# METHODS FOR THE ISOLATION OF CRYSTALLINE ALCOHOL DEHYDROGENASE

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Alcohol dehydrogenase (in the following: ADH) has been isolated in crystalline form from brewer's yeast by NEGELEIN and WULFF [1], as well as by KELETI [3], who employed a slightly modified method described for baker's yeast by RACKER [2]. Methods for the isolation of crystalline ADH from baker's yeast have been described by RACKER[2], as well as by WALLENFELS and SUND [4].

In this paper we present further methods for the isolation of ADH.

## 1. Preparation of crystalline ADH from baker's yeast

Except for some minor modifications, the procedure is a combination of the methods described by RACKER [2] and by WALLENFELS and SUND [4].

After washing the baker's yeast is dried at room temperature. To 1 kg of dried baker's yeast is added 3 litres of a 0.067 *M* pH 8 phosphate buffer. After autolysis at 37° C for 3.5 hours and standing at room temperature for 2 hours the solution is centrifuged. The supernatant is allowed to stand for 15 minutes in a water bath of 55° C. After cooling in ice and centrifuging, the supernatant is stored in a refrigerator overnight.

The supernatant is chilled to −2° to −5° C in an ice-salt mixture and is precipitated with acetone of the same temperature (50 ml of acetone to 100 ml of solution). When the temperature of the mixture is again below −2° C, it is centrifuged in chilled tubes. The supernatant is chilled again to −2° to−5° C and is precipitated with acetone of the same temperature (55 ml of acetone to 100 ml of solution). After the temperature of the mixture has sunk to below −2° C, it is centrifuged in chilled tubes. The precipitate is suspended in 250 ml ice water and dialysed for 3 hours against running tap water. After centrifuging, 36 g of solid $(NH_4)_2SO_4$ is added to each 100 ml of the supernatant, under cooling with ice and after 30 minutes, the supernatant is centrifuged in chilled tubes. The precipitate is dissolved in 50 ml of 0.025 per cent ethylene diamine tetraacetate (EDTA) (pH 8.2 to 8.5) at

room temperature, and to the solution is added 10 g of $(NH_4)_2SO_4$, without cooling. After centrifuging, the clear supernatant is placed into a refrigerator without adding to it any further amounts of $(NH_4)_2SO_4$. In the refrigerator the ADH will crystallize within 1 to 2 hours.

The turnover number of the crystalline baker's yeast ADH prepared by RACKER's [2] or by WALLENFELS and SUND's [4] method is 26 000 to 28 500. REDETZKI and NOWINSKI [5] have shown that the turnover number of the crystalline baker's yeast ADH prepared by RACKER's method [2] is increased by minute concentrations of o-phenantrolene, which forms complexes with the traces of heavy metal inhibiting the enzyme, its affinity to these being greater than that to the Zn linked to the protein. Higher concentrations of o-phenantrolene inhibit the activity of the enzyme, by binding the Zn linked to the protein, that is essential for the binding of the coenzyme [6]. REDETZKI and NOWINSKI[5] used a reaction mixture containing no trace of heavy metal and found that under such conditions the turnover number of the crystalline ADH is 42 900. In such a mixture the activity of the enzyme is inhibited by any minute amount of o-phenantrolene.

As measured in the usual reaction mixture [3], without the use of any special procedure, the turnover number of the crystalline baker's yeast ADH prepared by the above-described method is 40 800. The activity of the enzyme is inhibited by any minute concentration of o-phenantrolene. This indicates that by this method of preparation it can be avoided that the enzyme contain traces of heavy metal inhibiting its activity.

The yield agrees with that attainable by RACKER's method [2].

### 2. Preparation of crystalline ADH from brewer's yeast

The procedure is identical with that described above for baker's yeast, except the following.

After the second fractionation with aceton the precipitate is suspended in 750 ml ice water and dialysed against running tap water. The precipitate obtained

*Fig. 1.* Crystalline yeast ADH

by the $(NH_4)_2SO_4$ fractionation is dissolved in 100 ml of a 0.025 per cent EDTA solution.

The turnover number of the crystalline enzyme thus prepared varies from 50 000 to 56 000. Any minute concentration of o-phenantrolene added to the reaction mixture inhibits the enzyme.

As the different ADH crystals cannot be distinguished morphologically either according to their origin or to the method of preparation, the photograph of only one preparation is presented (Fig. 1).

## Appendix

Crystalline ADH can be isolated from brewer's yeast also by the following simple method.

After centrifugation, the washed brewer's yeast is dried air-dry at room temperature, autolysed with a 3-fold amount of $M/15$ $Na_2HPO_4$ at 37° C for 2 hours. The autolysate is allowed to stand for further 3 hours at room temperature. After centrifugation, the supernatant is brought to 0.45 saturation with solid $(NH_4)_2SO_4$, and centrifuged. The supernatant is stored overnight in a refrigerator, where a rich crystalline ADH precipitate will separate together with much amorphous material.

The turnover number of the ADH thus prepared (recrystallized once) varies from 22 000 to 29 000.

This preparation is not homogeneous and for this reason it can be used for technical purposes (demonstration of alcohol, DPN reduction, etc.) only.

## SUMMARY

Methods have been described for the isolation of crystalline ADH from baker's yeast and from brewer's yeast, respectively.

## LITERATURE

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