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-