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# Dynamic Frequency Shifts of Complexed Ligands: An NMR Study of D-[1-$^{13}$C,1-$^{2}$H]Glucose Complexed to the *Escherichia coli* Periplasmic Glucose/Galactose Receptor

Scott A. Gabel,* Linda A. Luck,† Lawrence G. Werbelow,‡ and Robert E. London*

*Laboratory of Structural Biology, MR-01, National Institute of Environmental Health Sciences, Box 12233, Research Triangle Park, North Carolina 27709; †Department of Biology, Science Center, Clarkson University, Potsdam, New York 13699; and ‡Department of Chemistry, New Mexico Institute of Mining and Technology, Socorro, New Mexico 87801

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**The $^{13}$C multiplet structure of D-[1-$^{13}$C,1-$^{2}$H]glucose complexed to the *Escherichia coli* periplasmic glucose/galactose receptor has been studied as a function of temperature. Asymmetric multiplet patterns observed are shown to arise from dynamic frequency shifts. Multiplet asymmetry contributions resulting from shift anisotropy–dipolar cross correlations were found to be small, with optimal fits of the data corresponding to small, negative values of the correlation factor, $\chi^{\text{CD-CSA}}$. Additional broadening at higher temperatures most probably results from ligand exchange between free and complexed states. Effects of internal motion are also considered theoretically, and indicate that the order parameter for the bound glucose is $\geqslant 0.9$.** 

Recent studies of doubly $^{13}$C,$^{2}$H-labeled proteins have revealed unusual, asymmetric $^{13}$C lineshapes (*1, 2*) which were ultimately interpreted to arise as a consequence of dynamic frequency shifts (*3*). The dependence of such lineshapes on both dipolar and quadrupolar interaction constants, as well as on dynamic variables, provides a new approach for extracting structural and dynamic information from NMR studies of macromolecular systems (*3–7*). Although such interactions will generally characterize any scalar-coupled spin $\frac{1}{2}$–spin $S$ ($>\frac{1}{2}$) pair, the deuterium nucleus provides a particularly favorable situation for observation of these effects due to the relatively small quadrupolar coupling constant, which results in sufficiently low deuterium spin–lattice relaxation rates such that the perturbed multiplet is not collapsed for typical rotational correlation times encountered. Additionally, the alignment of the principal axes of the deuterium quadrupolar and carbon–deuterium dipolar interactions maximizes the observed effect. NMR studies of ligands complexed with macromolecules have previously made use of double $^{13}$C,$^{2}$H labeling in order to reduce the dipolar broadening of the bound species (*8–10*). It was therefore of interest to determine whether the perturbed multiplet structures previously observed in an isotopically labeled protein could be observed for such doubly labeled ligands, and to determine the sensitivity of the lineshape to various dynamic parameters. The *Escherichia coli* periplasmic glucose/galactose receptor (GGR) represents a particularly useful system for exploring such effects due to its good stability and solubility characteristics. In this study, we report on the spectra obtained from the complex formed between GGR and D-[1-$^{13}$C,1-$^{2}$H]glucose.

A series of carbon-13 NMR spectra, obtained as a function of temperature, showing the spectral region containing the $^{13}$C resonances of the labeled glucose in the GGR–D-[1-$^{13}$C,1-$^{2}$H]glucose complex is shown in Fig. 1. As in the case of thioredoxin labeled with [2-$^{13}$C,2-$^{2}$H]glycine (*3*), the $^{13}$C spectrum arising from the glucose C-1 carbon is characterized by a perturbed multiplet arising from scalar coupling to the directly bonded $^{2}$H nucleus. Spectral simulations were performed using a scalar coupling constant $J_{\text{CD}} = (\gamma_{\text{D}}/\gamma_{\text{H}})^{1}J^{\beta}_{\text{CH}}$, where $^{1}J^{\beta}_{\text{CH}}$ is the observed scalar coupling constant for the $\beta$-D-glucose (*11*), assumed to be unchanged in the complex; an axially symmetric deuteron quadrupolar coupling, $e^2Qq/h = 170$ kHz; a $^{13}$C–$^{2}$H dipolar coupling constant of $\gamma_{\text{C}}\gamma_{\text{D}}\hbar\langle r^{-3}_{\text{CD}}\rangle/2\pi = 3.5$ kHz (corresponding to $r_{\text{CD}} = 1.09$ Å); and a quadrupole–dipolar (cross) correlation factor $\chi^{\text{Q.D}} = 1$. If the dipolar and quadrupolar relaxation tensors are axially symmetric, and the motion isotropic, $\chi^{\text{Q.D}} = (3\cos^2\theta_{\text{CD-Q}} - 1)/2$, where $\theta_{\text{CD-Q}}$ is the angle between the principal axes of the C–D bond and the principal axis of the deuterium quadrupolar tensor. The overall isotropic correlation time at 25°C was set at 20 ns based on our previous studies of fluorotryptophan-labeled GGR performed under similar conditions (*12*), and was assumed to vary in proportion to the viscosity of water as a function of temperature.

In performing the lineshape simulations it is also important to account for the effects of nearby protons. Since no resolved $^{13}$C–$^{1}$H couplings are observed for the glucose C-1 multiplet, it can be shown that cross-correlation effects involving $^{13}$C–$^{1}$H dipolar interactions do not affect the spectral lineshape to first order. The dipolar broadening effect can be treated as a random field interaction, by adding a



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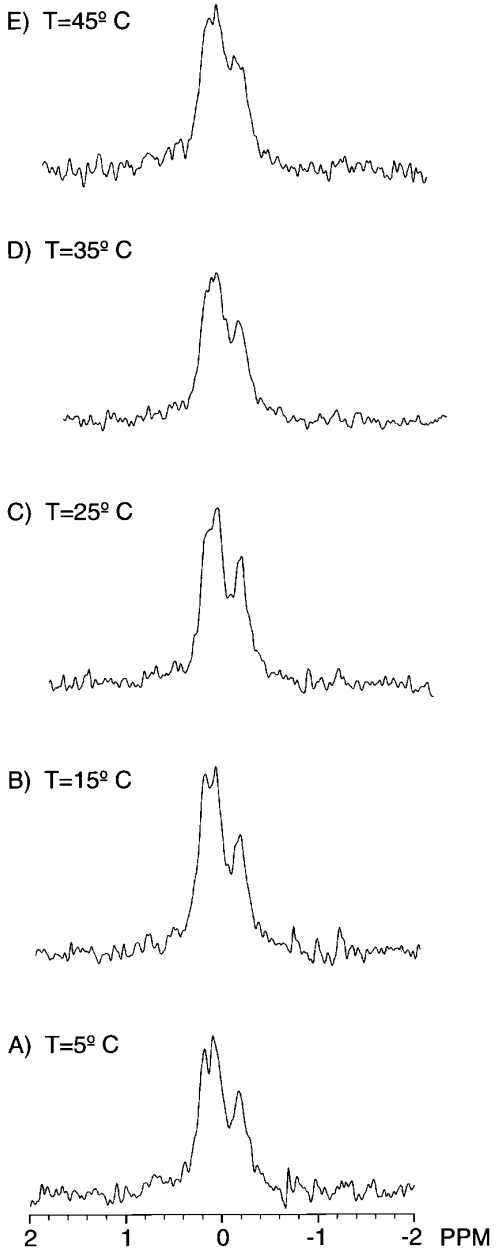


**FIG. 1.** Proton-coupled 125.76-MHz $^{13}C$ NMR spectra of the C-1 resonances from 0.5 m*M* D-[1-$^{13}C$,1-$^{2}H$]glucose (Omicron Biochemicals, Inc., South Bend, IN) complexed with the *E. coli* periplasmic glucose receptor, obtained on a General Electric GN-500 NMR spectrometer using a 10-mm broadband probe tuned to the $^{13}C$ resonance of 125.765 MHz, at the temperatures indicated. Other sample parameters are [GGR] = 1.2 m*M*, 10% $D_2O$ for the deuterium lock, 0.5 m*M* $CaCl_2$, 100 m*M* KCl, 10 m*M* Tris–HCl, pH 7.1. A 4-Hz Gaussian apodization function was used in signal processing. Other spectral parameters were 8K data points, 714 Hz sweep width, total recycle time of 3.52 s, and either 60,000 or 90,000 transients per spectrum. The spectra have been arbitrarily aligned to demonstrate changes in the multiplet structure.

term equal to $-\frac{2}{3}\Sigma_n J_{CH_n}(0)$ to each diagonal element of the **A** matrix (*3*) in order to model the dipolar contributions from protons in the protein and on the glucose. To simplify the calculations, the sum was replaced by a single, equivalent proton at a fixed $r_{CH}$ value selected to optimize the agreement with the observed spectra.

As first demonstrated by Shimizu (*13*) and recently discussed within the context of multiplets subject to dynamic frequency shift perturbations (*3*), cross correlations arising from chemical shielding anisotropy (CSA)–dipole interactions can significantly alter the appearance of the spectra, leading to linewidth differences among the multiplet peaks. The carbon-13 CSA $\Delta\sigma^C$ was set at 40 ppm based on reported values for structurally related molecules (*14*). If the CSA tensor is assumed to have axial symmetry and the motion isotropic, $\chi^{CD\text{-}CSA} = (3\cos^2\theta_{CD\text{-}CSA} - 1)/2$, where $\theta_{CD\text{-}CSA}$ is the angle between the principal axes of the $^{13}C$ CSA interaction and the $^{13}C-^{2}H$ dipolar interaction. It is important to recognize, however, that the lineshape calculation used here senses only the product $\chi^{CD\text{-}CSA}\Delta\sigma^C$. In the present study, $\chi^{CD\text{-}CSA}$ was varied as an adjustable parameter.

Spectral simulations were based on either the assumption of isotropic motion, described by correlation time $\tau_M$, or on a ''model free'' correlation function (*15*),

$$G(t) \propto S^2 e^{-t/\tau_M} + (1 - S^2)e^{-t/\tau}, \qquad [1]$$

which, in this simplest form, contains three parameters: an isotropic molecular correlation time, $\tau_M$, a generalized order parameter, $S^2$, and a correlation time characterizing internal motion, $\tau_i$, which enters via a reduced time, $\tau = \tau_M\tau_i/(\tau_M + \tau_i)$ (*15*). The cosine transform of this expression leads to the usual spectral density of form

$$J(\omega) \propto (1 - S^2)\frac{\tau}{1 + (\omega\tau)^2} + S^2\frac{\tau_M}{1 + (\omega\tau_M)^2}. \qquad [2]$$

Dynamic frequency shifts arise from the single-sided sine transform of the appropriate correlation function, leading to expressions of the form

$$L(\omega) \propto (1 - S^2)\frac{\omega\tau^2}{1 + (\omega\tau)^2} + S^2\frac{\omega\tau_M^2}{1 + (\omega\tau_M)^2}. \qquad [3]$$

For example, the imaginary spectral density term corresponding to the C–D dipolar–deuterium quadrupolar cross correlation has the form

$$L^{Q.D}(\omega) = \frac{3}{40}(e^2qQ_D)(\gamma_C\gamma_D\langle r_{CD}^{-3}\rangle) \times \left[(1 - S^2)\frac{\omega\tau^2}{1 + (\omega\tau)^2} + S^2\frac{\omega\tau_M^2}{1 + (\omega\tau_M)^2}\right]. \qquad [4]$$

It is important to recognize that the simplicity of Eq. [4] results because of the assumed colinearity between the prin-

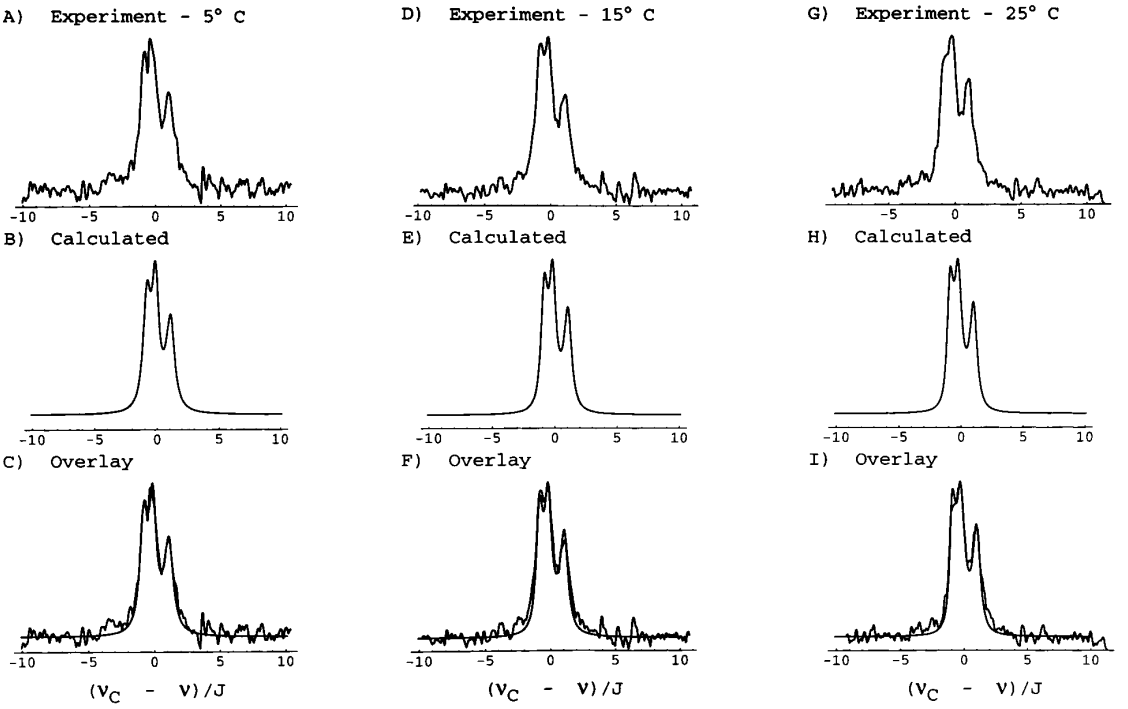


**FIG. 2.** Simulations of the observed $^{13}C$ resonances of D-[1-$^{13}C$,1-$^{2}H$]glucose complexed with GGR at 5, 15, and 25°C: Experimental spectra are shown in A, D, and G, respectively; theoretical simulations are shown in B, E, and H, and the overlays in C, F, and I. Other parameters used in the simulation are $e^2Qq/h$ = 170 kHz; $\gamma_C\gamma_D\hbar\langle r_{CD}^{-3}\rangle/2\pi$ = 3.5 kHz (corresponding to $r_{CD}$ = 1.09 Å); $\gamma_C\gamma_H\hbar\langle r_{CH}^{-3}\rangle/2\pi$ = 11.0 kHz (corresponding to $r_{CH}$ = 1.4 Å); $\Delta\sigma^C$ = 40 ppm; $\chi^{CD\text{-}CSA} = -0.4$; $^1J_{CD} = (\gamma_D/\gamma_H)160$ = 24.56 Hz; $H_0$ = 11.75 T (corresponding to $\nu(^1H)$ = 500 MHz). Motional parameters: $\tau_M$ = 20, 25.6, and 34.1 ns at 25, 15, and 5°C, respectively; $\tau_i$ = 10 ps; and $S^2$ = 0.9. Note that the units of the plot are not ppm, but multiples of the scalar coupling constant, $J$.

cipal axis of the deuteron's quadrupole coupling and the C–D bond axes (*16*). In general, there is no simple relationship between the cross-correlation and auto-correlation spectral density (*6, 17*).

Although optimal fits of the spectra obtained at different temperatures can be obtained by varying all of the parameters, it is more physically reasonable to vary the motional parameters while maintaining fixed structural parameters. For optimization of the spectral simulations, the $^{13}C$ spectrum obtained at the lowest temperature was most heavily weighted, since this spectrum exhibits the most clearly defined multiplet structure. Following this approach, optimal parameter values obtained were $r_{CH}$ = 1.4 Å and $\chi^{CD\text{-}CSA}$ = −0.4. Simulations and overlays of theoretical and experimental results obtained at 5, 15, and 25°C shown in Fig. 2 correspond to these parameters, and to motional parameters $S^2$ = 0.9, $\tau_i$ = 10 ps, and to the $\tau_M$ values indicated in the legend. However, simulations using $S^2$ values ranging from 0.9 to 1.0 gave essentially equivalent errors. Although the data obtained at 35 and 45°C are qualitatively consistent with the further collapse of the multiplet predicted on the basis of the change in overall correlation time, the quantitative fit is considerably poorer. Improved fits of the high-temperature data could be obtained by varying the value of $r_{CH}$. However, the primary reason for this discrepancy is most probably the greater exchange rate of the glucose at the higher temperature, leading to increased exchange broadening of the resonances. Values for the dissociation rate constant and equilibrium dissociation constant for D-glucose with GGR determined at pH 7.4, 20°C, are reported to be 1.38 $s^{-1}$ and 0.4 × $10^{-7}$ *M*, respectively (*18*). This would correspond to a linewidth contribution of 0.44 Hz at this temperature. These exchange contributions are consistent with the observed linewidths, and would be expected to increase with increasing temperature.

The effect of internal motion on the predicted lineshape is illustrated by a series of simulations corresponding to $\tau_M$ = 20 ns, $\tau_i$ = 10 ps, with the remaining parameters as in Fig. 2, and the indicated values of the order parameter, $S^2$, are shown in Fig. 3. From these simulations, it can be seen that increasing disorder results in a reduction of the multiplet asymmetry, as well as sharper component lines of the multiplet, so that the features will be more readily analyzed quantitatively, while leaving a significant degree of multiplet asymmetry. Of course, reducing $S^2$ to 0 also eliminates the contribution of the slower overall tumbling of the molecule, so that the multiplet asymmetry essentially disappears. Interestingly, the simulations indicate that since lowering $S^2$ reduces the linewidths along with the asymmetry, significant perturbations can be observed even at $S^2$ = 0.2.

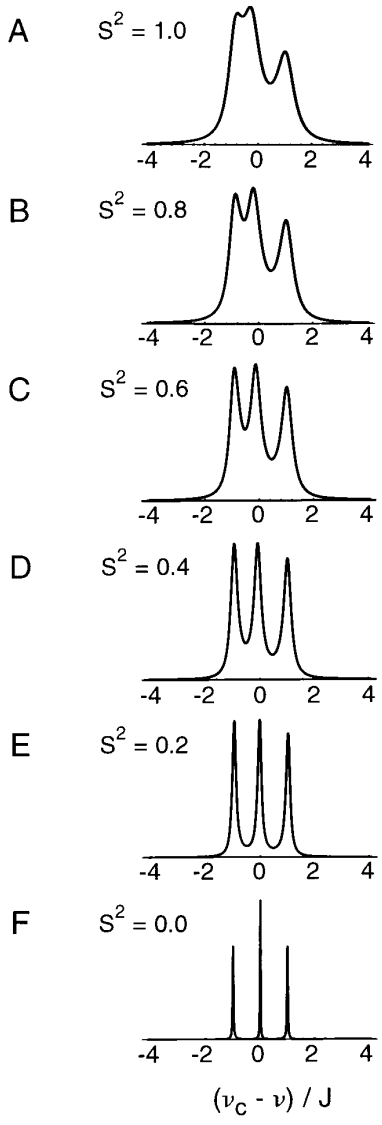


**FIG. 3.** Theoretical $^{13}C$ NMR spectra for the C-1 resonance of D-[1-$^{13}C$,1-$^{2}H$]glucose, with dynamics described by a ''model free'' spectral density. Parameters used for the simulation were the same as those in Fig. 2, with $\tau_M$ = 20 ns, $\tau_i$ = 10 ps, and the order parameters indicated.

Although simulations of the spectra as shown in Fig. 2 indicate that the observed lineshapes can be consistent with an order parameter less than 1, further reductions of the order parameter below 0.9 did not provide close simulations, due to the loss of multiplet asymmetry. The use of an $S^2$ value less than 1 for the simulations shown in Fig. 2 allows greater resolution of the two closely spaced downfield resonances of the multiplets, as observed at 5°C. However, approximately equal error values could also be obtained by reducing $r_{CH}$. Thus, the $S/N$ in these spectra did not allow quantitation of $S^2$ beyond the limit $S^2 \geqslant 0.9$. A high-order parameter for the GGR-complexed glucose is consistent with both the crystallographic $B$ factors (*19*) and in the $^{19}F$ relaxation times of fluorotryptophan-labeled GGR complexed with D-glucose (*12*); both approaches indicate that the complex is most highly ordered in the region surrounding the glucose molecule.

The present results demonstrate that asymmetric lineshapes can arise from tightly bound ligands as a result of dynamic frequency shift effects when the necessary isotopic composition, interaction constants, and geometries are present. Further, these lineshapes will in general be sensitive to both overall and internal motional effects, and hence provide useful sources of dynamic and structural information. Simulation of individual lineshapes can provide information on both structural and dynamic parameters, although the utility of the approach is ultimately limited by the $S/N$ which can be achieved.

## ACKNOWLEDGMENT

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