BIOCHEMISTRY

Acceleration of Enzyme Reactions in Ice

FREEZING is widely used by biologists and chemists for the purpose of slowing or stopping reactions. In the past few years we and others have shown that freezing can accelerate a number of catalysed reactions, including hydrolysis, aminolysis, dehydration, oxidation and peroxide decomposition[1–7].

The main part played by reactions catalysed by enzymes in biological processes raised the question of whether these reactions, also, could be accelerated under frozen conditions. If so, practical problems in the preservation of biological materials and theoretical problems concerning reactions in the structured-water environments of the living cell might be clarified.

The transfer reaction between an amino-acid ester and hydroxylamine which is catalysed by trypsin was selected for investigation. Reaction mixtures consisted of 0·005 M ester, 0·4 M hydroxylamine hydrochloride, 0·02 M potassium phosphate, and enzyme, and the entire system was adjusted to *p*H 7·5. Tubes were rapidly frozen in a solid carbon dioxide–acetone bath, transferred to constant temperature chambers, and thawed at selected intervals in a room temperature bath for 3–4 min. Chemical changes were negligible during freezing and thawing. Formation of hydroxamic acids was determined by acidification and complexing with ferric ammonium sulphate[4,5].

Three trypsin substrates, lysine ethyl ester, lysine methyl ester, and arginine methyl ester, all reacted with hydroxylamine more rapidly in a frozen solution at −23° C than in a liquid solution at +1° C (Fig. 1). The relative rates also differed in the two systems: lysine ethyl ester reacted faster than lysine methyl ester in the frozen solution but not the liquid solution.

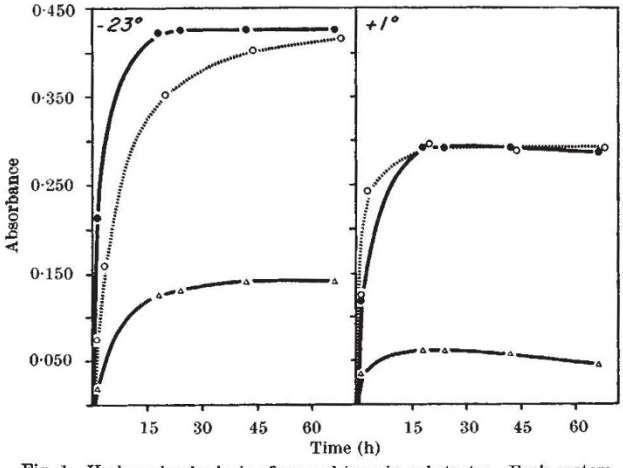

Fig. 1. Hydroxylaminolysis of several trypsin substrates. Each system contains 2 μg trypsin/ml. ●, L-Lysine ethyl ester; ○, L-lysine methyl ester; △, L-arginine methyl ester.

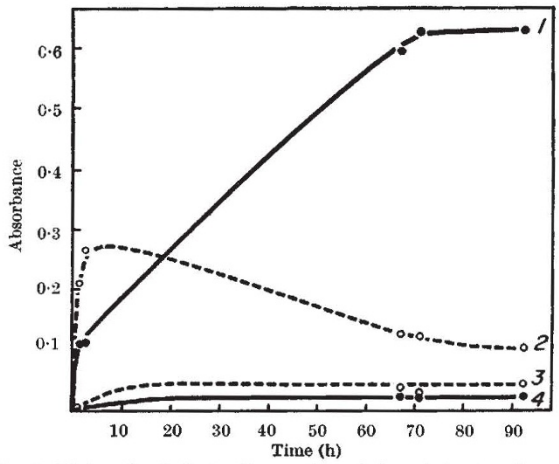

Fig. 2. Hydroxylaminolysis of benzoyl-L-arginine ethyl ester. Curve 1, −18° C, trypsin (2 μg/ml.); curve 2, +1° C, trypsin; curve 3, +1° C, no enzyme; curve 4, −18° C, no enzyme.

With benzoyl-L-arginine ethyl ester as substrate (Fig. 2), the rate of formation of hydroxamic acid catalysed by enzyme at 1° C initially exceeded that at −18° C, but then became negative, indicating hydrolysis to the amino-acid. This change did not occur in the frozen solutions.

It is thus apparent that enzyme reactions may speed up or change their pathway in the frozen state, despite decreased kinetic energy and probable restricted diffusion. Between 0° C and −70° C the liquid water component in frozen systems is highly structured[8]. We are now testing the idea that frozen solutions might offer a useful model for investigating how agents which make and break water structure influence biological processes.

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Inhibition by Puromycin of Incorporation of Tritiated Uridine into Nucleolar and Cytoplasmic Ribonucleic Acids

PUROMYCIN is a specific inhibitor of protein synthesis in bacterial and mammalian cells[1,2] and can therefore be used as a tool for investigating the dependence of various processes on concomitant synthesis of protein[3,4]. It has already been shown that the action of puromycin results in the depression of DNA (refs. 4–6) and RNA (refs. 6–8) synthesis in mammalian cells. These effects are thought to be due to secondary consequences of the interference with protein synthesis, rather than to a direct effect of puromycin on the synthesis of nucleic acids. Density gradient centrifugation of nucleic acids extracted from HeLa cells has shown that the molecular species of RNA most markedly affected by puromycin is the ribosomal[7] or ribosomal precursor type[8]. Correlation of this finding with the results of autoradiography reported here gives further support to the belief that the nucleolus is the site of production, or at least the site of accumulation, of the ribosomal types of RNA (refs. 9–12).

HeLa cells were grown as monolayers in multiple cultures on glass coverslips bound by metal rings[6]. The cells were incubated at 37° C in a humidified atmosphere of 5 per cent carbon dioxide in air. Eagle's minimal essential medium was supplemented with 8 per cent horse serum, 2 mM glutamine and 200 μg/ml. kanamycin. The cells were used in the exponential phase of growth at a cell density of approximately 100,000 cells per culture. Puromycin dihydrochloride (Nutritional Biochemical Company, Cleveland, Ohio) at a concentration of 100 μg/ml. (approximately 2×10^{-4} M) was applied for 30 min to establish full inhibition of protein biosynthesis. At the end of this period the medium was replaced with medium containing tritiated uridine (1 μc./ml. of a sample of specific activity of 5 c./mmole) as well as 100 μg/ml. of puromycin, and after 30 min the medium was again replaced, this time by a medium containing non-radioactive uridine at 1,000-fold excess over the radioactive