STUDY OF THE BINDING OF METAL IONS WITH PHOSPHORYLASE D FROM *Raphanus sativus* BY THE ESR METHOD

I. I. Abdrashitova, T. F. Aripov, and M. M. Rakhimov

UDC 577.150

In the present communication we give the results of a study of the binding of phosphorylase D from *Raphanus sativus* L. with Ca^{2+} and Mn^{2+} ions, which are activators for it and also with Pr^{+} ions, which inhibit it [1].

To a solution of the enzyme we added increasing amounts of Mn^{2+} ions, and it was then possible to observe the ESR signal of free Mn^{2+} ions only after the concentration of Mn in the solution had a reached a certain value, $[Mn]_0$, and then the signal rose in proportion to the concentration of Mn^{2+} ions. The initial $[Mn]_0$ section obviously characterizes the amount of ions that have been bound to the protein and therefore were not recorded under our conditions. For the two conformers D_S and D_Z obtained by separating the phosphorylase on a column with a biospecific sorbent [2] this amount was 38 and 28 ions per protein molecules, respectively.

To study the binding of Ca^{2+} ions with the enzyme, calcium was added to a solution of the enzyme saturated with Mn^{2+} ions ($[Mn] > [Mn]_0$). The ESR signal for the Mn scarcely changed. When a manganese salt was added to a solution of the enzyme saturated with Ca^{2+} ions it was possible to observe a slight decrease in $[Mn]_0$.

On the basis of these results and statements in the literature [3], according to which Mn^{2+} ions have higher covalent characteristics than Ca^{2+} ions and, consequently, more readily form bonds with proteins, it may be concluded that there are two types of sites for the binding of this protein with metal ions: 1) sites with which both Ca^{2+} and Mn^{2+} ions can be bound, and 2) sites with which Ca^{2+} ions cannot be bound while Mn^{2+} ions can be.

Similar experiments on the binding of Pr^{3+} ions with the protein (Fig. 1) showed that Pr^{3+} ions displace Mn^{2+} ions from their complex with phosphorylase D but if, on the other hand, phosphorylase D is previously bound with Pr^{3+} ions these ions are not displaced by Mn^{2+} ions. Thus, Pr^{3+} ions inhibit phosphorylase D by blocking the binding sites of the first type. The affinity of the praseodymium ions for phosphorylase D is higher than the affinity of Mn^{2+} and Ca^{2+} ions.

The measurements were performed on a Varian ESR spectrometer at an amplitude of modulation not exceeding 3.2 G and a power fed to the resonator of not more than 20 mW at room temperature.

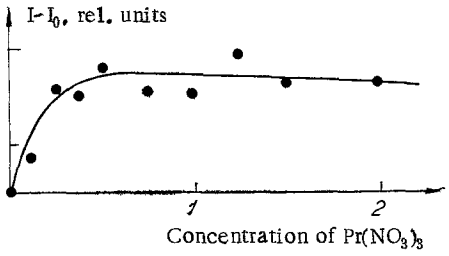

Fig. 1. Increase in the intensity of the manganese signal on the addition of a praseodymium salt to a solution of the enzyme saturated with manganese ions. Concentration of phosphorylase D 900 μg; $[MnCl_2]$ 0.5 mM.

V. I. Lenin Tashkent State University, Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 675-676, September-October, 1984. Original article submitted April 10, 1984.

 0009-3130/84/2005-0648$08.50

LITERATURE CITED

1. T. I. Kalendareva, M. M. Rakhimov, and S. Sh. Rashidova, Uzb. Khim. Zh., No. 6 (1980).
2. M. M. Rakhimov, R. Akhmedzhanov, Sh. R. Mad'yarov, and B. A. Tashmukhamedov, Biokhimiya, 47, 1454 (1982).
3. K. B. Yatsimirskii, Introduction to Bioorganic Chemistry [in Russian], Kiev (1976), p. 13.

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

I. I. Abdashitova, T. F. Arapov,
M. U. Babaev, and M. M. Rakhimov

UDC 577.150

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].

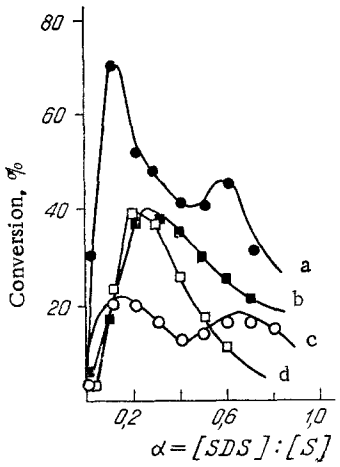

Fig. 1. Nature of the activation of phosphorylase D by Ca^{2+} ions (a, c) and Mn^{2+} ions (b, d): a, b) transesterification; c, d) hydrolysis ([S] = 5 mM, [SDS] = 1 mM, pH 5.6).

V. I. Lenin Tashkent State University. Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 676-677, September-October, 1984. Original article submitted April 10, 1984.

0009-3130/84/2005-0649$08.50