Preliminary communication

An n.m.r. study of the effects of fluorine substituents on the association between lysozyme and derivatives of 2-amino-2-deoxy-D-glucose

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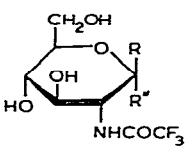
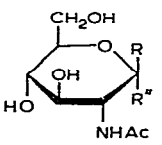
Previous studies from this laboratory[1] have shown that fluorine nuclear magnetic resonance (^{19}F n.m r.) parameters are generally more sensitive to changes in chemical environment than are proton (^{1}H) n m.r parameters In order to evaluate the potential of ^{19}F n m r. in studies of biochemically relevant systems, we have investigated the molecular association between lysozyme (salt-free, from hen egg-white, purchased from Worthington Biochemical Corporation) and some specifically fluorinated derivatives of 2-amino-2-deoxy-D-glucopyranose (D-glucosamine), this enzyme was chosen as a model system because of the detailed X-ray[2] and ^{1}H n.m r data[3] that were already available in the literature; since this work was completed, two other laboratories have also made ^{19}F n m.r. studies[4,5].

Four, *N*-trifluoroacetyl derivatives (**1**–**4**) of D-glucosamine were prepared by standard methods[6]. The ^{19}F chemical shifts of various concentrations (0 10–0 02 M) of these substances were measured in the presence of lysozyme (3 0 mM), 2-hydroxy-1, 1,1-trifluoroethane was used as an internal reference.





1 R′ = H, R″ = OH	**5** R′ = H, R″ = OH
2 R′ = OH, R″ = H	**6** R′ = OH, R″ = H
3 R′ = H, R″ = OMe	**7** R′ = H, R″ = OMe
4 R′ = OMe, R″ = H	**8** R′ = OMe, R″ = H

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Analysis[7] of the chemical-shift changes observed for **1** during such ^{19}F n.m.r experiments gave values (Table I) for the bound chemical-shift, Δ_B, and the binding constant, K_B. However, the lack of detectable changes in the shift of the ^{19}F resonances of **2**, **3**, or **4** effectively precluded any calculations of the binding for these substances. Nevertheless, separate series of competition experiments clearly indicated that both **3** and **4** compete directly, although less strongly, for the same binding site as **7**, a known inhibitor. Competition experiments between **1** and **2** indicated that, if **2** binds, it does so less strongly than **1**

TABLE I

VALUES OF BINDING CONSTANTS (K_B) AND BOUND CHEMICAL-SHIFTS (Δ_B) FOR DERIVATIVES OF D-GLUCOSAMINES

Compound	K_B *(mole^{-1})*	Δ_B *(p.p.m.)*
1	97	0.95
2	*a*	*a*
3	*a*	*a*
4	*a*	*a*
5	62 [b]	0.68 [b]
6	30 [b]	0.51 [b]
7	22.7	0.73
	19.2 [c]	0.55 [c]
8	30 [c]	0.54 [c]

[a] Within experimental error, there was no appreciable broadening or chemical shift of the ^{19}F resonance of these compounds as a function of concentration. [b] Data taken from Ref. 3a. [c] Data taken from Ref. 7

In addition to the binding constant for **1**, we have also determined the rates of association (k_1) and dissociation (k_{-1}) of its complex with lysozyme, the experiments were performed with a highly selective, pulse n.m.r. spectrometer[8], and the data were analysed by the method of Sykes[3c]. These rate constants, together with the literature values for **5**, are listed in Table II. In spite of the substantial experimental errors that are associated with such measurements, it seems that k_1 is higher for **1** than for **5**

TABLE II

RATES OF ASSOCIATION (k_1) FOR THE INTERACTION OF D-GLUCOSAMINE DERIVATIVES WITH LYSOZYME

Compound	k_1	k_{-1}
1	1.1×10^6	1.1×10^4
5 [a]	3.5×10^5	8.5×10^3

[a] Data taken from Ref. 3d.

In addition to the above numerical data, several important details of perspective clearly emerge from this study. First, although, for this system, ^{19}F chemical shifts are more sensitive to *inter*molecular changes in environment than are ^{1}H chemical shifts, the enhancement is not as marked as it is for *intra*molecular changes. Second, although a change in the chemical shift of a probe site of a small, reversible-inhibitor substance can be readily interpreted in terms of a molecular association, absence of any observed shift does not necessarily indicate an absence of molecular association. Finally, the expectation[9] that the introduction of a fluorine substituent can cause changes in the nature of a molecular association, clearly demonstrated here, implies that specifically fluorinated substrates cannot *necessarily* be used as direct models for normal (non-fluorinated) substrates.

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