19F NMR relaxation studies on 5-fluorotryptophan- and tetradeutero-5-fluorotryptophan-labeled E. coli glucose/galactose receptor

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Summary

19F NMR relaxation studies have been carried out on a fluorotryptophan-labeled E. coli periplasmic glucose/galactose receptor (GGR). The protein was derived from E. coli grown on a medium containing a 50:50 mixture of 5-fluorotryptophan and [2,4,6,7-2H4]-5-fluorotryptophan. As a result of the large γ-isotope shift, the two labels give rise to separate resonances, allowing relaxation contributions of the substituted indole protons to be selectively monitored. Spin-lattice relaxation rates were determined at field strengths of 11.75 T and 8.5 T, and the results were analyzed using a model-free formalism. In order to evaluate the contributions of chemical shift anisotropy to the observed relaxation parameters, solid-state NMR studies were performed on [2,4,6,7-2H4]-5-fluorotryptophan. Analysis of the observed 19F powder pattern lineshape resulted in anisotropy and asymmetry parameters of Δσ = -93.5 ppm and η = 0.24. Theoretical analyses of the relaxation parameters are consistent with internal motion of the fluorotryptophan residues characterized by order parameters S^2 of ~1, and by correlation times for internal motion ~10^-11 s. Simultaneous least squares fitting of the spin-lattice relaxation and line-width data with τ, set at 10 ps yielded a molecular correlation time of 20 ns for the glucose-complexed GGR, and a mean order parameter S^2=0.89 for fluorotryptophan residues 183, 127, 133, and 195. By contrast, the calculated order parameter for FTrp^{284}, located on the surface of the protein, was 0.77. Significant differences among the spin-lattice relaxation rates of the five fluorotryptophan residues of glucose-complexed GGR were also observed, with the order of relaxation rates given by: R_{1σ}^{183} > R_{1σ}^{127} ≈ R_{1σ}^{133} > R_{1σ}^{195} > R_{1σ}^{284}. Although such differences may reflect motional variations among these residues, the effects are largely predicted by differences in the distribution of nearby hydrogen nuclei, derived from crystal structure data. In the absence of glucose, spin-lattice relaxation rates for fluorotryptophan residues 183, 127, 133, and 195 were found to decrease by a mean of 13%, while the value for residue 284 exhibits an increase of similar magnitude relative to the liganded molecule. These changes are interpreted in terms of a slower overall correlation time for molecular motion, as well as a change in the internal mobility of FTrp^{284}, located in the hinge region of the receptor.

Introduction

19F NMR is increasingly used to probe structural and conformational features of proteins too large for 1H NMR analysis (Gerig, 1994, and references cited therein). Typically, such proteins are grown on media containing fluorinated amino acids, although other fluorination strategies have also been used. Despite early concerns about the significance of chemical shift anisotropy relaxation mechanisms for fluorinated macromolecules, surprisingly well-resolved spectra have often been obtained at fields up to 11.75 T (Luck and Falke, 1991a-c; Hoeltzli and Frieden, 1994) and for high molecular weights (Hull and Sykes, 1974,1975b; Browne and Otvos, 1976; Gerig et al., 1983;
Peersen et al., 1990; Hinds et al., 1992). In order to develop optimal labeling strategies and to characterize molecular dynamics, additional characterization of the relaxation behavior of fluorinated macromolecules would be useful. As initially demonstrated by Moreland and Carroll (1974), the measurement of the relaxation rates for simultaneously observed molecular isotopomers can provide unique insights into molecular dynamics and relaxation mechanisms. In such studies, all physical parameters for the different isotopically labeled species are identical, except for the interactions that vary with the isotopic species being observed. No analyses of this type appear to have been done for fluorine, presumably due to the difficulty of simultaneously introducing ¹³C and ¹⁹F labels, and to the fact that no other primary isotope effect would be achievable.

In this paper, we note that γ-isotope effects resulting from deuteration of vicinal protons are sufficiently large to permit the separation of ¹⁹F resonances, even in relatively large macromolecular systems. We have utilized this strategy with the glucose/galactose periplasmic receptor protein of E. coli, a soluble protein of MW = 33 370. Previous studies by Luck and Falke (1991a–c) show that the resonances corresponding to the five fluorotryptophan residues of 5FTrp-labeled GGR are well resolved, particularly for the glucose-ligated form of the receptor. The labeling protocol involves growth of the protein on a medium containing equal concentrations of 5-fluorotryptophan and [2,4,6,7-²H₄]-D,L-5-fluorotryptophan. This approach provides a theoretical basis for the separation of intraresidue and interresidue contributions to the relaxation parameters. Field-dependent relaxation measurements and solid-state NMR studies have been performed in order to obtain a better understanding of tryptophan dynamics.

Materials and Methods

D,L-5-fluorotryptophan was obtained from Sigma (St. Louis, MO). The [2,4,6,7-²H₄]-D,L-5-fluorotryptophan was either obtained from Isotec, Inc. (Miamisburg, OH), or was prepared using published procedures (Griffiths et al., 1976; Matthews et al., 1977) for tryptophan deuteration. The techniques used to generate and isolate the FTTrp-labeled receptor have been described previously (Luck and Falke, 1991a), the only modification being the substitution of a 50:50 mixture of the D,L-5-fluorotryptophan and [2,4,6,7-²H₄]-D,L-5-fluorotryptophan in the growth medium.

¹⁹F NMR measurements were done on the purified enzyme in a buffer containing 10% D₂O for the deuterium lock, 0.5 mM CaCl₂, 100 mM KCl, and 10 mM Tris-HCl, pH 7.1. ¹⁹F spectra at 470 MHz were obtained on a GN-500 NMR spectrometer (GE NMR, Fremont, CA), and 340 MHz spectra were obtained on an NT-360 spectrometer ( Nicolet Magnetics Corp., Fremont, CA). All NMR studies were performed at 25 °C. The R₁F values summarized in the tables are averages of two separate determinations, and the variation was generally <10%. In several cases, measurements were repeated 3-4 times on different enzyme preparations, and standard deviations ranged from 5 to 16%. T₁ measurements were done using an inversion-recovery sequence. In order to investigate the possible interconversion between folded and denatured enzyme, the fluorine resonance(s) of the latter were selectively inverted using a DANTE pulse sequence (Morrison and Freeman, 1978). CHARMm calculations were performed on a Silicon Graphics Indigo XS-4000 workstation.

Initially, dynamic variables were estimated assuming rigid, isotropic motion of the glucose–GGR complex. Subsequently, dipolar and chemical shift anisotropy contributions to the ¹⁹F relaxation of the fluorotryptophan-labeled complex were modeled using a ‘model-free’ spectral density formalism (Cipriano and Szabo, 1982), and the ranges of dynamic variables consistent with the observed relaxation parameters were determined. Finally, dynamic variables were optimized by minimizing an error function defined as the sum of the squares of the fractional differences between calculated and experimental relaxation parameters:

$$\text{Error} = \sum_{i} \left[ \left( \frac{P_{\text{exp}}(i) - P_{\text{calc}}(i)}{P_{\text{exp}}(i)} \right)^2 \right]$$  \hspace{1cm} (1)

The relaxation contributions of the indole protons were isolated by measurements of the relaxation-rate differences between the protonated and deuterated fluorotryptophan residues. Using the abbreviation R₁F(i) for the fluorine spin-lattice relaxation rate in the 5-fluorotryptophan (i = H) or deuterated 5-fluorotryptophan (i = D) residues at NMR frequency ν (in MHz), the three parameters used in the minimization were R₁F(H)¹⁰⁰ − R₁F(D)¹⁰⁰, R₁F(H)²⁰⁰ − R₁F(D)²⁰⁰, and the CSA line-width contribution, W²⁰⁰ CS₁²⁰⁰. Since the resulting error function was found to be fairly insensitive to the value of τc, the correlation time for internal motion, for values near 10 ps, τc was fixed at this value and only the molecular correlation time, τm, and the order parameter, S², were varied in the minimization.

Solid-state ¹⁹F NMR spectra of a powder sample of [2,4,6,7-²H₄]-D,L-5-fluorotryptophan were performed on a Varian UnityPlus 500 MHz spectrometer at 25 °C using a spin-echo pulse sequence. To ensure constant excitation over the fluorine bandwidth, short rf pulse lengths of 2 and 4 μs were used. Such an approach minimizes distortion due to the finite pulse power (Rance and Byrd, 1983). A total of 360 FIDs were averaged, left-shifted three times to center the echo about the time origin, and phased such that the real and imaginary components were purely absorptive and dispersive, respectively. A 1-kHz exponential line-broadening was applied prior to Fourier transformation. Theoretical solid-state powder spectra were