Crystalline Animal Alcohol Dehydrogenase 2

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Recently we reported purification and crystallization of alcohol dehydrogenase from horse liver [1]. In this paper we wish to describe the new method, which has given consistently good results, and some of the properties of the enzyme.

Preparation. One horse liver is ground and extracted with a double weight of distilled water for about 3 hours. We have generally used fresh livers, but frozen livers, 2—3 weeks old, give about the same yield.

The extract is heated to 52° C and kept at this temperature for 15 minutes [2], cooled to room temperature and centrifuged.

To the turbid red-brown solution is added ammonium sulphate to give a saturation of 0.5, and the solution centrifuged. The protein precipitate, which contains catalase, ferritin and the aldehyde oxidase activity, is discarded. The clear-red mother liquor contains the alcohol dehydrogenase activity.

The enzyme is precipitated when ammonium sulphate is added to give a saturation of 0.8. The precipitate is dissolved in 0.01 *M* phosphate buffer pH 7 and dialyzed against distilled water. The solution is then centrifuged, since some protein will denature on dialysis against water.

To the dialyzed solution is added phosphate buffer pH 7 untill the solution is 0.01 *M* with regard to phosphate. At this step the solution is clear red and contains about 150—200 mg of protein per ml.

The solution is now shaken vigorously with a mixture of alcohol and chloroform. To 1 liter solution we add 160 ml 99.6 % alcohol and 80 ml of chloroform. This procedure denatures nearly all of the hemoglobin. After centrifugation the alcohol and chloroform are evaporated under reduced pressure and the faint red solution is dialyzed against 0.01 *M* phosphate buffer pH 7.

The solution is again fractionated with ammonium sulphate and the precipitate between 0.50 and 0.80 % saturation is collected. The precipitate is dissolved in water and dialyzed against phosphate buffer as before.

The purity at this step is usually 0.06 to 0.1, the pure enzyme taken as 1.

The rest of the purification is carried out with alcohol at low temperature. We keep the temperature just above the freezing point of the mixture, though never below — 10° C. The protein concentration has varied from 10 to 50 mg per ml. As buffer solution we use 0.01 *M* phosphate at pH 7 throughout the whole procedure. The alcohol concentration referred to is volume percent 99.6 % alcohol.

From the first alcohol fractionation the precipitate between 20 and 60 percent alcohol is collected. The precipitate is dissolved in ice-cold phosphate. The purity at this step is 0.15—0.2.

The solution is then refractionated; this time the precipitates appearing at 30 and at 40 percent alcohol are collected separately and dissolved as before. Although the purity of these two fractions is about the same it is advantageous to separate them and refractionate them individually.

With the first fraction, the initial precipitate appearing at about 20 percent alcohol is discarded and the enzyme precipitated with 50 % alcohol. With the second fraction, the first precipitate is collected and the remaining protein is discarded. The two precipitates are dissolved as before and mixed. The purity is about 0.5—0.7.

On slow addition of alcohol to this solution the enzyme starts to crystallize at a alcohol concentration from 15 to 20 per-

Fig. 1. Crystalline alcohol dehydrogenase.

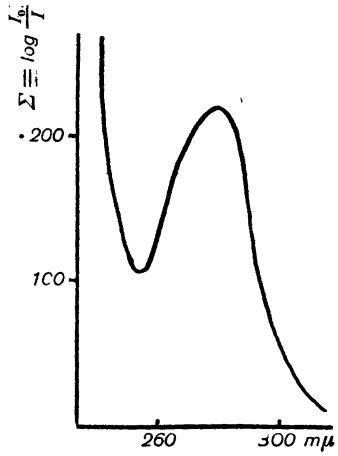

Fig. 2. Spectrum of alcohol dehydrogenase. 0.48 mg/ml; d = 1 cm.

cent. The purity of these crystals is about 0.8—0.9. We usually recrystallize three times before we get a pure enzyme. The enzyme solution is then dialyzed against water to remove all the alcohol from the solution.

The alcohol fractionations do not have to be carried out on the same day. We have kept different fractions for weeks in alcohol at low temperature without any loss in activity.

During crystallization all the activity leaves the solution and appears in the crystals. The protein is uniform on electrophoresis. The enzyme from different preparations has always shown the same properties. The crystals are shown in Fig. 1.

The total amount of enzyme present in the liver is difficult to determine on account of the large blank in the crude extract. However, as a result of determinations from many preparations we estimate the total amount of enzyme to be about 1 ‰ of the wet weight of the liver. A horse liver weighing 5.5 kg thus should contain 5.5 g of enzyme. From one horse liver we usually get 7—800 mg of crystalline enzyme. This yield can be increased considerably by refractionation of the discarded fractions.

Spectrum. The enzyme is a colorless protein. In the ultraviolet there is no other band than the usual protein band at 280 mμ (Fig. 2).

The band at 280 mμ is very low per mg of enzyme, about three times as low as that given for the yeast enzyme [3]. We use the extinction coefficient of 0.455 per mg of protein per cm at 280 mμ for determining the protein concentration in all our experiments.

Stability. The enzyme is stable from pH 5.4 to about 11. We store it in the form of crystals at low temperature or in solution in 30 % ammonium sulphate. Drying destroys the enzyme.

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