than values for ^1H (Elmsley, Feeney & Sutcliffe, 1966). Present studies are concerned with determining the values of these parameters for fluorine in distinctive configurations in a number of fluoromonosaccharides.

It has already been found that anomeric fluorine [F(1)] has a chemical shift (ϕ_c) of approx. 150 p.p.m. (with respect to trichlorofluoromethane) (Hall, Adamson, Johnson & Foster, 1970; Dwek, Kent, Kirby & Harrison, 1970) and that coupling with adjacent protons gives values of $J[\text{F}(1a)-\text{H}(2a)]$ 25 Hz and $J[\text{F}(1e)-\text{H}(2e)]$ 2 Hz. (Hall & Manville, 1967, 1968; Campbell, Dwek, Kent & Prout, 1969). Extended studies have shown that $-\text{CH}_2\text{F}$ in extracyclic positions, as in methyl 6-deoxy-6-fluoro- α -D-galactoside, has $\phi_c + 230$ p.p.m. similar to $-\text{CH}_2\text{F}$ in acyclic systems, e.g. fluoroacetamide where ϕ_c is +221.5 p.p.m. In contrast, $-\text{O}-\text{CF}_3$, as in an aglycone, has $\phi_c + 60$ p.p.m. The value of ϕ_c for F(2) is approx. 212 p.p.m. for a variety of fluoromonosaccharides, whereas F(3) in methyl 3-deoxy-3-fluoro- α -D-guloside has $\phi_c + 196$ p.p.m. The crystalline sugar reported as 3-deoxy-3-fluoro-D-glucose was found to give two peaks in g.l.c. (T_m 14.0 min and 16.8 min) in approximately equal amounts, and two ^{19}F shifts were observed of +194 and +200 p.p.m.

In the furanose series methyl 2,5-di-O-benzoyl-3-deoxy-3-fluoro- α -D-arabinoside has $\phi_c + 186$ p.p.m. whereas the corresponding 3-fluoroxylloside has $\phi_c + 202$ p.p.m. (in chloroform). Under high-resolution conditions the fluoroarabinoside showed a fine structure having $J[\text{F}(3)-\text{H}(3)]$ 46 Hz, $J[\text{F}(3)-\text{H}(2)]$ 7 Hz and $J[\text{F}(3)-\text{H}(4)]$ 10 Hz, and the xylo analogue had $J[\text{F}(3)-\text{H}(3)]$ 56 Hz, $J[\text{F}(3)-\text{H}(2)]$ 24 Hz and $J[\text{F}(3)-\text{H}(4)]$ 15 Hz. The *gem* F-H coupling constants of 53 Hz and 46 Hz for axial and equatorial fluorine respectively are similar to values reported for other positions in the ring (Hall & Manville, 1967; Hall *et al.* 1970).

Thus ^{19}F parameters, chemical shifts in particular, can provide significant stereochemical information about fluoromonosaccharides and open a way for the use of fluorine as a more general structural probe for biological macromolecules.

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N-Fluoroacetyl- α -D-glucosamine as a Molecular Probe of Lysozyme Structure by using [^{19}F]Fluorine Nuclear-Magnetic-Resonance Techniques

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In enzymic pathways involved in the biosynthesis of glycoproteins and mucopolysaccharides there is evidence that acylation of amino sugar intermediates is an important regulatory step (Kent, 1970). Chemical modification of such *N*-acyl groups, as in *N*-iodoacetyl- α -D-glucosamine, results in substantially altered biological properties, e.g. inhibition of glycoside transport and bacterial growth (Kent, Ackers & White, 1970; White & Kent, 1970; White, 1970). These properties appear to arise from the competitive occupancy by the haloacyl sugars of molecular sites specific for the glucose or *N*-acetylglucosamine configuration.

This hypothesis has been examined further on the basis of the close substrate analogy shown between metabolites and certain of their fluorinated derivatives (Kent, 1969). In particular, an investigation was made of the binding of *N*-fluoroacetyl- α -D-glucosamine by hen's-egg lysozyme, by using the large and distinctive ^{19}F chemical shifts that fluorocarbohydrates display (Dwek, Kent, Kirby & Harrison, 1970; Kent, Dwek & Taylor, 1971). *N*-Fluoroacetyl- α -D-glucosamine [m.p. 156–158°C, $[\alpha]_D^{25} + 29.4^\circ$ (c 1.0 in water, 3h); cf. Grieg & Leaback (1963): m.p. 189–192°C, $[\alpha]_D + 31^\circ$ (c 1 in water, 2h)], synthesized by dicyclohexylcarbodiimide condensation, has a ^{19}F chemical shift (ϕ_c) of +225 p.p.m. (with reference to trichlorofluoromethane at 33°C at 94 MHz in water or sodium acetate buffers between pH 5 and pH 7) comparable with extracyclic $-\text{CH}_2\text{F}$ [ϕ_c F(6) +230 p.p.m.]. At high resolution *N*-fluoroacetyl- α -D-glucosamine displays a 'deceptively simple' quartet of the ABX type as would arise from non-magnetic equivalence of the $-\text{CH}_2\text{F}$ protons.

In the presence of hen's-egg lysozyme in 50 mM-sodium acetate buffer, pH 7, *N*-fluoroacetyl- α -D-glucosamine shows a downfield shift of 13 Hz compared with enzyme-free controls. At high resolution the original quartet is observable with considerable band-broadening. Fluoroacetamide ($\phi_c + 221.5$ p.p.m.) gives its own distinctive triplet ($J[\text{F}-\text{H}]$ 53 Hz) when examined in the presence or absence of lysozyme; no change in chemical shift is observed in the presence of enzyme. Similarly no changes in ^{19}F n.m.r. parameters are observed with *N*-fluoroacetyl- α -D-glucosamine or fluoroacetamide in the presence of serum albumin. As will be reported elsewhere, haloacetylglucosamines are

more effective inhibitors of lysozyme than is *N*-acetylglucosamine, which is known to bind competitively with the C site (Blake *et al.* 1967) of the enzyme.

The observed changes in chemical shift can be attributed to a fast exchange between the free and enzyme-bound forms of *N*-fluoroacetyl- α -D-glucosamine.

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Structural and Activational Zinc in *Escherichia coli* Alkaline Phosphatase

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The ability of zinc to constrain the *Escherichia coli* alkaline phosphatase molecule against gross conformational change in urea (Trotman & Greenwood, 1969) has been applied to investigate the distinction between structural and activational zinc.

Enzyme metal content was manipulated by two methods: (1) wild-type enzyme was partially depleted of zinc by treatment with EDTA buffer for different periods of time before gel filtration; (2) apoenzyme prepared by extensive dialysis against EDTA followed by metal-free buffer was partially or wholly reconstituted by zinc sulphate in up to 20-fold molar excess, followed by gel filtration. Zinc binding was determined by atomic absorption spectrophotometry. Apoenzyme bound zinc in the proportion added, with maximum capacity 4g-atoms of Zn/mol.

Circular-dichroism spectra of each preparation recorded between 203 and 250nm before and after addition of urea to 6M were compared. The extent to which 6M-urea induced a major spectral change indicative of the transformation from native conformation towards random coil was expressed as

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the disappearance of the α -helix extremum, i.e. $[\theta]_{222\text{nm}, 6\text{M-urea}}/[\theta]_{222\text{nm}, \text{buffer}}$.

Mean conformational stability was 1 in preparations containing 4–2g-atoms of Zn/mol, falling linearly to the basal value of 0.2 at zero zinc content. Both zinc-depletion and zinc-reconstitution pathways were coincident. Excess of Mn^{2+} , Co^{2+} , Cu^+ or Cd^{2+} also restored conformational stability to apoenzyme.

Enzymic initial activity followed an opposite course, being substantially proportional to the amount of bound zinc in excess of 2g-atoms of Zn/mol; again depletion and reconstitution pathways were the same and the hysteresis suggested by the result obtained by Simpson & Vallee (1968) did not appear.

Complementary ultracentrifugation and circular-dichroism analyses confirmed the initiation of apoenzyme dissociation in 1M-urea, and of randomization between 1M- and 2M-urea, without disulphide reducing agent; the denatured subunit had $s_{20,w}$ 1.8S, molecular weight 4×10^4 .

The distinction between two pairs of zinc atoms is clear. Group I, comprising the first two atoms bound by apoenzyme and the last two removed by chelation, are so efficiently located as to constrain tertiary and quaternary conformation against initiation of denaturation, but do not alone facilitate activity. Group II, the last two atoms bound and the first two removed, do not demonstrate major structural involvement, but are essential participants in activity.

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Phosphorylated Phenolic Glucosides in Higher Plants

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Phenolic glycosides are very common higher-plant constituents (Harborne, 1964), but their physiological role has been continually debated since the end of the last century.

Low-molecular-weight phenolic compounds, such as quinol and resorcinol, are readily converted into the corresponding β -D-glucopyranosides when fed to plants (see, e.g., Pridham & Saltmarsh, 1963; Pridham, 1964), and, in addition, strongly acidic phenolic derivatives with high paper-electro-