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PRESSURE AND REACTIVITY OF PROTEINS, WITH PARTICULAR REFERENCE TO INVERTASE[1]

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In recent studies it has been shown that reactions involving proteins, both in living cells and in extracts, are often accompanied by large volume changes of activation or reaction, respectively: e.g., bacterial luminescence (4, 11, 12, 13, 16, 17, 19), sol–gel changes in myosin and other systems (21), bacterial growth and disinfection (18), denaturation of purified serum globulin and egg albumen (14, 15), inactivation of specific antitoxin (20), and specific precipitation (5). Under conditions favoring protein denaturation, such as increased temperature, alkaline pH, or the presence of certain drugs such as alcohol, the application of hydrostatic pressures up to some 700 atm. has been found to retard, or in some cases to reverse, the denaturation of the system concerned. Moreover, analyses of the kinetic data have indicated that the volume change in all these cases is in the direction of an increase, amounting to between 50 and 100 cc. per mole.

In addition to the above phenomena relating to protein denaturation, the catalytic reactions of certain enzymes, e. g., in bacterial luminescence, may also proceed with large volume increases of activation, on the order of 50 cc. per mole. The object of the present study has been to obtain evidence, through kinetic data, with regard to volume changes involved in the activity of invertase, both under optimal conditions for the reaction and under conditions of pH and increased temperature causing a partial inactivation of the enzyme. The results have extended the data concerning the pressure–temperature–pH relation of enzyme activity and have led to some considerations of a more general interest, which are briefly suggested in the discussion.

METHODS

Most of the experiments were carried out with Difco yeast invertase solution, diluted to 0.2 per cent, and a sucrose concentration of 10 per cent. Buffered solutions of the latter, in aliquot portions of 19 cc., were equilibrated at the desired temperature, and 1 cc. of the enzyme preparation added. After intervals of usually 30, 60, 90, and 120 min., one drop of 10 *N* sodium hydroxide was added to the appropriate specimen. This brought the pH to approximately 10.5, stopping the enzyme reaction and causing mutarotation to go rapidly to completion. The percentage of the original concentration of sucrose remaining at each interval of time was then determined by polarimetric analysis at 20°C. ± 1.5°.

There was evidence of an initial lag in rate of hydrolysis in all cases except

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