A CHEMICAL RELAXATION STUDY OF HUMAN PROSTATIC ACID PHOSPHATASE

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ABSTRACT Chemical relaxation methods and a dilution technique were applied to the study of the hydrolysis of *p*-nitrophenyl phosphate by human prostatic acid phosphatase. Although the reaction mechanism was not elucidated, rate constants and equilibrium constants were obtained for the reaction of enzyme and *p*-nitrophenol to form a complex. A slow, 2-sec relaxation effect which showed no concentration dependence was observed in various reaction mixtures, including some lacking the substrate and products of the hydrolytic reaction. The conclusion drawn is that there are two forms of the prostatic enzyme, which are normally in equilibrium with each other, but which undergo a relatively slow interconversion when this equilibrium is perturbed. A preliminary calculation indicates that these forms are present in the equilibrium ratio of 2:1.

INTRODUCTION

Prostatic acid phosphatase is a nonspecific phosphomonoesterase that will hydrolyze a wide range of orthophosphate monoesters, as well as creatine phosphate (Morton, 1958). The enzyme is found in comparable levels in normal prostate glands of human adults, in hypertrophic glands, and in primary carcinoma of the prostate and its metastases, as well as in seminal fluid (Schmidt, 1955; Gyorkey, 1964).

Chemical relaxation methods are those in which the response to an externally produced perturbation in one or more of the intensive parameters of a reaction system (temperature, pressure, concentrations of reactants, etc.) is analyzed to obtain rate constant data. The simplest form of perturbation is a jump that is so rapid that it may be considered a step function. For discussion of the methods and theory, see Eigen and DeMaeyer (1963), and Kustin, Shear, and Kleitman (1965).

The following abbreviations will be used: *p*-Nϕ = *p*-nitrophenol; *p*-NϕP = *p*-nitrophenyl phosphate; E, E′ = enzyme; Eϕ = enzyme-*p*-nitrophenol complex; and τ_ϕ, τ_e = relaxation times.

ENZYME PREPARATION

The enzyme was prepared from noncancerous, postmortem prostate glands obtained from the Boston City Hospital, using Boman's method (Boman, 1958) followed by a further chromatographic separation on DEAE-cellulose to remove remaining traces of hemoglobin. Small amounts of the nonionic detergent Tween 80, which protects the enzyme against glass surface denaturation (London and Hudson, 1953), were always present during the preparation and experimental procedures. Purification was followed, and the maintenance of activity determined, by an assay based on the change in optical density at 298 mμ accompanying the hydrolysis of *o*-carboxyphenyl phosphate.

EXPERIMENTAL CONDITIONS

All experiments were carried out in 0.1–0.2 M acetate buffer, pH 5, at 25°C. (In pH jumps, the pH varied between 4.5 and 5.5; in temperature-jump studies, the final temperature was about 20°C.) Indicators were *p*-Nϕ, enzyme-reactant complexes, and hemoglobin impurity, all with absorption maxima at about 420 mμ.

MICHAELIS CONSTANT

Initial rate studies gave a value for the Michaelis constant of 2×10^{-4} M, which agrees with the value given by Bunton, Silver, and Vernon (1957), also at pH 5 and 25°C, and is close to the values ($2.32–2.37 \times 10^{-4}$ M) obtained by Lundquist (1947) at pH 6 and 27°C.

TEMPERATURE JUMP

Temperature-jump studies were carried out on solutions prepared by mixing enzyme and *p*-NϕP. A relaxation effect in the range of 100 msec was seen and there were indications of a small effect in the range of 1 msec.

CONCENTRATION JUMP

The stopped-flow apparatus provides for the rapid mixing of solutions contained in two pushing syringes. Several types of concentration jump were used in the present study: some in which the solutions in the two pushing syringes differed in the concentration of one or more reactants; some in which the solutions were the same except that one had been diluted somewhat with buffer (partial dilution); and some in which the two solutions were prepared in buffers of different pH (pH jumps).

Two effects were clearly and repeatedly seen in concentration-jump studies of the entire hydrolytic reaction system. One effect had a relaxation time of about 100 msec, and the other, which was often much smaller in magnitude, had a relaxation time of about 2 sec (τ_e).

Concentration jumps were performed on solutions of enzyme and *p*-Nϕ and

showed an effect in the range of 100 msec. Under conditions in which [p-Nϕ] $\gg$ [E], a reaction of the type

$$E + p\text{-}N\phi \underset{k_{-\phi}}{\overset{k_\phi}{\rightleftharpoons}} E\phi$$

would lead to the relation

$$1/\tau_\phi = k_\phi[p\text{-}N\phi] + k_{-\phi}$$

where [p-Nϕ], the final concentration of free p-Nϕ in the mixed solution, is virtually equal to the total concentration of p-Nϕ in the mixed solution. A linear plot of $1/\tau_\phi$ versus $[p\text{-}N\phi]_{total}$ was obtained from the concentration-jump data: the second-order rate constant, $k_\phi = 1.8 \times 10^3$ mole^{-1} sec^{-1}, is given by the slope, and the first-order rate constant, $k_{-\phi} = 6$ sec^{-1}, by the intercept of the plot. Thus, the equilibrium constant, $K_\phi = k_\phi/k_{-\phi} = 300$ mole^{-1}.

A series of concentration-jump experiments on the entire reaction system showed that, within experimental error, τ_e did not vary with either the concentration of enzyme or the initial amount of substrate added to the reaction mixture. The average value of τ_e was 2.4 sec. This relaxation effect was then observed in concentration-jump experiments performed on solutions containing enzyme and p-Nϕ alone. It was not observed in partial dilution concentration jumps of solutions containing only enzyme (using hemoglobin impurity as the indicator), but was present in pH jumps of these solutions.

SERIAL DILUTION

The contribution of a molecular species to the optical density of a solution is proportional to the concentration of that species. Any deviations from linearity in a plot of optical density versus over-all concentration, during dilution by addition of more buffer, indicate chemical reactions. The serial dilution method can be applied to cases where any or all reactants and products absorb at a given wavelength. All that is required is that the partial derivative of the optical density with extent of reaction be different from zero. The curve of optical density versus concentration will move between two linear asymptotes which pass through the origin. Under appropriate conditions (only one reaction present), the dissociation constant for a complex can be obtained from the point where the curve cuts a line halfway between the two asymptotes.

Several kinds of observation led to the conclusion that there was at least one colored complex involving the enzyme and p-Nϕ, such that when a molecule of p-Nϕ binds to enzyme, the optical density of the solution increases. Thus, during dilution, as [p-Nϕ] decreases, the optical density of the solution will pass from the upper linear asymptote through the origin corresponding to 100% Eϕ complex to the lower linear asymptote corresponding to 0% Eϕ complex. The value of [p-Nϕ]

at the point where the experimental curve cuts the line halfway between the asymptotes (the point at which $[E\phi]/[E] = 1$) is the dissociation constant, the reciprocal of K_ϕ. (This is strictly correct only if there is only one free enzyme species and one $E\phi$ complex.)

The experiments were run in the Beckman DU spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif.), with the cuvette jacket thermostated at 25°C. The value determined for K_ϕ by this method was 100 mole^{-1}.

DISCUSSION

The observations described above indicate that the process responsible for the slow relaxation effect, τ_e, does not require the presence of substrate or product species. All the effects described are too slow to be part of the catalytic hydrolysis, since the smallest first-order rate constant in the forward direction, given by the turnover number, is at least 100/sec (London and Hudson, 1953; Lundquist, Thorsteinsson, and Buus, 1955). The fact that partial dilution concentration jumps will not perturb the equilibrium for this process, unless it is coupled to a second-order process ($E + p\text{-}N\phi \rightleftharpoons E\phi$), and the fact that τ_e shows no concentration dependence, indicate that the reaction is first order in both directions.

The simplest explanation of these results is that there are two interconvertible (allosteric) forms of enzyme, normally in equilibrium with each other in solution:

$$E \underset{k_{-e}}{\overset{k_e}{\rightleftharpoons}} E' \qquad 1/\tau_e = k_e + k_{-e}$$

A pH jump would perturb the equilibrium for this reaction by altering the surface charges and ionic atmospheres associated with this enzyme, which is suspected to have a high local surface charge density even at its isoelectric point (London and Hudson, 1953).

The discrepancy between the values of K_ϕ as determined by concentration jump and by serial dilution lends additional support to the idea that there are two forms ot enzyme. Partial dilution of the solution containing E and p-Nϕ would perturb the equilibrium for the interconversion if p-Nϕ had different affinities for the two forms. If we suppose that p-Nϕ combines with E, but not E′, to form Eϕ, and assume that the slower reaction may be ignored in treating the faster one, we obtain the result that

$$K_{\phi(\text{serial dil.})} = [E\phi]/[p\text{-}N\phi][E]_{\text{total}}$$

$$K_{\phi(\text{conc. jump})} \cong [E\phi]/[p\text{-}N\phi][E]$$

where $[E]_{\text{total}} = [E] + [E']$, and thus, at equilibrium,

$$[E']/[E] = k_e/k_{-e} = K_e \cong 2.$$

It may be mentioned that evidence has been obtained for similar sorts of slow interconversion of enzyme forms with a bacterial NADH oxidase (Worcel, Goldman, and Cleland, 1965), and with yeast D-glyceraldehyde-3-phosphate dehydrogenase (Kirschner et al., 1966).

It might be a fruitful line of investigation to study the effects of citrate (an activator) and L(+)-tartrate (an inhibitor) concentration jumps on the 2-sec relaxation time of prostatic phosphatase. It is possible that one form of the enzyme is catalytically active, and the other inactive, and that the slow interconversion is related to the biological control of activity. The high concentration of citrate in the semen of man and other primates with high acid phosphatase activity, and the finding of Anagnostopoulos (1953) that the activity of the enzyme approaches the same limiting value under the influence of all the activators studied, would seem to make this a plausible speculation.

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