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ACTIVATION OF PHOSPHORYLASE D FROM *Raphanus sativus* BY METAL IONS

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Phosphorylase D from the root-crop plant *Raphanus sativus* (garden radish) consists of an equilibrium mixture of two low-molecular-weight conformers D_s and D_γ and of a high-molecular-weight form which apparently contains both the D_s and D_γ conformers [1-3]. It must be mentioned that the D_s and D_γ conformers differ in their catalytic properties. In the present work we have investigated the comparative influence of Ca^{2+} and Mn^{2+}

The phosphorylase D was isolated from the garden radish as described previously [1]. As the substrate we used egg lecithin, [S] = 5 mM, and as the initiator of the reaction sodium dodecyl sulfate (SDS), and in the determination of the transferase activity 0.4 ml of methanol was present in the medium.

As the results of the experiments performed showed, Ca^{2+} ions are more than twice as effective in transesterification (Fig. 1, curves a and d) and Mn^{2+} ions are more effective in hydrolysis (Fig. 1, curves c and d). One of the reasons why these ions exhibit different activating capacities in hydrolysis and transesterification may be the molecular heterogeneity of the phosphorylase D in solutions. It is known that Ca^{2+} ions displace the equilibrium in the direction of the formation of the high-molecular-weight variety of phosphorylase D [4, 5]. It has been shown [3] that it is precisely this form of phosphorylase D that possesses the greatest transferase activity. Furthermore, it has been shown that in the presence of Ca^{2+} ions it is just the transferase function of the enzyme that is predominant [6].

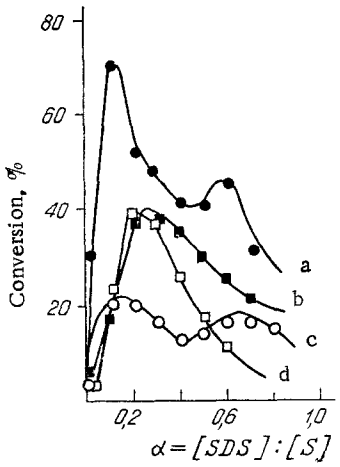

Fig. 1. Nature of the activation of phosphorylase D by Ca^{2+} ions (a, c) and Mn^{2+} ions (b, d): a, b) transesterification; c, d) hydrolysis ([S] = 5 mM, [SDS] = 1 mM, pH 5.6).

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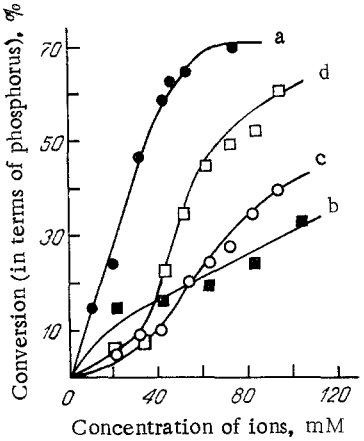

Fig. 2. Influence of α = [SDS]/[S] on the the activity of phosphorylase D: a) activation by Ca^{2+} ions, transesterification; b) activation by Mn^{2+} ions, transesterification; c) activation by Ca^{2+} ions, hydrolysis; d) activation by Mn^{2+} ions, hydrolysis ([S] = 5 mM, [$CaCl_2$] = 60 mM, pH 5.6).

Additional facts favoring this hypothesis are shown in Fig. 2, which gives the dependence of the activity on the parameter α (α = [SDS]/[S]), characterizing the composition of the mixed micelles and, consequently, the surface charge and the structure of the micelles [7]. On activation by Ca^{2+} ions, two maxima are observed. The positions of the maxima correspond to the properties of the high-molecular-weight form of radish phosphorylase D [3]. On activation with Mn^{2+} ions there is only one maximum (α = 0.2-0.25), which is characteristic for the D_γ conformer, possessing, as is well known, a predominantly hydrolase activity.

Thus, it has been shown that Mn^{2+} ions are specific for the D_γ conformer of phosphorylase D.

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