Analysis of Dynamics and Mechanism of Ligand Binding to Artocarpus integrifolia Agglutinin

A 13C AND 19F NMR STUDY*

M. V. Krishna Sastry‡, Musti Joginadha Swamy§, and Avadhesha Surolia

From the Molecular Biophysics Unit, U. G. C. Centre of Advanced Studies, Indian Institute of Science, Bangalore, 560 012 India

*(Received for publication, September 21, 1987, and in revised form, March 31, 1988)

Binding of 13C-labeled N-acetylglactosamine (13C-GalNAc) and N-trifluoroacetylglactosamine (19F-GalNAc) to Artocarpus integrifolia agglutinin has been studied using 13C and 19F nuclear magnetic resonance spectroscopy, respectively. Binding of these saccharides resulted in broadening of the resonances, and no change in chemical shift was observed, suggesting that the α- and β-anomers of 13C-GalNAc and 19F-GalNAc experience a magnetically equivalent environment in the lectin combining site. The α- and β-anomers of 13C-GalNAc and 19F-GalNAc were found to be in slow exchange between free and protein bound states. Binding of 13C-GalNAc was studied as a function of temperature. From the temperature dependence of the line broadening, the thermodynamic and kinetic parameters were evaluated. The association rate constants obtained for the α-anomers of 13C-GalNAc and 19F-GalNAc (kₐ = 1.01 x 10⁶ M⁻¹s⁻¹ and 6.08 x 10⁵ M⁻¹s⁻¹, respectively) are in close agreement with those obtained for the corresponding β-anomers (kₐ = 0.95 x 10⁶ M⁻¹s⁻¹ and 0.85 x 10⁵ M⁻¹s⁻¹, respectively), suggesting that the two anomers bind to the lectin by a similar mechanism. In addition those values are several orders of magnitude slower than those obtained for diffusion controlled processes. The dissociation rate constants obtained are 49.9, 56.9, 42, and 43 s⁻¹, respectively, for the α- and β-anomers of 13C-GalNAc and 19F-GalNAc. A two-step mechanism has been proposed for the interaction of 13C-GalNAc and 19F-GalNAc with A. integrifolia lectin in view of the slow association rates and high activation entropies. The thermodynamic parameters obtained for the association and dissociation reactions suggest that the binding process is entropically favored and that there is a small enthalpic contribution.

Lectins, due to their ability to bind to cell-surface carbohydrates, have become widely used tools for exploring the structure and dynamics of cell surfaces (1, 2). An elucidation of the specificity and mechanism of saccharide binding to the lectin is necessary for understanding the mechanism of their interaction with cell-surface receptors. Lectin-sugar interactions have been studied using UV absorption, fluorescence, stop-flow, and relaxation techniques. In such studies a chromophore or a fluorophore is usually attached to the ligand which may perturb the specificities of interactions. On the other hand nuclear magnetic resonance (NMR) spectroscopy is a convenient tool to study the dynamics of ligand binding to proteins (3). It has been particularly useful to study the nature of lectin-sugar interactions (4–8), using naturally occurring carbohydrates, by monitoring the changes in NMR parameters such as chemical shifts, change in line widths of the observed resonances, and spin-lattice relaxation times (7). In NMR spectroscopy the use of 13C and 19F nuclei in the studies of protein-ligand interactions is more useful than that of the 1H nucleus because the former nuclei have large chemical shifts and, therefore, yield simple spectra. However, the use of 13C is severely hampered because of its low natural abundance and poor sensitivity. Therefore, specific enrichment at a required position is necessary for effective use of this isotope.

Artocarpus integrifolia lectin was shown by us to be specific for 1-O-α-methylgalactose and T-antigenic disaccharide by fluorescence spectroscopy (9, 10). We have used N-acetylglactosamine (GalNAc)-specific, 13C-labeled at carbonyl carbon and N-trifluoroacetylglactosamine to study their binding to A. integrifolia lectin. The change in line widths of the resonances of α- and β-anomers of GalNAc upon binding to the lectin has been used to obtain the activation parameters for the binding of these anomers to A. integrifolia lectin. The results suggest that the binding of these anomers is characterized by large activation entropy and a two-step binding process.

**EXPERIMENTAL PROCEDURES**

**Materials**

2-Amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and D₂O were products of Sigma. 13C-Labeled acetate anhydride (labeled at carbonyl carbon) was obtained from Stohler Isotopes Chemicals, CA. Dowex 1 was supplied by National Aluminate Corp., Chicago. Chelex 100 was obtained from Bio-Rad. All other reagents were of analytical grade.

**Methods**

**Purification of A. integrifolia Lectin—**A. integrifolia lectin was purified by affinity chromatography on cross-linked guar gum as reported (9, 11). Protein concentration was estimated according to the method of Lowry et al. (12).

**Synthesis of 13C- and 19F-labeled N-Acetylgalactosamines—**N-Acetylgalactosamine specifically 13C-labeled at the carbonyl carbon of

*The abbreviations used are: GalNAc, N-acetylglactosamine; NTFAGalN, N-trifluoroacetylglactosamin; NTFAcN, N-trifluoroacetylchalcone; dansyl, 5-dimethylaminonaphthalene-1-sulfon}

14826
the acetamido group was synthesized according to Roseman and Ludewig (13), using 13C-labeled acetic anhydride in the presence of the carbonate form of Dowex 1. The purified sugar was recrystallized from ethanol.

N-Trifluoroacetylgalactosamine (NTFAGalN) and N-trifluoroacetylgalactosamine (NTFAGalN) were synthesized by following the procedures outlined by Wolfram and Coniglio (14).

Chemical Modification of Tyrosyl Side Chains of A. integrifolia Lectin by N-Acetylimidazole—Tyrosyl side chains of A. integrifolia lectin (5-6 mg/ml) were modified with 300-fold molar excess of N-acetylimidazole in 200 mM sodium phosphate buffer, pH 7.3, at 5 °C for 1 h. The protein was passed through a Sephadex G-25 column and dialyzed and concentrated by lyophilization.

Treatment for Demetalization of A. integrifolia Lectin—All glassware were washed with deionized water. Phosphate-buffered saline, pH 7.3, was prepared and passed through Chelex 100 (15) resin column. The A. integrifolia lectin was dialyzed initially against 1 mM EDTA and then against 3 mM EDTA twice. Then the lectin was dialyzed against deionized water and phosphate-buffered saline. The lectin was concentrated by lyophilization. None of the transition metal ions could be detected by atomic absorption (Perkin Elmer, Model 2380), either in the Chelex 100-treated buffer or in the protein used for NMR studies.

NMR Measurements—NMR samples were made in phosphate-buffered saline, pH 7.2. The resultant protein concentration in the samples was 1.252 mM (dimer) for 13C-GalNAc and 1.046 mM for NTFAGalN. The concentration of 13C-GalNAc and NTFAGalN were varied from 4 to 12 mM and 7 to 26 mM, respectively. All samples contained 0.03% azide for preventing bacterial growth and 25% D2O for field frequency locking of the spectrometer. 13C NMR spectra were recorded in tubes of 10-mm diameter on a Bruker WH-270 spectrometer at 67.8 MHz with quadrature detection. 19F spectra were obtained using 5-mm tubes on a Varian FT 80A spectrometer. Equimolar concentrations of p-dioxane and trifluoroethanol were used as internal reference for obtaining 13C and 19F NMR spectra, respectively. In the case of 13C NMR spectra, inverse gated decoupling was done with 3.0-s delay time in order to avoid undue heating of the sample. 13C spectra with good signal to noise ratio were obtained within 6-8 h of accumulation. Experiments were carried out at different temperatures using a Bruker B-ST 100/700 temperature control unit. Line broadening of resonances was measured at half-height of the resonance under observation. Observed resonances were corrected for magnetic field inhomogeneity by using the width at half-height of the resonances of p-dioxane and trifluoroethanol in 13C and 19F NMR experiments, respectively.

RESULTS

The 13C and 19F NMR spectra of 13C-GalNAc and NTFAGalN in the absence and in the presence of A. integrifolia lectin are shown in Figs. 1 and 2, respectively. The resonances to the upfield were assigned as β- and those of the downfield as α (16). The resonances of both the anomers broadened only in the presence of the lectin. We argue that this line broadening emanates from the specific binding of the saccharide(s) to the lectin. This was established by the following criteria. 1) The line broadening varies as a function of total concentration of the labeled saccharide. 2) In the presence of an excess unlabeled competing saccharide (in this case 1-O-α-methylgalactose), the line broadening was substantially diminished (~80-85%). 3) The line width of the resonances of 13C-GalNAc as well as NTFAGalN remains unchanged when a chemically modified protein was used. 4) The line width of resonances of α- and β-anomers of 13C-GlcNAc as well as NTFAGlcN remains unchanged in the presence of 1.4 mM A. integrifolia lectin. These four control experiments not only clearly demonstrate the fact that the line broadening occurs only as a consequence of specific binding of these anomers (α and β) to the lectin but also emphasizes the fact that the phenomenon of line broadening is not due to a change in viscosity of the sample due to the addition of large macromolecules or due to the presence of metal ions sticking to the surface of the protein. Identical line width for the α- and β-anomers of NTFAGalN was observed when protein subjected to demetalization was compared with untreated protein of identical concentration. Moreover, the line width of the α- and β-anomers remained unchanged when the spectrum was recorded in presence of 3 mM EDTA. These observations taken together with our failure to detect transi-
tion metals in the protein suggest that metal ions have no role in the line broadening of the anomers as observed in the present study. In the present study both the anomers, $\alpha$ and $\beta$ of $^{13}$C-GalNAc, as well as NTFAGalN, are in equilibrium with the protein. Thus it is possible to evaluate the kinetic parameters for each of the anomers as outlined below.

As the observed line widths are characteristic of the anomer present, it is possible to estimate the ratio of $\alpha$- and $\beta$-anomers by measuring the area under each resonance. The possible equilibria within the sample tube are

$$ P + L \rightleftharpoons K_{21} P_\alpha $$

$$ P + L \rightleftharpoons K_{21} P_\beta $$

From the above equations, the total protein concentration present in the sample is given by

$$ [P]_t = [P]_f + [P]_\alpha + [P]_\beta $$

where $[P]_t$ is total protein concentration, $[P]_f$ is free protein (or unbound), $[P]_\alpha$ and $[P]_\beta$ represent protein bound to the $\alpha$- and $\beta$-anomers, respectively, of the ligand L.

From Equations 1 and 2

$$ K_a = \frac{k_{21}}{k_{12}} \quad K_a = \frac{k_{21}}{k_{12}} $$

where $K_a$ and $K_b$ are association constants for the interaction of $\alpha$- and $\beta$-anomers with the lectin, respectively. $k_{21}$, $k_{12}$, and $k_{11}$ and $k_{22}$ are association and dissociation rate constants of $\alpha$- and $\beta$-anomers, respectively.

The bound fraction ($f$) of $\alpha$- and $\beta$-anomers can be defined as

$$ f = \frac{[P]_\alpha}{[\alpha]} = K_a[P]_f $$

$$ [P]_f = [P]_t + [P]_\alpha + [P]_\beta = [P]_t(1 + K_a[\alpha] + K_b[\beta]) $$

$$ [P]_\beta = \frac{[P]_f}{1 + K_a[\alpha] + K_b[\beta]} $$

Substituting the $[P]_f$ in Equation 5

$$ f = \frac{K_a[P]_f}{1 + K_a[\alpha] + K_b[\beta]} $$

Similarly for $\beta$

$$ f = \frac{K_b[P]_f}{1 + K_b[\beta] + K_a[\alpha]} $$

The line broadening of a small molecule due to its binding to a macromolecule can be treated according to the method of Swift and Connick (17). For a molecule undergoing chemical exchange between two sites, i.e., between free and bound to a macromolecule, the spin-spin relaxation rate is given by

$$ \frac{1}{T_2} = \frac{f}{T_2 + 1} $$

where $f$ is the fraction of small molecule bound to the macromolecule (in this case it is $\alpha$- and $\beta$-anomers bound to the A. integrifolia lectin), $\tau_m$ is the residence time of the respective anomer in the protein binding site, and $T_m$ is the spin-spin relaxation time in the bound environment. In the fast exchange limit ($T_m \gg \tau_m$) one observes the average width of the free and bound saccharide, while in slow exchange limit ($\tau_m \gg T_m$) the line broadening is governed by the exchange rate $1/\tau_m$, which is equal to the dissociation rate constant $k_{-1}$ of the anomer-protein complex (3). In this study, it has been observed that the anomers are in slow exchange with the protein as the line width at half-height of the $\alpha$- and $\beta$-anomers increases with increase in temperature (Fig. 3). The net change in line width at half-height ($1/T_2$) of the resonance is given by

$$ \frac{1}{T_2} = \frac{1}{T_1} - \frac{1}{T_3} $$

where $1/T_1$ is the observed line width in the presence of the protein, and $1/T_3$ is the line width measured for the resonance in the absence of the protein. Substituting for $f$ with respective fraction of bound with $f_\alpha$ or $f_\beta$ in Equation 9

$$ \frac{1}{T_{2b}} = \frac{K_a[P]_f}{(\tau_a + T_m)(1 + K_a[\alpha] + K_b[\beta])} $$

This equation is essentially the same as the one derived by Novan et al. (18). A rearrangement of the above equation gives

$$ T_{2b} = \frac{(\tau_a + T_m)}{K_a[P]_f} \cdot (1 + K_a[\alpha] + K_b[\beta]) $$

$$ T_{2b} = \frac{(\tau_a + T_m)}{K_a[P]_f} + \frac{(\tau_a + T_m)}{K_b[P]_f} \cdot (K_a + K_b - R_s \cdot [\alpha]) $$

where $R_s = [\beta]/[\alpha]$ is the ratio of the anomers in equilibrium.

If a plot of anomer concentration versus respective line width ($T_{2b}$) is drawn, the negative intercept on x axis gives the dissociation constant $K_{d} = K_a$ for the anomer and the negative y intercept yields the residence time $\tau_m$ (18).

Representative plots are shown in Figs. 4 and 5 for the $\alpha$-anomer of $^{13}$C-GalNAc and NTFAGalN, respectively. The association rate constant was evaluated by the relationship $k_{+1} = K_a \times k_{-1}$. The values obtained for the binding of $\alpha$- and $\beta$-anomers of $^{13}$C-GalNAc as well as NTFAGalN to A. integrifolia lectin are listed in Table I.

Experiments were performed at temperatures ranging from 10 to 20°C. The association constants and dissociation rate constants were evaluated as mentioned above. From the temperature-dependent association and dissociation rates (Figs. 6 and 7), the thermodynamic parameters for the binding of $\alpha$- and $\beta$-anomers of $^{13}$C-GalNAc to A. integrifolia lectin were
**13C and 19F NMR Study**

**FIG. 4.** Plot of the concentration of α-anomer of $^{13}$C-GalNAc versus the reciprocal line broadening, $T_B$, at 20°C.

**FIG. 5.** Plot of the concentration of α-anomer of $^{19}$F-GalNAc versus the reciprocal line broadening, $T_B$, at 21°C.

**TABLE I**

| Sugar anomer | $T$  | $K_a$  | $k_{+1}$ | $k_{-1}$ |
|--------------|------|--------|----------|----------|
|              | °C   | M$^{-1}$ | M$^{-1}$.s$^{-1}$ | s$^{-1}$ | |
| $^{13}$C-GalNAc |      |         |           |          | |
| α-Anomer     | 10   | 3.33    | 0.87      | 26.2     | |
|              | 15   | 2.5     | 0.93      | 37.1     | |
|              | 20   | 2.0     | 1.01      | 49.9     | |
| β-Anomer     | 10   | 2.5     | 0.88      | 36.0     | |
|              | 15   | 2.0     | 0.94      | 47.1     | |
|              | 20   | 1.7     | 0.95      | 56.0     | |
| $^{19}$F-GalNAc |      |         |           |          | |
| α           | 21   | 1.6     | 0.898     | 42.0     | |
| β           | 21   | 1.5     | 0.045     | 43.0     | |

obtained using the following formulations (19) (listed in Table II).

$$E_a = \Delta H + RT$$

$$\Delta G = \Delta H - T\Delta S$$

$$\ln(K/T) = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln(k'/h)$$

where $\Delta H$, $\Delta G$, and $\Delta S$ are enthalpy, free energy, and entropy, respectively, $k$ is the appropriate rate constant, $k'$ is the Boltzman constant, and $h$ is the Planck's constant.

**DISCUSSION**

The control experiments as described under "Results" show that the observed broadening is due to specific binding of α-

**FIG. 6.** $\ln(k_{+1}/T)$ and $\ln(k_{-1}/T)$ values obtained for the binding of α-anomer of $^{13}$C-GalNAc to A. integrifolia lectin, plotted against inverse of temperature (1/T).

**FIG. 7.** $\ln(k_{+1}/T)$ and $\ln(k_{-1}/T)$ values obtained for the binding of β-anomer of $^{13}$C-GalNAc to A. integrifolia lectin, plotted against inverse of temperature (1/T).

**TABLE II**

| Parameter | $\alpha$-Anomer | $\beta$-Anomer |
|-----------|------------------|----------------|
| $\Delta G_{en}$ | 43.65 | 43.64 |
| $\Delta G_{ef}$ | 58.84 | 57.8 |
| $\Delta G^o$ | -15.2 | -14.16 |
| $\Delta H_{en}$ | 8.68 | 8.186 |
| $\Delta H_{ef}$ | 45.85 | 38.226 |
| $\Delta H^o$ | -37.3 | -30.04 |
| $\Delta S_{en}$ | -119.38 | -121 |
| $\Delta S_{ef}$ | -46.896 | -70.1 |
| $\Delta S^o$ | -72.48 | -51.3 |
and \( \beta \)-anomers of \(^{13}\)C-GalNAc as well as NTFAGalN to \( A. \ integrifolia \) agglutinin.

The rate parameters can be determined more accurately by stop-flow or temperature-jump studies; however, these methods were used by the introduction of chromogenic reporter group(s) in the saccharide which may influence some perturbation and/or significantly alter the mechanism. On the other hand the \(^{13}\)C- and \(^{19}\)F-labeled ligands represent more closely the naturally occurring carbohydrates and, hence the information obtained is near to the physiological situation. Moreover, in this study it has been possible to elucidate the dynamics of binding of \( \alpha \)- and \( \beta \)-anomers to the lectin, which was not feasible with the former techniques. On the other hand the elucidation of the dynamics of sugar binding to lectins by following the changes in the intrinsic fluorescence of the protein has not been possible due to a lack of correlation between the ligand binding and fluorescence change.

The temperature-dependent changes in the line widths of the resonances reveal that these anomers are in slow exchange with the protein \( (r_m \gg T_m) \). If the anomers were in fast exchange with the protein one would expect a decrease in line width with increase in temperature, the line broadening effects are governed by the residence time \( (r_m \gg T_m) \) of the anomers at the binding site, and hence the kinetic parameters for the interaction of the lectin with sugar could be evaluated as per the procedure of Swift and Connick (17). This condition enabled the determination of the residence times of the anomers in the protein-binding site \( (r_m = 1/k_r) \). The association constants for both \(^{13}\)C-GalNAc and NTFAGalN are in good agreement with the earlier studies using fluorescence spectroscopy (9, 10). It can be seen from Table I that the association constants as well as the association and dissociation rate constants obtained for the \( \alpha \)- and \( \beta \)-anomers of \(^{13}\)C-GalNAc are similar to those obtained for the \( \alpha \)- and \( \beta \)-anomers of NTFAGalN. The thermodynamic parameters, \( \Delta H^0 \) and \( \Delta S^0 \), obtained for the two anomers of \(^{13}\)C-GalNAc are, however, at some variance with those obtained earlier \( (\Delta H^0 = -55.73 \text{ kJ} \cdot \text{mol}^{-1}; \Delta S^0 = -119 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}) \) by the fluorescence method (see Table II). This could arise because of the difference in the principles in the two techniques (in the NMR method determination of association constant is direct, whereas in the fluorescence method it is indirect). In order to verify the values using another direct method, we have determined these values for the interaction of GalNAc (mixture of anomers) by monitoring the ligand-induced changes in the protein fluorescence. The values obtained \( (\Delta H^0 = -44.87 \text{ kJ} \cdot \text{mol}^{-1}; \Delta S^0 = -87.58 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}) \) are in better agreement with the values obtained from the NMR experiments (for example \( \Delta H^0 = -57.3 \text{ kJ} \cdot \text{mol}^{-1} \) and \( \Delta S^0 = -72.48 \text{ J} \cdot \text{mol}^{-1} \) for the \( \alpha \)-anomer). A reasonable agreement between the thermodynamic data obtained for the binding of GalNAc by intrinsic fluorescence and those determined by NMR measurements suggests that NMR parameters reflect the events related with the association of the lectin with the anomers. Examination of Table II reveals that the association of these anomers with the lectin is entropic and the activation enthalpy needed is small \( (\Delta H_{\text{act}} = 6.88 \text{ kJ} \cdot \text{mol}^{-1} \) and \( 8.18 \text{ kJ} \cdot \text{mol}^{-1} \) for the \( \alpha \)- and \( \beta \)-anomers, respectively). The large enthalpy of dissociation \( (\Delta H_{\text{off}} = 45.85 \text{ kJ} \cdot \text{mol}^{-1} \) and \( 48.3 \text{ kJ} \cdot \text{mol}^{-1} \) for the \( \alpha \)- and \( \beta \)-anomers, respectively) could be due to an energetic requirement to break hydrogen bonds between the protein and the saccharide during the dissociation process. The situation found here is similar to that observed for the binding of \(^{13}\)C-labeled 1-O-\( \alpha \)-methylgalactose and its \( \beta \)-methyl derivative to peanut agglu-

\( ^{13} \)C and \(^{19} \)F NMR Study

The apparent association constant becomes

\[
\frac{[PL]_0[PL]}{[P][L]} = K_1 + k_1k_2 \quad K_1K_2 = [K_1(1 + K_3)]
\]

The apparent association rate constant is then given by

\[
k_{\text{on}} = \frac{K_1K_2}{k_{-2}} = k_{-2}K_1
\]
The apparent association rate constant remains almost constant with temperature, indicating that $k_{-1}$ does not increase appreciably with increase in the temperature, whereas $k_{1}$ diminishes, thus resulting in a negligible net change in $k_{ ass}$ with change in temperature and low values of change in enthalpy.

An alternative explanation for the slow association reaction assumes the presence of two conformational states of the protein (P and $P^*$), only one of which ($P^*$) binds to the saccharide. The saccharide binding induces a shift in the equilibrium between the two conformers toward the one which binds the protein as shown below:

$$P \rightarrow P^*, \quad P^* + L \rightarrow P L^*$$

However, such a mechanism is very unlikely here in view of the linearity of the $\ln(K/T)$ versus $(1/T)$ plots (Figs. 6 and 7). This mechanism is also ruled out by the fact that similar values of $k_{1}$ and $k_{-1}$ were obtained when protein concentration was varied between 0.3 and 2 mM. If this mechanism were to be operative then one would have expected an increase in $k_{1}$ with increase in the concentration of the lectin.

The data obtained here suggest a two-step mechanism based on the following observations. The first step (i.e. formation of PLd), which is probably a diffusion controlled reaction, would be a loose association of the saccharide with the protein. The second step is a mutual fitting of the saccharide in the lectin-binding site. The step is associated with a large activation entropy, due to the requirement of a specific orientation of the reactants for the association process. Our failure to detect the intermediate complex [PL], could be due to the fact that $k_{32} > k_{12}$ and, therefore, it is possible to observe only the overall reaction (28).

It is interesting to compare the binding of $\alpha$- and $\beta$-anomers to the A. integrifolia lectin with that of the binding of NTFAGlcN to concanavalin A (6) and the binding of GlcNAc to lysozyme (29). In the case of GlcNAc binding to lysozyme, the acetamido groups of $\alpha$- and $\beta$-anomers of GlcNAc experience a magnetically nonequivalent environment in the enzyme binding site resulting in a chemical shift of the acetamido resonances to varying degrees (29), whereas in the case of binding of $\alpha$- and $\beta$-anomers of NTFAGlcN to ConA, no shift in the resonances of either of the anomers was observed.

In the present study, the $\alpha$- and $\beta$-anomers and $^{13}$C-GalNAc as well as NTFAGalN do not show any chemical shift differences between free and bound states. This observation in conjunction with the similar association and dissociation rate constants for the binding of $\alpha$- and $\beta$-anomers of $^{13}$C-GalNAc as well as NTFAGalN to A. integrifolia lectin suggests that the acetamido groups of $\alpha$- and $\beta$-anomers experience a magnetically equivalent environment in contrast to the binding of $\alpha$- and $\beta$-anomers of GalNAc to lysozyme.

Acknowledgments—We thank the Sophisticated Instruments Fa-

REFERENCES

1. Lis, H., and Sharon, N. (1986) Annu. Rev. Biochem. 55, 35-67
2. Goldstein, I. J., and Hayes, C. E. (1978) Adv. Carbohydr. Chem. Biochem. 35, 127-340
3. Dwek, R. A. (1975) Nuclear Magnetic Resonance in Biochemistry: Applications Enzyme Systems, Clarendon Press, Oxford
4. Brewer, C. F., Sternlicht, H., Marcus, D. M., and Grollman, A. P. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 1007
5. Brewer, C. F., Sternlicht, H., Marcus, D. M., and Grollman, A. P. (1973) Biochemistry 12, 4448-4457
6. Alter, G. M., and Magnuson, J. A. (1974) Biochemistry 13, 4038-4045
7. Brewer, C. F., and Brown, R. D., III (1979) Biochemistry 18, 2555-2562
8. Neurohr, K. J., Young, N. M., Smith, I. C. P., and Mantsch, H. H. (1981) Biochemistry 20, 3499-3504
9. Krishna Sastry, M. V., Banerjee, P., Patanjali, S. R., Swamy, M. J., Swarnalatha, G. V., and Surolia, A. (1986) J. Biol. Chem. 261, 11726-11733
10. Krishna Sastry, M. V., and Surolia, A. (1986) Biosci. Rep. 6, 863-860
11. Suresh Kumar, G., Appukuttan, P. S., and Basu, D. (1982) J. Biochem. (Bangalore) 4, 257-261
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
13. Roseman, S., and Ludoweig, J. (1954) J. Am. Chem. Soc. 76, 301-302
14. Wolfrom, M. L., and Conigliaro, P. J. (1969) Carbohydr. Res. 11, 63
15. McKee, D. J., and Frieden, E. (1971) Biochemistry 10, 3880-3883
16. Millet, F., and Raftery, M. A. (1972) Biochemistry 11, 1639-1643
17. Swift, J. J., and Connick, R. E. (1962) J. Chem. Phys. 37, 307-320
18. Navaz, G., Shulman, R. G., Wyluda, B. J., and Yamane, T. (1970) J. Mol. Biol. 51, 15-30
19. Laidler, K. J. (1979) Theories of Chemical Reaction Rates, pp. 41-55, R. E. Krieger Publishing Co. Inc., Huntington, NY
20. Clegg, R. M., Loontiens, F. G., and Jovin, T. M. (1977) Biochemistry 16, 167-170
21. Lanir, A., and Avon, G. (1971) Biochemistry 10, 1024-1032
22. Lewis, S. D., Shafer, J. A., and Goldstein, I. J. (1976) Arch. Biochem. Biophys. 172, 689-695
23. Clegg, R. M., Loontiens, F. G., Sharon, N., and Jovin, T. M. (1983) Biochemistry 22, 4797-4804
24. Podder, S. K., Surolia, A., and Bachhawat, B. K. (1978) FEBS Lett. 85, 313-316
25. Swamy, M. J., Krishna Sastry, M. V., Khan, M. I., and Surolia, A. (1986) Biochem. J. 234, 515-522
26. Sykes, B. D. (1969) Biochemistry 8, 1110-1116
27. Neurohr, K. J., Mantsch, H. H., Young, N. M., and Bundle, D. R. (1982) Biochemistry 21, 498-503
28. Jardetzky, O., and Roberts, G. C. K. (1981) in NMR in Molecular Biology (Horecker, B., Kaplan, N. O., Mormur, J., and Scherga, H. A. eds) pp. 425-428, Academic Press, New York
29. Dahlquist, F. W., and Raftery, M. A. (1968) Biochemistry 7, 3269-3277

13C and 19F NMR Study