A Model Peptide with Enhanced Helicity†

Gene Merutka,‡ William Shalongo, and Earle Stellwagen*

Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242

Received July 25, 1990; Revised Manuscript Received December 6, 1990

ABSTRACT: The sequence of a model monomeric peptide, acetylA(EAAAK)$_3$Aamide was altered to expedite measurement of peptide concentration and to enhance its fractional helical content. Replacement of the N-terminal alanine residue with a tryptophan residue provides a convenient chromophore for measurement of peptide concentration without diminishing the helical content. Replacement of the three lysine residues with arginine residues enhances the helical content without loss of their electrostatic contributions. Increasing the number of EAAAR sequence units in the peptide acetylW(EAAAR)$_n$Aamide from three to five indicates that the spectral features anticipated for a completely helical peptide are closely approached.

The analysis of the effect of amino acid replacements on the helix/coil equilibrium of a small model peptide would be improved if the circular dichroism of the helical form and the coil form of the peptide were observable in aqueous solution. While the coil form can be stabilized by addition of denaturant at elevated temperature, solvent conditions have not been clearly identified for stabilization of the helical form. In this paper, we describe efforts to stabilize the helical form of a model peptide using an alternative approach, namely by manipulation of the peptide sequence.

The sequence of the parent peptide used in these studies, acetylA(EAAAK)$_3$Aamide, was originally designed by Marqusee and Baldwin (1987) to contain residues with a high statistical preference for occurrence in protein helices, to form three glutamate/lysine ion pairs in the helical form, and to stabilize the helix macrodipole. Replacements (Merutka et al., 1990) were made in the parent peptide at two sequence positions. First, the N-terminal alanine residue was replaced with a tyrosine residue to facilitate rapid and precise measurement of peptide concentration by using the unique absorbance of its chromophoric side chain. While the measurement of peptide concentration was improved, the fractional helical content of the peptide was diminished. The statistical helical preference of the amino acids (Argos & Palau, 1982) suggest that placement of a tryptophan at the N-terminus should introduce a chromophoric side chain for peptide concentration measurement without suffering the loss in helical content generated by introduction of an N-terminal tyrosine. Secondly, the central alanine residue at position 9 was replaced in turn with the remaining 19 residues to measure the helix propensity of each residue at this position in the peptide. The fractional helical content of the 20 peptides was greatest when position 9 was occupied by an alanine, an arginine, or a glutamate residue. These observations suggest that replacement of the three lysine residues in the parent peptide with three arginine residues should increase the helical content while retaining the designed electrostatic interactions.

In this paper we examine the cumulative effects of placement of a tryptophan residue at the N-terminus, replacement of the lysine residues by arginine residues, and changes in the number of sequence repeats on the helicity of the parent peptide. We find that peptides with the sequence acetylW-(EAAAR)$_n$Aamide having $n > 3$ have dichroic features very similar to those anticipated for a completely helical peptide.

Materials and Methods

Peptide Preparation. The peptides employed in this report were synthesized by the simultaneous multiple peptide synthetic procedure described by Houghten et al. (1986), fractionated by reversed-phase chromatography, and analyzed by FAB mass spectrometry and analytical reversed-phase chromatography as described previously (Merutka & Stellwagen, 1990). The mass/charge ratio of the main molecular ion of each peptide was within one mass unit of that expected for the singly protonated peptide. The analytical chromatographic elution profile observed for each peptide preparation was characterized by a single peak accounting for at least 95% of the material eluted by a 10% acetonitrile gradient in 0.1% trifluoroacetic acid developed over 100 min. The peptides containing three or more sequence repeats were at least 99% pure as judged by isocratic HPLC in an acetonitrile/water mixture containing 0.1% trifluoroacetic acid. Each of these peptides was also chromatographed on a calibrated Toyo Soda G2000SW gel-filtration HPLC column equilibrated with 6 M guanidinium chloride. Each peptide eluted as a single symmetrical component whose maximal ordinate corresponded to the expected number of residues.

Spectral Measurements. Circular dichroism measurements were made with an Aviv Associates model 6ODS spectropolarimeter, and absorbance measurements were made with an Aviv Associates model 14DS spectrophotometer, each located in the Protein Structure Facility at the University of Iowa. Each peptide solution was equilibrated in a thermostatable cell holder in the spectropolarimeter until the ellipticity at 222 nm was constant prior to recording of a dichroic spectrum. The initial ellipticity at 0 °C was regained within 1% following heating of a peptide solution above 90 °C and cooling to 0 °C. All ellipticity measurements in this report are expressed as mean residue ellipticity, $[\theta]$, having the units deg cm^2 dmol^{-1}. The concentration of peptides with an N-terminal trypophan was obtained from their absorbance at 280 nm by using the extinction coefficient of 5559 M^{-1} cm^{-1} reported for tryptophan

† This investigation was supported by U.S. Public Health Service Program Project Grant HE 14388 from the National Heart, Lung, and Blood Institute and by National Science Foundation Biological Instrumentation Program Grant DMB 8413658.

* To whom correspondence should be addressed.

‡ Present address: Department of Molecular Biology, Research Institute of Scripps Clinic, 1666 North Torrey Pines Road, La Jolla, CA 92037.

0006-2960/91/0430-4245$02.50/0

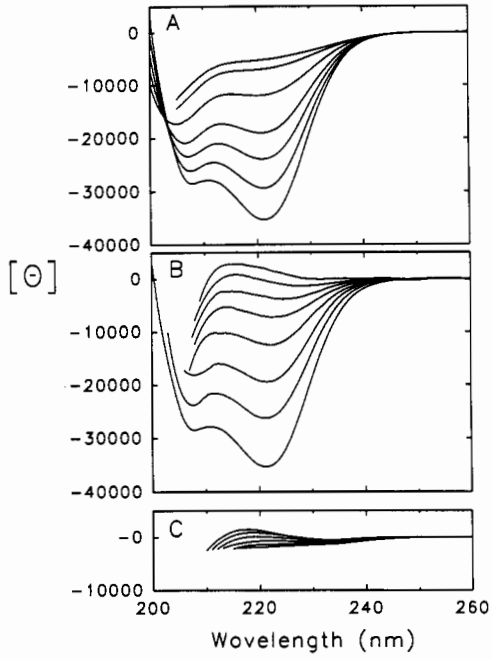

FIGURE 1: Dichroic spectra of the peptide acetylW(EAAAR)$_3$Aamide. (A) The dichroic spectrum of a 27 μM solution of the peptide at temperatures, reading upward at 222 nm, of 0, 15, 26, 36, 54, 76, and 96 °C. (B) The dichroic spectrum of a 29 μM solution of the peptide at 0 °C in guanidinium chloride concentrations, reading upward at 222 nm, of 0, 1, 2, 3, 4, 5, 6, and 7 M. (C) The dichroic spectrum of a 217 μM solution of the peptide in 7 M guanidinium chloride at temperatures, reading upward at 222 nm, of 95, 80, 60, 40, 21, and 0 °C. All solvents contained 10 mM NaCl and 1 mM phosphate buffer, pH 7.0.

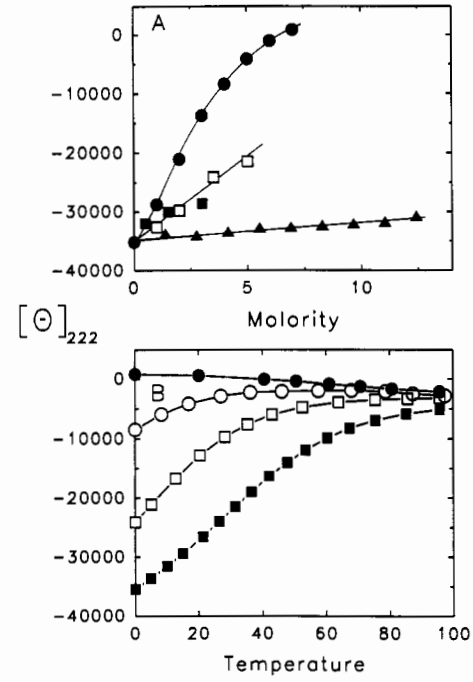

FIGURE 2: The effect of additives on the mean residue ellipticity of the peptide acetylW(EAAAR)$_3$Aamide at 222 nm. (A) Measurements made at 0 °C in the presence of guanidinium chloride (●), NaCl (□), ammonium sulfate (■), and trifluoroethanol (▲). (B) Measurements made in solutions containing 7 M guanidinium chloride (●), 4 M guanidinium chloride (○), 3.5 M NaCl (□), and 10 mM NaCl (■). All measurements were made in solutions containing 26–29 μM peptide, 10 mM NaCl, and 1 mM phosphate buffer, pH 7.0.

at neutral pH (Milhalyi, 1968). This extinction coefficient is virtually identical with that reported for *N*-acetyltryptophan methyl ester (Fasman, 1976) and was selected because of its greater precision. Mean residue ellipticity values measured for multiple solutions of the same peptide in the same solvent varied by about ±500 deg cm^2 dmol^{-1}.

Results

Residue Replacement. The far-ultraviolet dichroic spectrum of a solution of the peptide acetylW(EAAAR)$_3$Aamide in 10 mM NaCl at 0 °C and pH 7.0 has minima at 208 and 222 nm as shown in Figure 1A. Such a spectrum is characteristic for a peptide having a high fractional content of helical residues (Holzwarth & Doty, 1965). As the temperature of the solution is increased, the spectrum becomes characteristic for an unfolded protein in excess denaturant (Dearborn & Wetlaufer, 1970). Similar spectral changes with increasing temperature have been reported for related peptides in the same solvent such as acetylA(EAAAK)$_3$Aamide (Marqusee & Baldwin, 1987; Merutka & Stellwagen, 1990) and acetylY(EAAAK)$_3$Aamide (Merutka & Stellwagen, 1990). These spectral changes can be considered to represent a thermally dependent helix/coil transition.

The mean residue ellipticity of the peptide acetylW(EAAAR)$_3$Aamide at 222 nm, pH 7.0, and 0 °C is −35 200 ± 400 deg cm^2 dmol^{-1} based on seven measurements. This value is independent of peptide concentration over the range 0.43–256 μM, suggesting that it is not reflective of peptide association. The mean residue ellipticity values for the peptide acetylW(EAAAK)$_3$Aamide in the same solvent is −29 400 ± 600 deg cm^2 dmol^{-1} based on four measurements. This comparison indicates that replacement of the three lysine residues with three arginine residues enhances helicity significantly. The mean residue ellipticity for the peptide acetylA(EAAAK)$_3$Aamide in the same solvent is 30 200 ± 1700 deg cm^2 dmol^{-1} (Marqusee & Baldwin, 1987; Merutka & Stellwagen, 1990). The indole chromophore should contribute no more than +1200 deg cm^2 dmol^{-1} to the mean residue ellipticity of a 17-residue peptide at 222 nm (Auer, 1973; Brahms & Brahms, 1980). Accordingly, an N-terminal tryptophan provides a convenient chromophore for peptide concentration determination without diminishing peptide helicity.

Effect of Solvent Additives. Addition of guanidinium chloride to a solution of the peptide acetylW(EAAAR)$_3$Aamide maintained at 0 °C and pH 7.0 generates changes in the dichroic spectrum similar to those observed upon increasing the temperature, as shown in Figure 1B. In contrast to the variable temperature spectra, the isothermal spectra observed in guanidinium chloride do not exhibit an apparent isodichroic point at 203 nm. This is likely due to the effect of guanidinium chloride on the ellipticity of the peptide bond. This effect was found to be 272 deg cm^2 dmol^{-1} per molarity of guanidinium chloride for the coil form of the peptide acetylW(EAAAR)$_3$Aamide at 222 nm and 90 °C. Comparison of the spectra in Figure 1A,B indicates that the dichroic spectrum of the peptide solution at 96 °C in 10 mM NaCl is not equivalent with the dichroic spectrum observed at 0 °C in 7.0 M guanidinium chloride. This nonequivalence is due to the effect of temperature on the dichroic spectrum of the coil form of the peptide as illustrated in Figure 1C.

The dependence of the mean residue ellipticity at 222 nm of solutions of the peptide acetylW(EAAAR)$_3$Aamide at 0 °C and pH 7.0 on the concentration of several other additives is illustrated in Figure 2A. The addition of each of these additives, including the helix stabilizing reagents trifluoroethanol and ammonium sulfate (Nelson & Kallenbach, 1989; von Hippel & Schleich, 1969), diminishes the negative ellipticity of the peptide solution. The thermal dependence of the ellipticity of the peptide at 222 nm in the presence of sodium

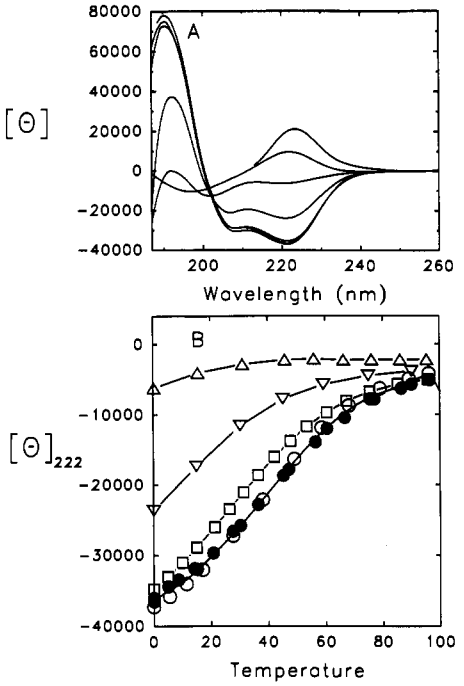

FIGURE 3: The effect of the number of EAAAR sequence repeats on the dichroic properties. (A) Spectra, reading downward at 222 nm, for acetylWamide and for peptides, acetylW(EAAAR)$_n$Aamide, having n values of 0, 1, 2, 3, 4, and 5. All solutions were maintained at 0 °C and contained between 28 and 84 μM peptide, 10 mM NaCl, and 1 mM phosphate buffer, pH 7.0. (B) The thermal transitions observed for the peptides having n values of 1 (△), 2 (▽), 3 (□), 4 (●), and 5 (○). All measurements were obtained at 222 nm in solutions containing between 28 and 84 μM peptide in 10 mM NaCl and 1 mM phosphate buffer, pH 7.0.

chloride and guanidinium chloride is illustrated in Figure 2B. The thermal dependence becomes linear in 7 M guanidinium chloride having a $d[\theta]/dT$ of −33 deg cm^2 dmol^{-1} °C^{-1}. This value is within the range observed for a acetylated amidated tripeptide (Mattice, 1974) and for longer peptides and proteins in excess denaturant (Shoemaker et al., 1987; Goodman & Kim, 1989; Nojima et al., 1977, 1978). Accordingly, we assume this value represents the thermal dependence of a residue in the coil form of the peptide acetylW(EAAAR)$_3$Aamide. As shown in Figure 2B, the thermal transitions of this peptide in 10 mM NaCl, in 3.5 M NaCl, and in 4 M guanidinium chloride each appear to approach the thermal dependence of the coil form of the peptide.

Number of Sequence Repeats. Peptides with the sequence acetylW(EAAAR)$_n$Aamide having n ranging from 0–5 are designated WER0–WER5, respectively. The far-ultraviolet dichroic spectra of these peptides and of acetylWamide in 10 mM NaCl at pH 7.0 and 0 °C are illustrated in Figure 3A. The spectrum for acetylWamide illustrates the dichroic maximum of the B_a band of the indole side chain at 223 nm (Auer, 1973) having a mean residue ellipticity of +21 300 ± 500 deg cm^2 dmol^{-1} at 222 nm. The contribution of the single indole side chain to the dichroic spectra of the peptides is diminished as the number of repeating EAAAR units increases. This contribution is so small in peptides having three or more repeats that an isodichroic point at 202 nm becomes apparent. The mean residue ellipticity at 222 nm corrected for the tryptophan dichroism appears to asymptotically approach a value of about −40 000 deg cm^2 dmol^{-1} as the number of repeating sequences is increased.

The thermal transitions observed at 222 nm for solutions of peptides WER1–WER5 in 10 mM NaCl at pH 7.0 are shown in Figure 3B. The thermal stability of the peptides

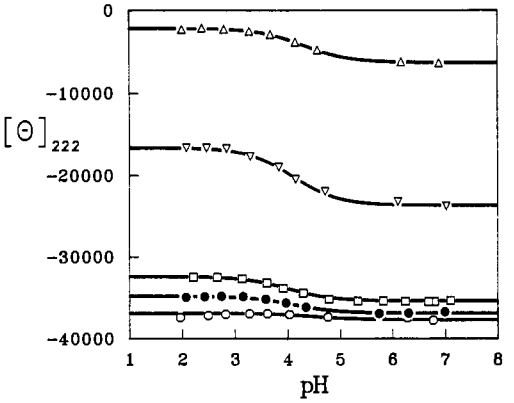

FIGURE 4: The effect of pH on the mean residue ellipticity of acetylW(EAAAR)$_n$Aamide at 222 nm. Measurements are illustrated for peptides having n values of 1 (△), 2 (▽), 3 (□), 4 (●), and 5 (○). All measurements were obtained in solutions containing between 15 and 68 μM peptide and 10 mM NaCl maintained at 0 °C.

correlates with their relative ellipticity values observed at 0 °C. The thermal transitions for the peptides WER4 and WER5 have a distinct sigmoidal shape as if limiting ellipticity values were being approached at each end of the observed temperature span.

Acidification of peptides WER1–WER5 in 10 mM NaCl at 0 °C generates systematic reversible changes in the dichroic spectra characteristic of a decrease in the fractional helical content. The dependence of the ellipticity observed at 222 nm on pH is illustrated in Figure 4. The observed changes can each be fit with a single transition having an apparent pK of 4.1 ± 0.1, no cooperativity, and a variable difference in ellipticity. These transitions likely represent the protonation of the three glutamate residues present in each peptide. The difference in ellipticity accompanying the protonation of the glutamate residues diminishes from 7100 to 800 deg cm^2 dmol^{-1} as the number of repeats increases from 2 to 5.

Discussion

Several observations suggest that peptides with the sequence acetylW(EAAAR)$_n$Aamide having $n > 3$ are nearly completely helical in 10 mM NaCl at 0 °C and pH 7.0. First, the dichroic spectra of these peptides, shown in Figure 3A, are very similar to model helical spectra obtained by measurement of long homopolypeptides or by deconvolution of dichroic spectra of proteins of known structure (Greenfield & Fasman, 1969; Saxena & Wetlaufer, 1971). Second, the spectra appear to approach a limiting ellipticity as the number of sequence repeats is increased. Third, the ellipticity of these peptides cannot be increased following addition of helix-forming reagents such as trifluoroethanol or ammonium sulfate, as shown in Figure 2A. Fourth, ionization of the three glutamyl side chains generates only a modest increase in ellipticity, as shown in Figure 4, as if the protonated forms of these peptides were themselves nearly completely helical.

The mean residue ellipticity of a helical residue can be estimated by analysis of a peptide melting curve such as that illustrated for the peptide WER4 in Figure 3B as a two-state helix/coil transition. The illustrated curve was fit to a regular sigmoid, assuming the thermal dependence of a coil residue is −33 deg cm^2 dmol^{-1} °C^{-1} and allowing the thermal dependence of a helical residue to float freely. The best fit has mean residue ellipticity values for a coil residue and a helical residue at 0 °C of +400 and −40 600 deg cm^2 dmol^{-1}, respectively, and a thermal dependence for a helical residue of +27 deg cm^2 dmol^{-1} °C^{-1}. These ellipticity values are within the range observed for coil and helical residues (Gratzer, 1978). The

thermal dependence of the helical residue is within the range of values obtained from the native baseline dependence of helical proteins such as cytochrome *c* and apomyoglobin (Privalov et al., 1989). Very similar values are obtained by analysis of the melting transition of peptide WER5. These analyses are consistent with the view that peptides WER4 and WER5 are very nearly completely helical in aqueous solution at neutral pH and 0 °C.

Registry No. AcetylW(EAAAR)Aamide, 132776-13-7; acetylW-(EAAAR)$_2$Aamide, 132776-14-8; acetylW(EAAAR)$_3$Aamide, 131684-39-4; acetylW(EAAAR)$_4$Aamide, 132776-15-9; acetylW-(EAAAR)$_5$Aamide, 132776-16-0; acetylA(EAAAK)$_3$Aamide, 113852-19-0.

References

Argos, P., & Palau, J. (1982) *Int. J. Pept. Protein Res. 19*, 380-393.

Auer, H. E. (1973) *J. Am. Chem. Soc. 95*, 3003-3011.

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

Dearborn, D. G., & Wetlaufer, D. B. (1970) *Biochem. Biophys. Res. Commun. 39*, 314-320.

Fasman, G. D. (1976) *Handb. Biochem. Mol. Biol.*, 3rd ed., Vol. I, p 186.

Goodman, E. M., & Kim, P. S. (1989) *Biochemistry 28*, 4343-4347.

Gratzer, W. B. (1978) *Tech. Protein Enzyme Biochem. B108*, 1-43.

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

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

Houghten, R. A., DeGraw, S. T., Bray, M. K., Hoffman, S. R., & Frizzell, N. D. (1986) *Biotechniques 4*, 522-528.

Marqusee, S., & Baldwin, R. L. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8898-8902.

Mattice, W. L. (1974) *Biopolymers 13*, 169-183.

Merutka, G., & Stellwagen, E. (1990) *Biochemistry 29*, 894-898.

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

Mihalyi, E. (1968) *J. Chem. Eng. Data 13*, 179-182.

Nelson, J. W., & Kallenbach, N. R. (1989) *Biochemistry 28*, 5256-5261.

Nojima, H., Ikai, A., Oshima, T., & Noda, H. (1977) *J. Mol. Biol. 116*, 429-442.

Nojima, H., Hon-Nami, K., Oshima, T., & Noda, H. (1978) *J. Mol. Biol. 122*, 33-42.

Privalov, P. L., Tiktopulo, E. I., Venyaminov, S. Y., Griko, Y. V., Makhatadze, G. I., & Khechinashvili, N. N. (1989) *J. Mol. Biol. 205*, 737-750.

Saxena, V. P., & Wetlaufer, D. B. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 969-972.

Shoemaker, K. R., Fairman, R., York, E. J., Stewart, J. M., & Baldwin, R. L. (1987) *Proc. Tenth Peptide Symp.*, 15-20.

von Hippel, P. H., & Schleich, T. (1969) in *Structure and Stability of Biological Macromolecules* (Timasheff, S. N., & Fasman, G. D., Eds.) pp 417-574, Marcel Dekker, Inc., New York.

^{19}F NMR Studies of the D-Galactose Chemosensory Receptor. 1. Sugar Binding Yields a Global Structural Change†

Linda A. Luck and Joseph J. Falke*

Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215

Received September 21, 1990; Revised Manuscript Received November 27, 1990

ABSTRACT: The *Escherichia coli* D-galactose and D-glucose receptor is an aqueous sugar-binding protein and the first component in the distinct chemosensory and transport pathways for these sugars. Activation of the receptor occurs when the sugar binds and induces a conformational change, which in turn enables docking to specific membrane proteins. Only the structure of the activated receptor containing bound D-glucose is known. To investigate the sugar-induced structural change, we have used ^{19}F NMR to probe 12 sites widely distributed in the receptor molecule. Five sites are tryptophan positions probed by incorporation of 5-fluorotryptophan; the resulting ^{19}F NMR resonances were assigned by site-directed mutagenesis. The other seven sites are phenylalanine positions probed by incorporation of 3-fluorophenylalanine. Sugar binding to the substrate binding cleft was observed to trigger a global structural change detected via ^{19}F NMR frequency shifts at 10 of the 12 labeled sites. Two of the altered sites lie in the substrate binding cleft in van der Waals contact with the bound sugar molecule. The other eight altered sites, specifically two tryptophans and six phenylalanines distributed equally between the two receptor domains, are distant from the cleft and therefore experience allosteric structural changes upon sugar binding. The results are consistent with a model in which multiple secondary structural elements, known to extend between the substrate cleft and the protein surface, undergo shifts in their average positions upon sugar binding to the cleft. Such structural coupling provides a mechanism by which sugar binding to the substrate cleft can cause structural changes at one or more docking sites on the receptor surface.

While important structural features of sensory and signaling proteins have now been elucidated in a number of systems, much less is known about the structural changes these proteins undergo when they are activated. Such structural changes play a key functional role, since they in turn trigger changes in the interaction between an activated protein and its target proteins or nucleic acids. The present study illustrates a ^{19}F NMR approach that can be used to probe structural changes within

† This investigation was supported by the National Institutes of Health Grant R01-GM40731 and by a University of Colorado CRCW Junior Faculty Development Award (J.J.F.).

* Address correspondence to this author.

0006-2960/91/0430-4248$02.50/0