^{19}F Magnetic Resonance Spectroscopic Investigation of the Binding of 2-Deoxy-2-trifluoroacetamido-α-D-glucose to Lysozyme

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Summary The ^{19}F n.m.r. signal of 2-deoxy-2-trifluoroacetamido-α-D-glucose, but not the β-anomer, shows an upfield shift in the presence of lysozyme.

IT has been shown that when both anomers of 2-acetamido-2-deoxy-D-glucose are bound to lysozyme there are upfield shifts in the ^1H n.m.r. signals of the *N*-acetyl groups.[1-3] We find that there is a similar shift of the fluorine signal of 2-deoxy-2-trifluoroacetamido-α-D-glucose† but that certain significant differences suggest that this compound is bound differently to 2-acetamido-2-deoxy-α-D-glucose.

Mutarotated 2-deoxy-2-trifluoroacetamido-D-glucose has a similar inhibiting effect to 2-acetamido-2-deoxy-D-glucose on the rate of hydrolysis of the cell walls of *Micrococcus lysodeikticus* catalysed by lysozyme (Hens' egg white lysozyme from Boehringer). Thus under standard conditions this reaction was 24% inhibited in the presence of $2{\cdot}5 \times 10^{-2}$ M-2-acetamido-2-deoxy-D-glucose and 20%

† M.p. 199—202°, $[\alpha]_D^{30} = 38{\cdot}0 \rightarrow 13{\cdot}6°$ (*c*, 1·0 in H_2O).

inhibited in the presence of $1\cdot8 \times 10^{-2}$M-2-deoxy-2-trifluoroacetamido-D-glucose. An aqueous mutarotated solution of 2-deoxy-2-trifluoroacetamido-D-glucose has ^{19}F-signals at 339·6 and 312·0 Hz‡ downfield from external trifluoroacetic acid arising from the α- and β-anomers respectively. The signal of the α-anomer moves upfield in the presence of lysozyme ($3\cdot9 \times 10^{-3}$ M) and the extent of this shift depends on its concentration. The signal of the β-anomer moves downfield *ca.* 4 Hz and this shift is concentration independent. When tetra-*N*-acetylchitotetraose is added at a concentration 1·2 times that of the lysozyme the ^{19}F signals of the α- and β-anomers appear 0·7 and 3·9 Hz downfield from their original position in the absence of enzyme. These downfield shifts presumably arise from a medium or bulk-susceptibility effect since downfield shifts were also found in the presence of chymotrypsin. The upfield shift of the α-anomer which is suppressed by tetra-*N*-acetylchitotetraose must therefore arise from binding in the active site.

The variation of the shift of 2-deoxy-2-trifluoroacetamido-α-D-glucose with its concentration was analysed by the method of Sykes[3] and values of 78 Hz for the differences in chemical shift between unbound and bound 2-deoxy-2-trifluoroacetamido-α-D-glucose and $9\cdot1 \times 10^{-3}$ mol l^{-1} for the binding constant were calculated. The analogous values for 2-acetamido-2-deoxy-α-D-glucose determined under identical conditions were 87 Hz and $2\cdot04 \times 10^{-2}$ mol l^{-1}. The similarity of the shift differences suggest that although 2-deoxy-2-trifluoroacetamido-α-D-glucose is bound in the active site it is situated differently from 2-acetamido-2-deoxy-α-D-glucose since if they were situated identically and experience identical changes in magnetic environment a much larger chemical shift should be found with the fluoro-compound. This conclusion is supported by the observation that methyl 2-deoxy-2-trifluoroacetamido-α-D-glucoside§ shows only a concentration-independent downfield shift in the presence of lysozyme whereas methyl 2-acetamido-2-deoxy-α-D-glucoside shows an upfield shift similar to that shown by the free sugar.[1,2] Presumably the situation in which the *N*-trifluoroacetyl compound is bound leads to an unfavourable interaction with the enzyme when there is a methoxy-group at C-1.

Kent and Dwek have recently reported an investigation of the binding of 2-deoxy-2-fluoroacetamido-α-D-glucose to lysozyme using ^{19}F n.m.r. spectroscopy. It is interesting that there is a downfield shift in the presence of the enzyme in contrast to our results with 2-deoxy-2-trifluoroacetamido-α-D-glucose.[5]

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‡ All measurements were carried out at 94·1 MHz. The signals were assigned on the basis of the ^{1}H and ^{19}F n.m.r. spectra of the mutarotated and non-mutarotated *N*-trifluoroacetyl-α-D-glucosamine.

§ M.p. 192—193°; $[\alpha]_D^{25} = +84\cdot6°$ (*c*, 0·8 in H_2O).

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