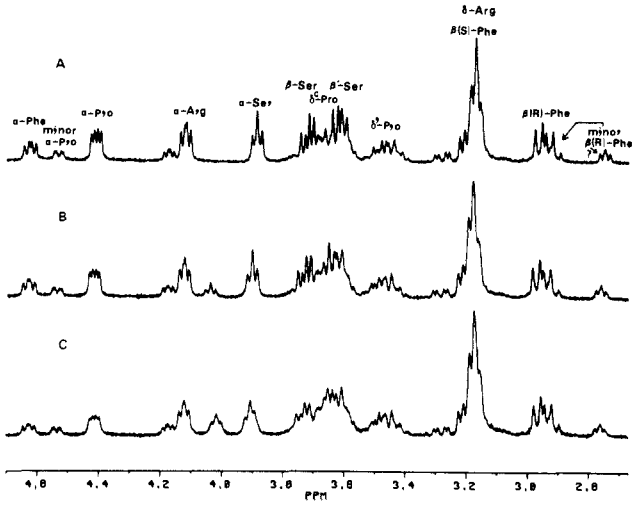

Figure 6. ^{1}H NMR spectrum of the downfield portion of the aliphatic proton region of SerProPheArg, 23°. Each spectrum was acquired with 400 60° pulses over a 4000 Hz spectral width by using 16 384 data points and with solvent suppression during a 3-s relaxation delay. They were transformed after zero-filling to 32 768 data points and application of −0.2 Hz line broadening. A. 2.47 mM peptide in "100%" ^2H_2O, pH 8.4, without SDS. B. Same as A but with 0.570 mM SDS added, pH 8.4. The new peak at 4.07 ppm is from the H1 resonance of SDS. C. Same as A but with 1.10 mM SDS added, pH 8.4. All pH values are meter readings.

^{1}H NMR spectra of SerProPheArg at molar ratios of SDS:peptide from 0.12 to 0.96:1 showed shifts only of certain resonances as illustrated in Figure 6 for the α-proton region. The chemical shifts differences were greatest at and near the Ser residue, suggesting that, when complexed, the sulfate group of SDS was located near the N-terminus. The protons showing the largest shifts (Figure 7) were H1 of SDS, α-, β-, and β′-Ser, β^t- and γ^t-Pro, the Phe ring protons (not shown), and the minor β(R)-Phe resonance. In fact the shifts for the para proton of Phe with increasing ratio of SDS:peptide paralleled, and was of comparable magnitude, to those for the β^t- and γ^t-Pro resonances. Shifts for the ortho and meta protons also were comparable in magnitude but instead of moving upfield after an initial downfield shift, they continued to move downfield more like the behavior of the minor β(R)-Phe resonance (Figure 7). Interestingly, the interaction of the internal chemical shift reference compound, tetradeuterio-(trimethylsilyl)propionate, with BK predominantly shifted the same residues[10] as did complexation of SDS with SerProPheArg.

Those resonances showing little, if any, effect of complexation with SDS were α-Pro, the major β(*R*)-Phe, and all the resolved Arg protons except the β′-Arg. A larger shift in the tentatively assigned minor β(*R*)-Phe resonance than for the major β(*R*)-Phe signal suggested that the interaction with SDS was different for the two forms of the peptide. Such an effect was reminiscent of the preferential interaction of SDS with the cis conformer of [^{13}C-2-Gly^6]BK. Where significant shifts were observed, the nature of the change with increasing ratios of SDS:peptide suggested a cooperative process wherein the initial reaction produced little change in δ, except for H1 of SDS, but abruptly increased followed by a period of less dramatic change that sometimes was in the opposite direction. This may have reflected the two-step process of complex formation followed by self-association as deduced earlier from Job plot data.[13]

Even though the interaction with SDS selectively shifted some of the peptide protons, measured line widths (Table III) increased essentially uniformly for all resonances, including 1 H of SDS (Figure 6). Broadening was not due to a viscosity effect since the highest concentration of SDS used was only 2.4 mM, and a 5.2 mM solution of SDS alone had given sharp lines. Also, the proton signals of *t*-Boc-Gly and *t*-Boc-*p*-fluoro-D-Phe were unaffected by this concentration and molar ratio of SDS. Thus, broadening more likely resulted from reduced rotational correlation times for the self-association products of the SDS–peptide complex

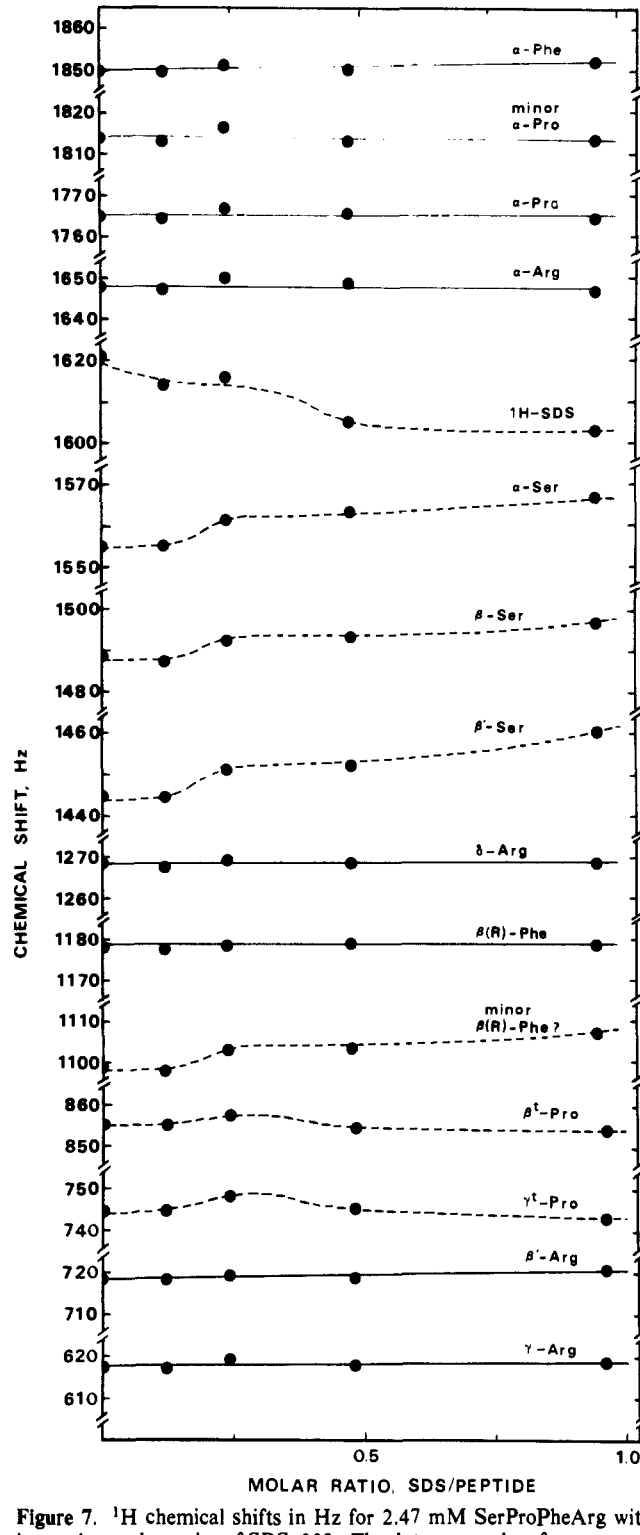

Figure 7. ^{1}H chemical shifts in Hz for 2.47 mM SerProPheArg with increasing molar ratios of SDS, 23°. The data were taken from spectra acquired and processed as described in Figure 6. Shifts for the resonances below 860 Hz are from one of the multiplet peaks. All other shifts are for the resonance position.

or from an intermediate exchange rate among one or more of the complexes involved.

Changes were also visible in the apparent coupling constants for some of the well-resolved resonances (Table III). Those showing substantial changes were $J_{\alpha\beta}$ for Ser, $J_{\alpha\beta}$ and $J_{\alpha\beta'}$ for Arg, $J_{\gamma^t\delta^t}$ for the major Pro species, and $J_{\alpha\beta'}$ for the minor Pro form of the peptide. For each of these the apparent *J* decreased, indicating a change toward a higher relative contribution from gauche forms[25] in those side-chain conformations when complexed

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Table III. Changes in Apparent Coupling Constants and Line Widths with Increasing Molar Ratios of Sodium Dodecyl Sulfate/SerProPheArg

resonance	coupling constant/ line width, Hz	SDS/peptide 0	0.12	0.24	0.48	0.96
α-Ser	$J_{\alpha\beta} = J_{\alpha\beta'}$	6.5	6.5	6.2	5.7	5.7
	$\Delta\nu_{1/2}$[a]	3.5	3.8	*b*	*b*	*b*
β-Ser	$J_{\beta\beta'}$	11.1	11.1	11.3	11.3	
	$J_{\alpha\beta'}$	6.0	6.1	5.8	6.0	
	$J_{\alpha\beta}$	7.0	7.0	7.3	6.6	
α-Pro	$J_{\alpha\beta'}$	8.7	8.9	7.6	7.6	6.7
(minor)	$J_{\alpha\beta}$	2.5	2.6	2.2	2.7	2.2
α-Pro	$J_{\alpha\beta'}$	8.4	8.5	8.0	8.0	8.3
	$J_{\alpha\beta}$	4.2	4.2	3.8	4.2	3.3
δ[t]-Pro	$J_{\delta\delta}$t	16.8	16.4	16.6	16.4	*b*
	J_{γ}t$_{\delta}$t	11.0	10.7	9.0	8.2[c]	8.3[c]
	J_{γ}c$_{\delta}$t	9.0	8.8	7.7	8.2	8.3
α-Phe	$J_{\alpha\beta(R)}$	9.1	9.3	8.2	8.5	8.3
	$J_{\alpha\beta(S)}$	6.5	5.8	6.7	6.4	6.4
	$\Delta\nu_{1/2}$[a]	3.4	3.4	4.2	4.1	3.7
β(*R*)-Phe	$J_{\beta(S)\beta(R)}$	14.1	14.1	14.1	14.0	13.9
	$J_{\alpha\beta(R)}$	9.2	9.1	9.2	9.3	9.8
	$\Delta\nu_{1/2}$[a]	3.6	3.9	4.1	4.7	4.9
β(*R*)-Phe	$J_{\beta(S)\beta(R)}$	13.0	13.2	*b*	*b*	*b*
(minor)	$J_{\alpha\beta(R)}$	6.5	6.6	6.4	5.8	6.1
	$\Delta\nu_{1/2}$[a]	3.1	3.3	4.4	5.4	*b*
α-Arg	$J_{\alpha\beta'}$	7.5	7.7	6.1[b]	6.3[b]	6.1[b]
	$J_{\alpha\beta}$	4.8	4.7	6.1	6.3	6.1
	$\Delta\nu_{1/2}$[a]	3.5	3.8	4.4	5.8	*b*
H1-SDS	$J_{^1H^2H}$		6.8	6.2	5.9	5.4

[a] Measured for the outermost peaks of the multiplet. [b] Coalesced to an apparent triplet. [c] No measurable differences in the α-proton couplings.

with SDS. Although SDS selectively shifted and changed the coupling constants of different minor resonances than it had among the major peaks, the relative intensities of the minor peaks remained at ca. 15% up to 0.96:1 SDS:peptide. This apparent lack of preferential interaction of SDS with the cis form of the tetrapeptide was in contrast to what was seen for [Gly[6]]BK by ^{13}C and ^{19}F NMR at 7.5:1 SDS:BK. The CD spectra of SerProPheArg, however, indicated further changes at a 7.5:1 SDS:peptide molar ratio. Thus, any potential preferential binding to the cis form may not yet have been detectable at 0.96:1 SDS:peptide.

Discussion

In contrast to naturally occurring BK, ^{13}C NMR spectra of the [99% ^{13}C-2-Gly[6]]BK analogue indicated a high cis/trans ratio for Pro[7] which was confirmed in the ^{19}F NMR spectra of [Gly[6],*p*-fluoro-Phe[8]]BK (Table I). (The finding that *p*-fluoro-Phe[8] sensed the cis–trans isomers of Pro[7] is in accord with the model shown in Figure 6 of London et al.[19] to account for the ^{13}C NMR parameters of [Gly[6]]BK.) The ^1H decoupled ^{13}C line width calculated for the cis conformer, however, was consistently 30–40% greater than that for the trans form. Since the CD spectra of the [Gly[6]]peptide obeyed Beer's law, the greater line width for the cis form apparently was not due to differences in conformer self-associations. Neither was it likely due to less motional freedom in the cis form since the spin–lattice relaxation time (T_1) was 12% larger at 25° for the cis Gly C-2 carbon of [20% ^{13}C-Gly-1,2]BK in 60% methanol–40% ^2H_2O than for the *trans*-methylene carbon.[19] Temperature-dependence data for the cis–trans isomerization suggested that the difference resulted mostly from subpopulations of cis conformers undergoing intermediate or slow interconversions. This conclusion also is supported by the ^{19}F NMR data on [Gly[6],*p*-fluoro-Phe]BK which did not show significant differences in line widths probably because the time scale defining intermediate exchange rates, for comparable Δppm, differs by nearly 4-fold from that for ^{13}C.

For [Gly[6]]- and [Gly[6],*p*-fluoro-Phe[8]]BK, the extent of interaction with nonmicellar SDS also differed for the two isomers (Table I). (The exclusive use of nonmicellar SDS concentrations was considered appropriate since it is the SDS monomer that interacts with proteins.[26]) In addition to the selective shifts of the isomers, complexation with SDS increased the cis/trans ratio of [Gly[6]]BK for both analogues. This sensitivity to cis/trans conformations implies a strong structural bias for the interaction of SDS with a peptide. Such specificity has not been generally appreciated, perhaps because SDS is such a powerful denaturant of proteins.

The amount of broadening due to complexation compared to that from interconversion rates among subpopulations was not obtainable with the present results. But, temporarily assuming that all the broadening in the presence of SDS was due to the greater effective mass of the associated species, the increases in $\Delta\nu_{1/2}$ for [^{13}C-2-Gly[6]]BK can be used to place an upper limit on the size of the complex. Since protonated carbons are relaxed almost exclusively by dipolar coupling,[27] an increase in $\Delta\nu_{1/2}$ at 100.6 MHz for ^{13}C from 6.9 to 16.9 Hz implies a 4.8-fold increase in volume for the trans species based on the Stokes relationship for spheres. Similarly, increases in $\Delta\nu_{1/2}$ for the cis form (9.4–18.9 Hz) gives a maximum volume increase of 3.6-fold. Considering the volume of an SDS molecule to be about 1/5 of that for BK, the line width data suggests that the trans complex could contain no more than four and the cis form complexes no more than three peptide molecules. But, the complex probably contains fewer peptide molecules since, based on the ^{19}F NMR line width data, not all the broadening is due to slower motions.

^{19}F data for [Gly[6],*p*-fluoro-Phe[8]]BK showed much less increase in $\Delta\nu_{1/2}$ upon the addition of the same molar ratio of SDS. Since the fluorine nucleus normally is relaxed predominantly by the chemical shift anisotropy mechanism, it should be even more sensitive to reduced rotational correlation times. Furthermore, the *p*-fluoro-Phe nucleus should reflect the motions of the molecule as a whole even if the Phe ring were rotating rapidly since it would be coincident with that axis of rotation. (That was the case at least for the *p*-carbon of Gly-Pro-Phe wherein its T_1 was not significantly different from that of α-Phe, while the ortho and meta carbons had longer T_1's.[19]) Thus, the ^{19}F data are consistent with a complex containing *fewer* than three to four peptide molecules. Consequently, the data on the two analogues, when taken together, suggest that a major contribution to the increases in $\Delta\nu_{1/2}$ for the ^{13}C Gly analogue–SDS complex is due to intermediate exchange rates or unresolved chemical shift heterogeneity from several slowly interconverting states. Smaller complexes would also be compatible with the Job plot data[13] which were well-fitted to a multiple equilibrium model involving 1:1, 2:1, and 2:4 BK:SDS species.

ΔCD of native vs SDS complexed peptides demonstrated a much greater similarity between BK and SerProPheArg than between BK and its N-terminal tetrapeptide.[13] This similarity was confirmed by the near identities of the chemical shifts of the Pro, Phe, and Arg residues of SerProPheArg and the corresponding residues of BK. Therefore, studies of SerProPheArg and its interaction with SDS were included to better define the nature of the interaction with BK. In contrast to BK, however, the ^1H NMR of SerProPheArg showed numerous minor peaks some of which were assignable. The presence of cis–trans isomerization in SerProPheArg is compatible with the known propensity for X-L-Pro-L-Phe sequences to show substantial contributions from cis conformers.[28] Interactions of SDS with SerProPheArg up to about 1:1 SDS/peptide, however, did not change the cis/trans ratio as had a 7.5:1 ratio of SDS:[Gly[6]]BK.

Complexation with SDS selectively shifted certain resonances of the tetrapeptide. The largest shifts (downfield) occurred for the α- and β-Ser peaks with only minor shifts observable for the Arg resonances. That fact suggested that the sulfate group of SDS was oriented nearer the N-terminal end of the tetrapeptide and that the shifts largely resulted from changes in pK_a of the α-amino group. From protonated to neutral states of the α-amino group of serine the α-proton shifts upfield −0.49 ppm, while the

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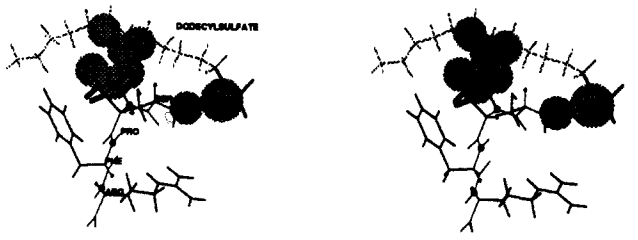

Figure 8. Relaxed stereomodel for the interaction of SerProPheArg with SDS constructed with the Mendyl program (Tripos Associates, St. Louis, MO) based on the observed changes in chemical shifts and coupling constants. (The small filled circles locate the peptide bond nitrogens.)

β-protons shift −0.20 and −0.23 ppm.[29] Thus, downfield shifts of 0.030 ppm for α-, 0.021 for β-, and 0.036 for β'-Ser between native peptide and 0.96:1 SDS:peptide are in the direction predicted from an increase in pK_a as would be expected from the presence of a nearby negatively charged group. However, since the interaction perturbs the β-protons more than would be expected from pK_a effects alone, additional factors probably are involved.

Among the other shifts produced by the SDS–peptide interaction, the largest was the H1 resonance of SDS itself (−0.046 ppm) suggesting a local perturbation such as that of a nearby positively charged group. The nature of the changes in δH1 of SDS with increasing ratios of SDS:peptide appeared complex, similar to what might be expected from interactions near a positively charged group whose pK_a was changing and whose state of protonation was affecting the peptide's conformation. SDS also rather substantially shifted β^t- and γ^t-Pro and the ring Phe resonances and decreased Pro $J_{\gamma\delta^t}$ by 25%. Smaller downfield shifts were observed for α-Phe and β'-Arg. The lack of shifts in the β^c- and γ^c-Pro resonances indicated that interaction with SDS did not disrupt the unique environment for these Pro[7] (in BK) protons (neither did a change in pH from 7.4 to 8.4, Table II).

A proposed stereomolecular graphic model for the SDS–SerProPheArg complex incorporating as much of these data as possible is illustrated in Figure 8. This model assumes that the sulfate group of SDS interacts electrostatically with the α-amino group of Ser and that the middle methylene groups of SDS interact with the β^t- and γ^t-Pro protons. In this model the alkyl chain does not interact directly with the Phe ring, and, thus, the shifts observed for those protons are attributed to an indirect effect resulting from the suggested Pro-Phe ring interactions or to changes in rotomer populations as suggested by the coupling constant results.

Finally, it seems reasonable that the changes in the chemical environment of the Phe ring(s) detected by NMR also contribute significantly to the changes in CD of BK and SerProPheArg upon interaction with SDS.[13]

Acknowledgment. This work was supported in part by the National Institutes of Health Research Grant GM 28793 (J.R.C.) and HL 26284 (J.M.S.). We also thank Kansas State University Professors Joseph Paukstelis for providing time on the NMR and R. Krishnamoorthi for his assistance with the COSY experiment and acknowledge the excellent technical assistance of Robert O. Coombs and Karl Hahn (University of Colorado Medical School). This is Contribution No. 89-375-J from the Kansas Agricultural Experiment Station.

Registry No. BK, 58-82-2; [99% ^{13}C-2-Gly^6]BK, 124419-26-7; [Gly^6,*p*-fluoro-Phe^8]BK, 124419-27-8; SerProPheArg, 16875-08-4; SDS, 151-21-3.

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Oligomerization Equilibria and Dynamics of 2,2-Di-*n*-butyl-1,3,2-dioxastannolanes

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Contribution from the Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J3. *Received March 22, 1989*

Abstract: The ^{119}Sn and ^{13}C NMR spectra of solutions of 2,2-di-*n*-butyl-1,3,2-dioxastannolane (**1**) and a number of its symmetrical derivatives in nonpolar solvents have been studied as functions of temperature and concentration. The compounds studied included (1*S*,6*S*)-8,8-di-*n*-butyl-7,9,8-dioxastannabicyclo[4.3.0]nonane (**2**), obtained from di-*n*-butyltin oxide and (*S*,*S*)-1,2-cyclohexanediol. A new method of resolution of *trans*-1,2-cyclohexanediol has been developed. Solutions of **1**, **2**, and related derivatives of disecondary diols were found to contain mixtures of oligomers that have been identified as dimers, trimers, tetramers, and pentamers. In contrast to earlier work, no evidence for the presence of monomers was obtained. The compositions of the mixtures are extremely temperature dependent; trimers and tetramers are the major constituents below −20 °C, but dimers increasingly dominate as the temperature is raised. Thermodynamic parameters for the equilibria of **1** and **2** have been measured. A derivative of a ditertiary diol, 2,2-di-*n*-butyl-4,4,5,5-tetramethyl-1,3,2-dioxastannolane, exists in nonpolar solvents predominantly as a dimer over the temperature range studied, from −60 to +80 °C. Activation parameters for a process in this dimer that causes the ^{13}C NMR signals of the pairs of nonequivalent methyl carbons to coalesce have been determined by total line shape analysis. A series of related reversible associative processes involving dimers, trimers, tetramers, pentamers, and possibly monomers and hexamers accounts for the changes observed in the NMR spectra with temperature.

2,2-Di-*n*-butyl-1,3,2-dioxastannolanes have been shown to be very useful intermediates for achieving selective reactions of diols or polyols.[1] Reactions that result in monoacylation, monoalkylation, or monooxidation of diols can be performed in high yield via an intermediate of this type, and the reaction is also often regiospecific or highly regioselective. As a result, reactions involving these intermediates and the related tri-*n*-butylstannyl ether intermediates have become part of the standard armory of methods employed when blocking groups are required for diols or polyols.[1] The source of the regioselectivity has been related to the oligomeric structures assumed by the tin-containing derivatives although the precise cause has not been clearly identified.[1] In addition, the considerable selectivity observed in the formation of macrocyclic

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