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

By C. N. A. TROTMAN* and C. GREENWOOD. (*School of Biological Sciences, University of East Anglia, Norwich NR8 88C, U.K.*)

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

* Present address: Department of Physiology, University of Newcastle upon Tyne Medical School, Newcastle upon Tyne NE1 7RU, U.K.

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

By SUSHILA NANAYAKKARA, J. B. PRIDHAM and MARGARET A. YOUNG. (*Department of Biochemistry, Royal Holloway College, University of London, Englefield Green, Surrey, U.K.*)

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-

phoretic mobilities in low-pH buffers are also produced. With resorcinol fed to germinating *Vicia faba* or *Pisum sativum* seeds the major acidic product yields *m*-hydroxyphenyl β -D-glucoside and P_i when treated with dilute alkali, and complete acid hydrolysis produces resorcinol, glucose and P_i in equimolar quantities. Feeding germinating seeds with resorcinol and [32 P] P_i results in incorporation of label into the compound. Methylation and hydrolysis gives 2,3,4-tri-*O*-methylglucose and hence the derivative would appear to *m*-hydroxyphenyl β -D-glucopyranoside 6'-phosphate. Analogous phosphorylated compounds are formed when higher-plant tissues are fed with other simple phenols, phenolic alcohols or phenolic aldehydes.

o-Hydroxybenzyl alcohol is converted into *o*-hydroxybenzyl β -D-glucoside 6'(?)-phosphate by plant tissues. A compound with similar chromatographic and electrophoretic properties occurs naturally in *Filipendula ulmaria* and in *Salix* spp.

Aerobacter aerogenes has been reported to utilize phenolic glucosides by a system involving phosphorylation followed by a hydrolytic cleavage of the products that is catalysed by specific 'phospho- β -glucosidases' (Schaeffer & Schenkein, 1968). It is possible that higher plants may metabolize phenolic glucosides by a similar mechanism with phosphorylation preceding hydrolysis and not vice versa.

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Aggregated States of Mitochondrial Structural Protein

By R. N. AWBERY, D. N. LEES and L. J. ROGERS.
(Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, U.K.)

Recently there has been much interest in the category of proteins, termed 'structural' or 'organizer' proteins, that are thought to form the backbone of membranes (see, e.g., Lenaz, 1968; Criddle, 1969). Present opinion is that structural protein may be not a homogeneous species but a group of proteins of similar structure and physicochemical characteristics (Green, Haard, Lenaz & Silman, 1968; Lejsek & Lusena, 1969).

In sedimentation-velocity studies on mitochondrial structural protein solubilized in 0.1% sodium dodecyl sulphate (Criddle, Bock, Green & Tisdale, 1962) the protein sedimented as a single

symmetrical boundary, most preparations having a sedimentation coefficient of 1.9–2.2S. However, in some 20% of the preparations studied the sedimentation coefficient was rather higher (2.6–2.8S).

We have examined the sedimentation properties of mitochondrial structural protein prepared from rat liver by a method developed in this laboratory. Preparations were studied under a variety of conditions of detergent composition and concentration, buffer composition, temperature and pH, and the results reported are based on some 90 sedimentation-velocity experiments. For most of the studies the detergent used was sodium dodecyl sulphate.

Species of mitochondrial structural protein sedimenting at $s_{20,w}^0$ values of 2.6 and 3.4S were identified and also species sedimenting at 5.7, 8.3 and 11.5S, the present results on these latter not permitting extrapolation to infinite dilution. Tentative identification of a species sedimenting between 2.6 and 3.4S is reported, preliminary results suggesting that the 2.6S, 3.4S and intermediate form may represent different conformational forms of monomeric structural protein. In all experiments carried out the presence and proportion of aggregates of mitochondrial structural protein, favoured by low concentration of sodium dodecyl sulphate, was dependent on the conditions during solubilization; dissociation to smaller aggregates promoted by dilution was irreversible under the conditions used. Some 3.4S species was nearly always present in preparations containing aggregates, but at concentrations below 3.5mg/ml such preparations evidenced only the 3.4S species sedimenting as a single symmetrical boundary, as did the other monomeric species at all concentrations.

The amino acid composition of our structural protein preparation agrees closely with that reported by other workers for mitochondrial structural protein. Investigations as to the degree of heterogeneity of the preparation are in progress, the need to distinguish between possible heterogeneity of the structural protein and presence of aggregated species being borne in mind.

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