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The reactivation of apodopamine β-monooxygenase by vanadyl ions

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Vanadyl ions may be used for reactivation of apodopamine β-monooxygenase. Maximal activity of the enzyme was achieved at a 350–400-fold molar excess of vanadyl ions, whereas for maximal reconstitution with copper, an 8–10-fold molar excess of copper was necessary. At higher concentrations of vanadyl as well as of copper, inhibition was observed.

Dopamine β-monooxygenase; Vanadyl ion; Copper ion

1. INTRODUCTION

Dopamine β-monooxygenase (EC 1.14.17.1) is the copper-containing enzyme catalyzing the conversion of dopamine to norepinephrine. Copper may be removed from dopamine β-monooxygenase by using metal chelators, and in order to reconstitute the catalytic activity of the enzyme, ionic copper should be added [1–4]. Attempts at reconstituting the apoenzyme using other metals, such as Ni(II), Co(II), Mn(II), Zn(II), Fe(II), Fe(III) and Mo(VI), have been reported to be unsuccessful [1,5].

Here we have investigated the use of vanadyl ion, VO(II), in the reactivation of apodopamine β-monooxygenase. There were several reasons for initiating this study. Firstly, vanadium is considered to be an essential trace element participating in the regulation (by stimulation or inhibition) of many enzymatic processes [6–9]. Secondly, vanadyl ion is known to have a high affinity for some proteins and enzymes [9,10]. Thirdly, dopamine β-monooxygenase has also been shown to have some catechol oxidase activity [11,12], vanadium having been reported to stimulate the nonenzymatic oxidation of catecholamines [13]. Finally, the autooxidation of vanadyl to vanadate is accompanied by the formation of superoxide radicals, O_2^-, which are also considered to be intermediates, and possibly even cofactors, of dopamine β-monooxygenase [14]. Thus, it would be expected that vanadyl ions are able to activate apodopamine β-monooxygenase.

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2. MATERIALS AND METHODS

The soluble form of dopamine β-monooxygenase was purified from chromaffin granules of bovine adrenal medulla essentially according to Saxena and Fleming [15]. Preparations obtained were electrophoretically homogeneous. Apodopamine β-monooxygenase was obtained by incubation of the enzyme with 5 mM diethyldithiocarbamate. Excess chelator was removed by filtration of the mixture through an Amicon PM-30 membrane in the presence of 0.5 M NaCl, followed by dialysis vs 10 mM sodium acetate (pH 6.2). Final purification of the apoenzyme was carried out by chromatography on DEAE-cellulose under the conditions used for isolation of the holoenzyme.

Copper was assayed chemically with tetraethylthiuram disulphide [16]. According to this method, the residual copper content in preparations of apodopamine β-monooxygenase was less than 3% of the value in the holoenzyme. Complete removal of diethyldithiocarbamate from the apoenzyme was confirmed by paper chromatography. Enzymatic activity of dopamine β-monooxygenase was assayed according to Blackburn et al. [1] on a Beckman 0260 oxygen analyzer equipped with a Clark-type oxygen electrode and an Omniscribe B-5000 recorder. The reac-

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tion mixture (2 ml) contained 0.2 M sodium acetate (pH 5.0), 15 mM tyramine, 20 mM ascorbate, 50 μg/ml of catalase and the enzyme. To reactivate the apoenzyme, vanadyl or copper ions were added to the reaction mixture. The enzyme concentration was determined by absorbance at 280 nm using $A_{280} = 12.4$ for a 1% solution [17]. To study the properties of the enzyme reconstituted by vanadyl ions, aliquots of $VOSO_4 \cdot 6H_2O$ solution were added to the apoenzyme dissolved in 0.2 M acetate buffer (pH 5.0). Excess vanadyl was removed by ultrafiltration of the solution through an Amicon PM-10 membrane. The same procedure was used for reconstitution of the enzyme with Cu(II).

EPR spectra were recorded on a Varian E-4 instrument at 77 K (conditions for EPR spectroscopy: microwave frequency, 9.11 GHz; microwave power, 10 mW; modulation amplitude, 10 G).

Distilled water used for preparation of all stock solutions of buffers, proteins and other chemicals was passed through Bio-Rex resin (Bio-Rad); all stock solutions were additionally treated with this resin. Catalase obtained from bovine liver was pretreated with EDTA to remove traces of copper. Stock solutions (10 mM) of $VOSO_4 \cdot 6H_2O$ (Wako) were prepared at pH 3.5 immediately before use. According to the atomic absorption data, obtained on an AAA-S1 instrument (Carl Zeiss, Jena), the admixture of copper in vanadyl sulphate used was 4 parts per 10000.

3. RESULTS AND DISCUSSION

Although it has been reported that vanadyl ions are able to stimulate auto-oxidation of some catecholamines [13], we found that vanadyl itself does not activate the reaction of monooxygenation (hydroxylation) of tyramine. However, when vanadyl ions were added to the reaction mixture containing apodopamine β-monooxygenase rapid reactivation of the enzyme occurred (fig.1). Preincubation of the apoenzyme with vanadyl ions was not necessary for reactivation. Maximal reactivation was observed at a molar ratio of vanadyl to enzyme tetramer of 350–400, whereas with copper ions it was achieved at a ratio of copper to enzyme tetramer of 8–10. Although vanadyl was less effective than copper as an activator of dopamine β-monooxygenase, we found that the maximal level of the specific activity of preparations reconstituted with vanadyl or copper ions was practically equal. Thus, besides copper, vanadium is the only metal which is able to reconstitute the activity of apodopamine β-monooxygenase. At higher concentrations of vanadyl as well as copper ions, inhibition of the enzyme activity was observed (fig.2). Thus, there are certain similarities in the effects of both metals on dopamine β-monooxygenase.

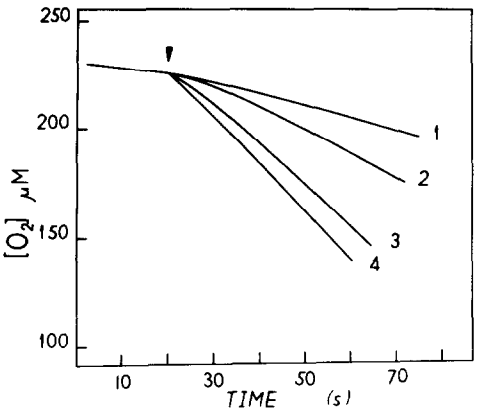

Fig.1. Reactivation of apodopamine β-monooxygenase by vanadyl ions. The arrowhead indicates the additions of vanadyl sulphate to the reaction mixture containing the apoenzyme (0.14 μM). Kinetic traces 1–4 were obtained in the presence of 10, 20, 30 and 40 μM vanadyl ions, respectively.

However, experiments along this line did not allow us to draw any conclusions as to the mechanisms of the effects observed. Therefore, we subsequently investigated the effect of different concentrations of vanadyl ions on the activity of holodopamine β-monooxygenase. The results obtained (fig.3) clearly indicate that at low concentra-

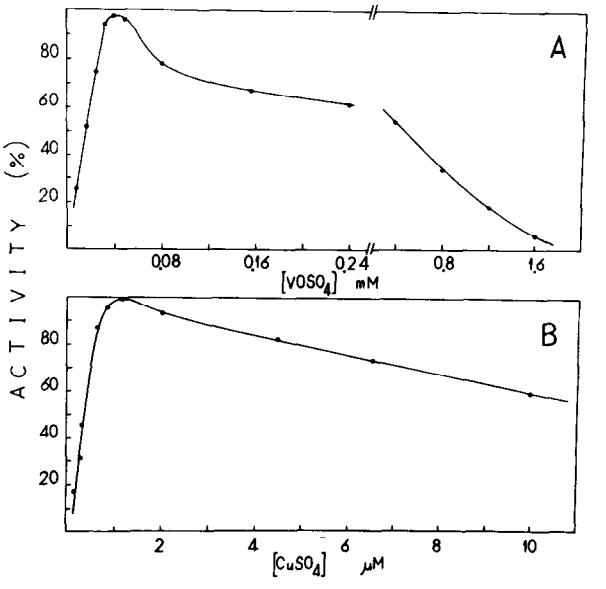

Fig.2. Dependence of relative activity of dopamine β-monooxygenase on concentrations of vanadyl (A) and copper (B) ions; [apodopamine β-monooxygenase]: 0.14 μM.

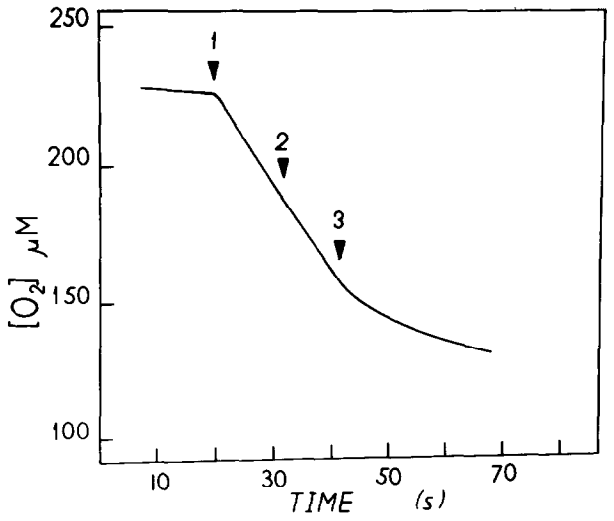

Fig.3. Inhibition of holodopamine β-monooxygenase by vanadyl ions. Arrowheads indicate additions of (1) 0.15 μM holoenzyme, (2) 30 μM vanadyl sulphate, (3) 1.2 mM vanadyl sulphate.

tions, vanadyl ions had no effect on the activity of copper-containing dopamine β-monooxygenase, whereas at high levels, inhibition was observed. It may be concluded from these experiments that vanadyl ions have low affinities to the enzyme as compared with copper ions. To determine the number of vanadyl-binding sites, the apoenzyme was incubated with a 400-fold molar excess of vanadyl for several minutes, unbound and weakly bound vanadyl ions being thereafter removed from the reaction mixture by ultrafiltration and exhaustive washing with acetate buffer (pH 5.0). Fig.4 shows the EPR spectrum of a preparation thus obtained. From a quantitative treatment of this spectrum, the preparation contains 4 vanadyl ions per enzyme tetramer. It is very attractive to suggest that VO(II) and Cu(II) ions are bound to the same catalytic sites of the enzyme. However, at the present state of knowledge, this suggestion would inevitably be speculative. On the other hand, it is important to note that during storage (at 4°C) of the preparation reconstituted with vanadyl ions a drop in EPR signal intensity was observed, indicating auto-oxidation of vanadyl to vanadate. This decrease may be reversed by addition of ascorbate which is known to be the cofactor in the dopamine β-monooxygenase reaction [18]. Thus, autooxidation of enzyme-bound vanadyl to vanadate is reversed in the ascorbate-containing reaction mixture used for determination of the ac-

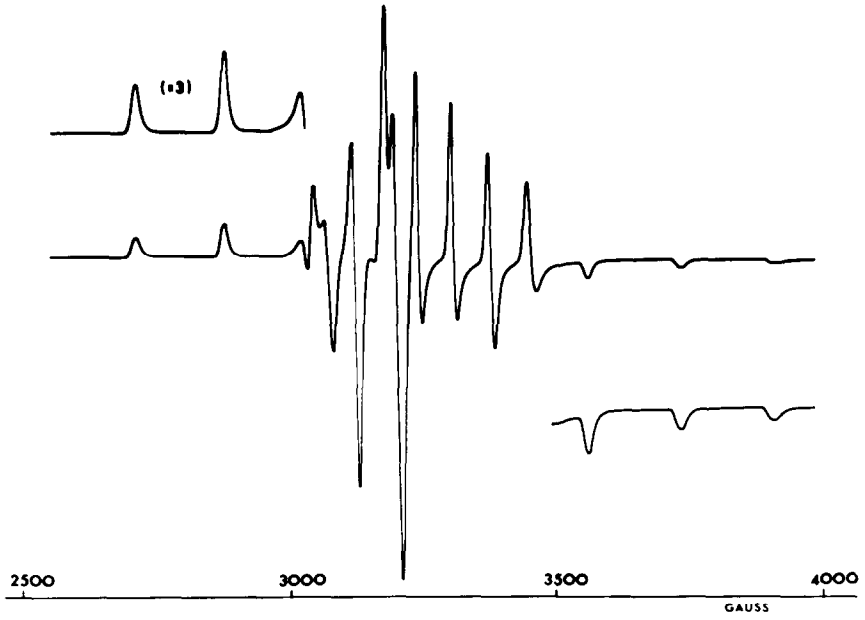

Fig.4. EPR spectrum of apodopamine β-monooxygenase reconstituted by vanadyl ions. Low- and high-field parts of the EPR spectrum were recorded separately at 3-times higher gains.

tivity of dopamine β-monooxygenase. Hence, the ability of vanadyl to reconstitute apodopamine β-monooxygenase may be connected with its reverse oxidoreductive transformations as well as being influenced by the following conditions which are favourable for stabilization of vanadyl ions: binding of the ions to the protein moiety; reducing media; and mildly acidic pH.

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