ANALYTICAL-BAND CENTRIFUGATION OF THE ACTIVE FORM OF PIG KIDNEY DIAMINE OXIDASE

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Received March 25,1974

Summary: In this communication it is shown that pig kidney diamine oxidase undergoes an association-dissociation reaction which is under the influence of the concentration of oxygen, one of the substrates. The sedimentation constant of the active unit was measured using the analytical-band centrifugation of the active enzyme-substrate complex.

It was suggested that amine oxidases (copper proteins) could be composed of one or more monomers with an approximate molecular weight of 80,000-100,000 (1). For pig kidney diamine oxidase (diamine-oxygen oxidoreductase, EC 1.4.3.6.) a molecular weight of 87,000 was calculated on the basis of copper analysis (2). Later on a molecular weight of 185,000 was reported, corresponding to a content of two g-atoms of copper per mole of enzyme (3).

Diamine oxidase was purified as described elsewhere (4). By using gel filtration we found that diamine oxidase exhibits two polymerization states which are under the influence of oxygen, one of the substrates (Fig. 1). A molecular weight of about 185,000 was found for the form obtained in an air saturated solution (form I), and a molecular weight close to 725,000 was determined for the form which is present in a practically oxygen-free medium (form IV). Assuming that the basic unit is form I, it may be suggested that form IV is composed of four units. These results are consistent with the ultracentrifugation data: $s^{o}_{20,w}$ = 9.86 S for form I (Figs. 2 and 3A) and $s_{20,w}$ = 19.6 S for form IV (value obtained for a concentration of 3.2 mg/ml) (Fig. 3B).

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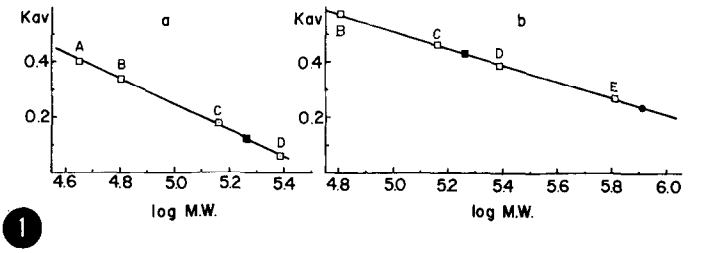

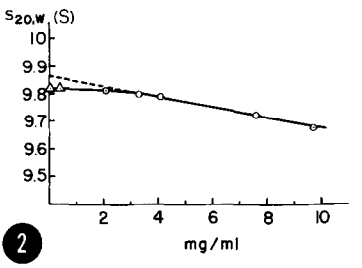

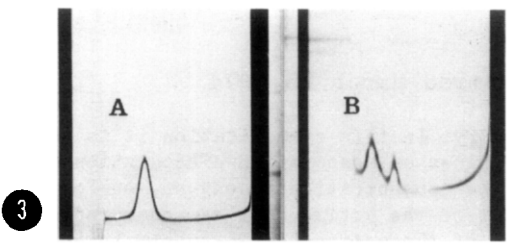

Fig. 1: Molecular weight estimation of diamine oxidase by gel filtration on Sephadex G-150 (a) and Sepharose 6B (b). The columns, 2.5 x 55 cm (a) and 2.5 x 50 cm (b), were equilibrated with 0.1 M triethanolamine-HCl buffer, pH 7.3. Squares (■) indicate the enzyme positions when buffer was air saturated. The circle (●) shows the enzyme position when buffer was treated as follows: degasing at room temperature under vacuum during 30 min, flushing with nitrogen for 5 min and re-degasing during 10 min; the solution was kept under nitrogen during elution. Filtrations were performed at 4° with ascendant elution. Reference proteins were ovalbumin (A), hemoglobin (B), lactate deshydrogenase (C), catalase (D), thyroglobulin (E).

Fig. 2: $s_{20,w}$ as a function of the concentration of diamine oxidase in 0.1 M triethanolamine-HCl buffer, pH 7.3 (air saturated). The dependence of the concentration of the sedimentation coefficients determined by conventional ultracentrifugation (⊙) can be described by the following equation: $s_c = 9.86\ (1 - 0.002\ c)$. Determinations made by analytical-band centrifugation (△) give a $s^o_{20,w}$ value of 9.82 S. Each value is the average of two or three measures.

Fig. 3: Sedimentation patterns of diamine oxidase. Diamine oxidase (9.7 mg/ml) in 0.1 M triethanolamine-HCl buffer (pH 7.3). (A) in air saturated solution; (B) after flushing solution with nitrogen (the cell was filled under nitrogen atmosphere). Runs were carried out at 52,640 rpm and 8°. Photographs were taken at about 37 min (A) and 15 min (B) after reaching speed with a phase-plate angle of 60° and 50° respectively. Sedimentation is from left to right. In (B) the two forms can be observed because of a too high residual oxygen concentration due to a gentle treatment compared to the one made for gel filtration (Fig. 1); concentrations were calculated by integration of the area under the gradient curve (6.5 mg/ml for the slower form and 3.2 mg/ml for the faster one).

To determine the polymerization state of diamine oxidase when it is fully active (in presence of the two substrates, diamine and oxygen) analytical-band centrifugation of the active enzyme-substrate complex was more useful than con-

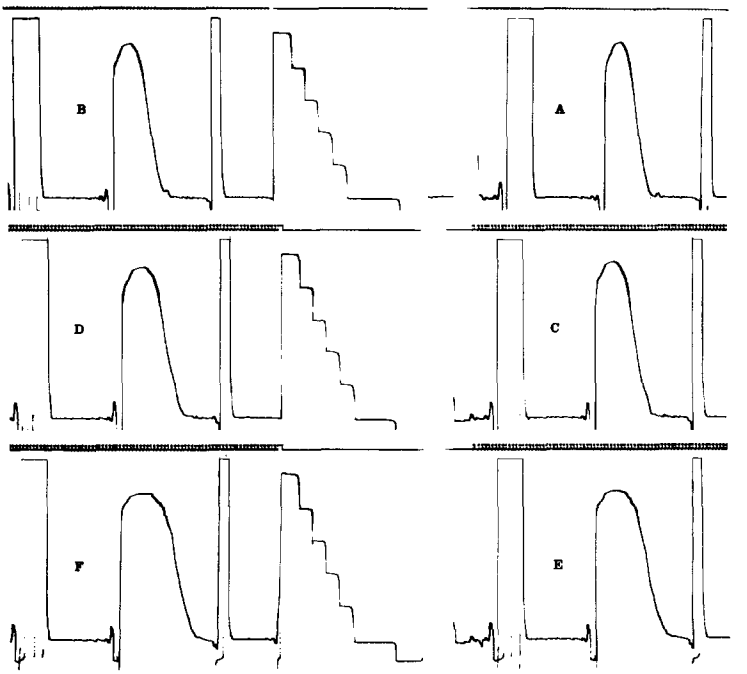

Fig. 4: Band centrifugation of the active form of pig kidney diamine oxidase. Centrifugation runs were performed at 56,000 rpm and 35°. Scans of this figure were taken (from A to F) at -1, 1, 3, 5, 9 and 11 min after reaching speed, at 430 nm. For this run enzyme solution was 50 µg/ml. Other details are given in text.

ventional ultracentrifugation (5). The method was performed as described by Cohen and Mire (6). The sedimentation experiments were carried out using a valve-type synthetic-boundary cell in a Spinco model E analytical ultracentrifuge equipped with a monochromator and a photoelectric scanner. Substrate solution (400 µl) containing 0.25 M triethanolamine-HCl buffer (pH 7.3), 4 mM o-aminobenzaldehyde and 15 mM putrescine was introduced into the sector space of the cell and 25 µl of enzyme solution were introduced into the cup. Centrifugation runs were carried out at 35° and 56,000 rpm using an An-D rotor. Enzyme solution transfer occured between 8,000 and 10,000 rpm. Around the time of the transfer, moderated acceleration rates were applied. Maximum speed was reached in about 20 min after layering the enzyme solution onto the substrate solution. Enzyme activity was followed by observing the formation of one product of the reaction at 430 nm (7). The method is based on the coupling reac-

tion of Δ^1-pyrroline formed with o-aminobenzaldehyde (8, 9). Scans were taken at intervals of two minutes. Typical figures obtained with this method are shown in Figure 4. Sedimentation coefficients were calculated using the method described by Cohen and Hahn (10), and values were corrected to standard conditions.

A value of 9.82 S is in agreement with the $s^{o}_{20,w}$ value found by classical ultracentrifuge method for form I. Therefore it can be concluded that the active unit has the same polymerization state as the free enzyme in air saturated solution. In the same way, it is shown that only one of the two substrates, oxygen, affects the polymerization state.

Acknowledgments: This work was supported by a NRC grant (no A-4963). The encouragement of Dr. J. L. Lavoie is acknowledged. I would like to thank Dr. R. H. Côté and Dr. S. Moorjani for the use of their facilities. The author was a recipient of a Canada Council fellowship.

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