The temperature dependence of the enolase reaction with different activating metal ions

In attempts at developing a method for the measurement of initial velocities of the enolase reaction at high enzyme concentrations by the use of low temperatures, rates were sometimes recorded which were much lower than those predicted on the basis of the ARRHENIUS equation[1]. Such low-temperature "inhibitions" have frequently been observed both in physiological processes and in individual enzyme reactions[2,3]. KISTIAKOWSKY AND LUMRY[4] claim that they are artifacts due to interactions between the enzyme and components of the reaction system, while KAVANAU[5] has offered an interpretation involving intramolecular changes in the enzyme, *e.g.* the formation of hydrogen bridges. The data on the enolase reaction to be presented here favor the latter explanation and also suggest that the activating metal ion is involved in determining the structure of the active site of the enzyme.

The active enolase molecule is a complex between the enzyme protein (*Pr*) and an activating metal ion (*M*)[6]. At high concentrations of both metal ion and substrate (*S*) all the enzyme is in the form of the complex *PrMS*. The formation of products from this complex is the rate-limiting step[6,7], so that the velocity constant (k) for this reaction can be determined from the initial velocity (v_0) since $v_0 = k \times [Pr]$. The values of v_0 at different temperatures between 0° and 37° were measured by a technique to be described elsewhere[8]. Arrhenius plots[1] of the results with Mg^{++}, Mn^{++} and Zn^{++} as activating ions are given in Fig. 1. With Mg^{++} an inhibition at low temperatures was frequently, but not always, observed. (The lack of reproducibility is probably due to the technique employed in which the enzyme, kept at room temperature, was added to the cold substrate solution. This question is being investigated further.) With the Zn^{++} enzyme, no curvature in the Arrhenius plot was obtained. Since Mg^{++} must be present at a much higher concentration than Zn^{++}, the concentration of the corresponding anion ($SO_4^{=}$) was also higher, while otherwise the reaction mixtures were identical. Thus, if the interpretation of KISTIAKOWSKY AND LUMRY[4] should apply to the enolase reaction, the inhibition of the Mg^{++} enzyme at low temperatures must be due to $SO_4^{=}$. However, the addition of Na_2SO_4 to the reaction mixture caused no inhibition of the Zn^{++} enzyme as seen in Fig. 1.

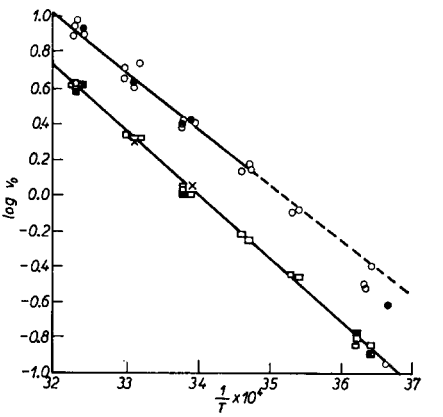

Fig. 1. The temperature dependence of the enolase reaction with different activating ions. All measurements were made in phosphate buffer, pH 6.75, $\mu = 0.05$. The enzyme concentration was about 10 μg/ml in all cases and the substrate concentration $2.4 \cdot 10^{-3}$ *M dl*-2-phosphoglyceric acid in all cases except the points marked (●) where it was $3.6 \cdot 10^{-3}$ *M*. Activators: (○) and (●) $8 \cdot 10^{-3}$ *M* Mg^{++}; (□) $3 \cdot 10^{-5}$ *M* Zn^{++}; (■) $3 \cdot 10^{-5}$ *M* $Zn^{++} + 8 \cdot 10^{-3}$ *M* Na_2SO_4; (×) $2 \cdot 10^{-4}$ *M* Mn^{++}.

In estimating the activation energies (E) of enzymic reactions, it is important that the substrate concentration is sufficiently high so that the observed changes in reaction rate are due solely to changes in one specific rate constant and not to variations in the Michaelis constant with temperature[9]. This condition is fulfilled here, since an increase in substrate concentration did not cause a change in the slope of the Arrhenius plot, as shown in Fig. 1. The estimated values of E for the reactions catalyzed by the Mg^{++} and Zn^{++} enzyme, together with the mean error of the estimate, are given in Table I. In calculating E with Mg^{++} as activator, only the points on the solid part of the line were used. The considerably greater error in E with Mg^{++} compared to the value with Zn^{++} is due to the smaller temperature range and the greater difficulty in estimating the initial velocities with the more active Mg^{++} enzyme.

The data in Fig. 1 confirm the earlier finding[6,7] that the Mg^{++} enzyme is more active than the Mn^{++} or Zn^{++} enzyme. However, the ratio between the rates with the different activators is not that which would be predicated on the basis of the activation energies. Since the number of collisions of *PrMS* molecules with each other and with solvent molecules must be the same regardless of the nature of *M*, collision theory[10] gives $k_{Zn}/k_{Mg} = (P_{Zn}/P_{Mg})\exp[-(E_{Zn}-E_{Mg})/RT]$, where P represents probability factors, R the gas constant, and T the absolute temperature. The values k_{Mg} and k_{Zn} at 22° are given in Table III. (These values were calculated from the maximum initial velocities actually observed and are thus not corrected for inhibition by substrate[6]; this effect is, therefore, included in the factor P.) The value of k_{Zn}/k_{Mg} is 0.34. If $P_{Zn} = P_{Mg}$, this ratio would be

obtained when $(E_{Zn}-E_{Mg}) \simeq 600$ cal. × mole $^{-1}$. However, the difference is actually 1900 ± 600 cal. × mole, which means that the ratio P_{Zn}/P_{Mg} is about 8:5.

In terms of the transition state theory[10], P is related to the entropy of activation. The standard free energy ($\Delta F^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), and entropy ($\Delta S^{\ddagger}$) of activation were calculated from the experimental values of k and E. In these calculations the transmission coefficient was assumed to be unity, which usually holds for reactions in solution[10,11], and unit molar concentration was chosen as the standard state. The values of $\Delta F^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ at 22° given in Table I.

TABLE I

KINETIC PARAMETERS FOR THE ENOLASE REACTIONS AT 22° IN PHOSPHATE BUFFER, pH 6.75, $\mu = 0.05$

Activator	k (sec.$^{-1}$)	E (cal. × mole^{-1})	$\Delta F^{\ddagger}$ (cal. × mole^{-1})	$\Delta H^{\ddagger}$ (cal. × mole^{-1})	$\Delta S^{\ddagger}$ (cal. × mole^{-1} × degree^{-1})
Mg^{++}	114	14600 ± 600	14600	14000	− 1.9
Zn^{++} (Mn^{++})	39	16500 ± 200	15100	15900	+ 2.6

To explain the low-temperature inhibition and the different entropies of activation in the reactions catalysed by the Mg^{++} and Zn^{++} enzyme, it is suggested that the enolase molcule can exist in (at least) two distinct forms, *En* and *En'*, which are in equilibrium with each other. In the active complex, the form of the enzyme is *En'* which has a less ordered (more probable) structure than *En*. In the Mg^{++} enzyme, the form *En'* predominates at high temperatures, while the equilibrium is shifted towards *En* at low temperatures, which results in inhibition. On the other hand, Mn^{++} and Zn^{++}, due to their greater strength of interaction with the protein, stabilize the more ordered form *En* also at the higher temperatures. Thus, with these ions the formation of the active complex involves a transition from *En* to *En'*, which would explain the positive entropy of activation. The magnitude of the entropy change is too small to involve a major conformation change[12] as suggested by KAVANAU[5]. The structural difference between *En* and *En'*, therefore, probably involves the active site only. This is also indicated by the effect of the nature of the activating ion, whose influence must be restricted to groups at or close to the active site.

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Photosynthetic activity of fragments of *Spirogyra* chloroplasts

Two types of chloroplasts occur in plants. As a rule higher plants contain grana-bearing chloroplasts while in some algae for instance these plastids are grana-free. In the former chloroplasts lamellar structure is principally restricted to grana embedded in a proteinaceous stroma, but the latter ones are composed of lamellae throughout the whole body and do not contain stroma substance. The platelet-shaped *Mougeotia* chloroplasts and the spirally built *Spirogyra* ones are examples of the lamellate type.