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Effects of Ions on the Activity of Enzyme Systems

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The observed activity of many enzyme systems is a function of the ion population of the medium. Recent kinetic studies from Theorell's laboratory [1-3] have emphasized the role of anions in determining the rates of certain enzyme reactions. In an effort to determine the generality and the order of magnitude of these ion effects, several additional systems have been studied. The activity of the glucose-6-phosphate dehydrogenase system was markedly (as much as six fold) stimulated by low concentrations of various ions, while at higher concentration an inhibition was observed *. The magnitude of the stimulation and the onset of inhibition depended upon an interrelationship between the concentration of the substrate, TPN, and the concentration of the specific ions present. The older experiments [4,5], which indicated only a strong inhibition by phosphate are probably a result of a very low effective concentration of the substrate. Studies with various salts indicated that the observed effects were primarily determined by the particular anion species present. The order of magnitude of stimulation is roughly

$$F^- > PO_4^{=} > Cl^- > Br^- > SO_4^{=} > I^- > SCN^-.$$

Large anions like glycyl-glycine and TPN are poor activators, but the substrate, glucose-6-phosphate is an effective "anion activator". The differences in stimulation produced by various cations was generally related to the magnitude of contribution to the ionic strength of the medium. The overall results favor the concept of a non-specific ionic effect rather than an interpretation which involves a heavy metal requirement [6,7].

The observed activity of certain coupled systems, for example the Zwischenferment-old yellow enzyme system and the succinate oxidase system is also related to the kind and concentration of anions present.

These effects have been interpreted according to the general principles of solvation of chemical reactions.

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* Hans Klenow, Institute of Cytophysiology, University of Copenhagen, has independently observed marked stimulation of this enzyme by various ions. (Personal communication.)

The Variation in the Structure of Water on Gelation

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X-ray diffraction patterns of various gels were studied at 20° C in a Guinier camera employing Cu$K\alpha$ radiation. The intensity curves, obtained in the usual way from photometer determinations of film densities, were corrected for polarization and absorption.

Dilute gels (0.3—2.0 %) of certain polyelectrolytes (desoxyribonucleic acid, carboxymethylcellulose) and of a low molecular weight substance (dibenzoylcystin) give similar diffraction patterns with small but characteristic differences from that of pure water at the same temperature. The major peak at $(\sin\Theta) / \lambda = 0.16$ Å^{-1} shifts toward smaller angles and the height of the minor peak at 0.23 Å^{-1} increases relative to the properties of pure water. There also seems to be a tendency for the minor peak to shift toward larger angles.

From diffraction data it is known that the structure of water changes with temperature [1]. With decreasing temperature the tendency of a water molecule to bond itself tetrahedrically to four other neighboring molecules increases. This is observed as a shift in the major peak towards smaller angles and of the minor peak towards larger angles and in an increase in the height of the minor peak.

Thus the changes on gelation are qualitatively similar to those obtained in pure water when the temperature is decreased; that is, the gel-forming substances produce a decrease in the "structural temperature" of the water. The effect is opposite to that found with electrolytes which are known to increase the "structural temperature" [2].

The experimental results confirm the Forslind theory of gelation [3] according to which the thermal vibrations in the water lattice are reduced through a specific coupling to the non-aqueous component of the gel. The stabilization of the water is the result of a structural similarity between the ideal, four-coordinated water lattice and the positions of the hydrogen bond forming atoms on the surface of the non-aqueous phase.

An identical theory was developed in order to explain certain physico-chemical properties of desoxyribonucleic acid solutions [4]. Dielectric properties, viscosity and proton magnetic resonance data were explained through assuming the existence of large hydration shells with a higher degree of water lattice order than that prevailing in pure water. The present X-ray diffraction data are in agreement with the theory that certain asymmetric macromolecules can stabilize the water lattice over distances of some hundred Å.

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Carboxymethyl Chitin, a New Substance Suitable for the Determination of Chitinase Activity

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The chitinases have not hitherto been investigated as extensively as could be expected for enzymes that break down such a common substance as chitin. This substance occurs in large quantities both in several animals (*e. g.*, crustaceans, insects and molluscs) and in various plants (*e. g.*, fungi). Chitinases are also known from various sources, *e. g.*, the "liver" of snails, insect larvae, sperm, a variety of bacteria, molds, and almonds.

The following substrates have been used for the determination of chitinase activity: native chitin, regenerated chitin (precipitated by diluting a solution of chitin in cold concentrated hydrochloric acid), chitosan (deacetylated chitin), chitotriose and chitobiose. As chitin is insoluble in water, it is not suitable as a substrate for the determination of enzymic activity. Chitosan is better for it is soluble in buffer solutions with pH less than about 6.5. In more alkaline solutions it is insoluble and hence not suitable. It is surely not the same enzyme [1,2] that attacks chitin and its oligosaccharides (*cf.* amylase and maltase).

A new chitin derivate, not described previously, carboxymethyl chitin, is suggested now as a substrate for the determination of chitinase activity. It has not the disadvantages of the substances used previously.

The preparation of carboxymethyl chitin. Raw chitin is prepared in the well-known way, *e. g.*, by treating shells of crustaceans with a dilute acid. The chitin is dissolved in supersaturated cold hydrochloric acid and precipitated by adding water or alcohol. The regenerated chitin is heated on a water bath with chloroacetic acid and a strong solution of sodium hydroxide in the way well-known for the preparation of carboxymethyl cellulose. Native chitin does not react with sodium hydroxide and chloroacetic acid under these conditions. The reaction product is dissolved in water and after neutralization the sodium salt of carboxymethyl chitin is precipitated by adding alcohol.

The determination of chitinase activity. A solution of carboxymethyl chitin is rather viscous, and so it is possible to run the determination of chitinase activity viscosimetrically in the well-known way [3]. This is advantageous