## BBA Report

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# Subunits of aldose reductase from Rhodotorula

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SUMMARY

Gel filtration and sodium dodecyl sulfate–polyacrylamide disc gel electrophoresis indicate that aldose reductase (polyol:NADP$^+$oxidoreductase, EC 1.1.1.21) from Rhodotorula is a dimer with a molecular weight of 61 000. The enzyme may be dissociated into two unequal subunits, only one of which has catalytic activity. NADP$^+$ appeared to stabilize the dimer.

In a previous report we have described the purification of aldose reductase (polyol: NADP$^+$ oxidoreductase, EC 1.1.1.21) from a strain of Rhodotorula[1]. After gel electrophoresis of the purified enzyme only one band of protein was observed. However, when $10^{-3}$ M sulfhydryl reagents were present in the gel the enzyme dissociated into two protein bands of approximately equal intensity.

Detailed analysis of aldose reductase from any tissue source has yet to be reported, although the enzyme has been isolated and partially characterized from lens, liver, muscle, adrenals and other tissues. Because of its stability and reproducibility of kinetic measurements, the yeast enzyme probably represents the best model for kinetic and molecular studies of this system. The present communication proposes a subunit structure for the yeast enzyme.

Materials were of the highest grade commercially available. The aldose reductase assay has been described[1].

*Sodium dodecyl sulfate–disc gel electrophoresis*

The standard procedure of Williams and Reisfeld[2] was modified in the following manner[3]: 10% acrylamide was used in the bottom gel; 0.1% sodium dodecyl sulfate was included in both gels. Protein samples to be electrophoresed were incubated with 1% sodium dodecyl sulfate at 45° for 1 h. These could be stored at 4° and used as needed.

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Gels were stained for protein with Coomassie Blue and destained in 12.5% acetic acid.

The procedure of Andrews[4] was used to determine molecular weights by column chromatography in Sephadex gels.

*Isolation and kinetics of aldose reductase subunits*

In a previous report[1] the molecular weight of aldose reductase was reported as 61 000 g/mole. This value was determined on both Sephadex G-100 and G-200 for a partially purified preparation of the enzyme. When these experiments were repeated using the same buffering system with the highly purified enzyme preparation, aldose reductase activity was detected in a single peak which corresponded to a molecular weight of 36 800 (Fig. 1). Two more experiments gave the same results. When a partially purified enzyme or enzyme from Step 6 of the purification procedure (which contained 1% ampholine) was chromatographed, a molecular weight of 60 000 was observed (Fig. 1). These results were clarified by kinetic studies of the two samples of enzyme. Kinetics of the lower molecular weight peak were classical; *i.e.* double-reciprocal plots were linear and Hill plots had a slope of 1.0. In the experiments which gave the low-molecular-weight activity, the fractions corresponding to a molecular weight of 22 000– 26 000 were pooled, and an aliquot of this sample was mixed with the low-molecular-weight enzyme (2:1). After a 1-week incubation at 4° the kinetics of this mixture were shown to resemble those of the native enzyme

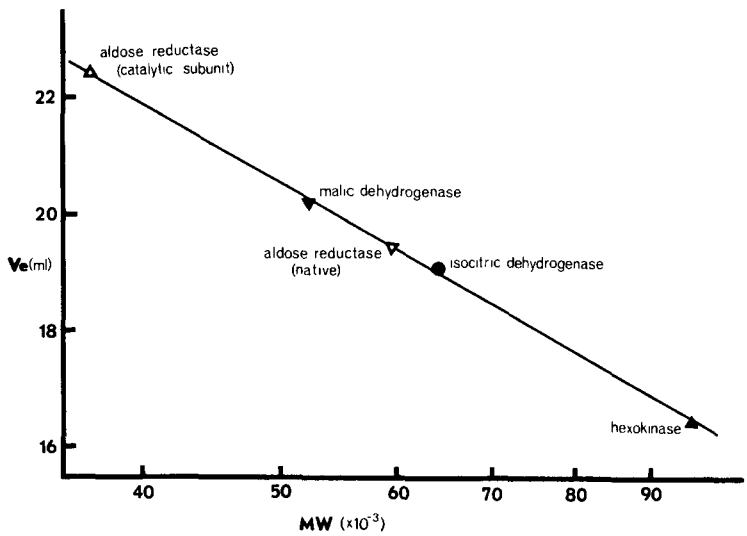


Fig. 1. Determination of molecular weights on Sephadex G-100; a plot of elution volume (Ve) *vs.* log molecular weight. Sephadex G-100 was swollen in 0.05 M Tris–HCl buffer, pH 7.5, and poured into a reservoir at the top of a jacketed (4°) column (60 cm x 0.9 cm). A bed height of about 50 cm was established and the column was eluted with 0.05 M Tris–HCl buffer, pH 7.5. The void volume was 12.5 ml, as determined using blue dextran 2000. Three marker enzymes were used for calibration of the column: hexokinase (96 000); isocitric dehydrogenase (64 000); and malic dehydrogenase (52 000). Native aldose reductase was eluted in 19.4 ml, corresponding to a molecular weight of 60 000. The catalytic subunit was eluted in 22.3 ml, corresponding to a molecular weight of 36 800. When the column was equilibrated and eluted with 125 $\mu$M $NADP^+$ present in the buffer, the enzyme eluted in 19.2 ml,corresponding to a molecular weight of 60 600.

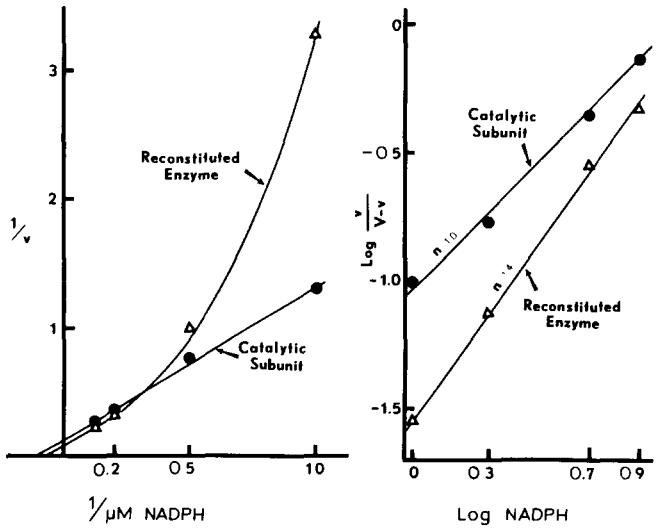


Fig. 2. Double-reciprocal and Hill plots for the catalytic subunit of aldose reductase and the reconstituted enzyme (see text). The standard assay was used at pH 7.5 with NADPH concentrations ranging from 1 $\mu$M to 8 $\mu$M, and D,L-glyceraldehyde at 10 mM in all reactions.

(ref. 1, Fig. 2). Hence, this preparation was called the reconstituted enzyme while the low-molecular-weight enzyme was termed the catalytic subunit (Fig. 2).

When the same G-100 column was equilibrated and eluted with 125 $\mu$M $NADP^+$ in the buffer, the highly purified enzyme eluted as the native enzyme with a molecular weight of 60 600 (Fig. 1).

*Determination of subunit molecular weights*[5]

Six globular proteins of known molecular weights were used as markers in sodium dodecyl sulfate–polyacrylamide disc gel electrophoresis (Fig. 3). A sample of aldose reductase (from Step 6 of the purification procedure) was also treated with 1% sodium dodecyl sulfate and used to determine the molecular weight of its subunits. Three separate experiments were performed and two subunits of aldose reductase were detected with average molecular weights of 22 300 and 36 600. The results of one experiment are shown in Fig. 3.

Three lines of evidence indicate that the aldose reductase activity obtained after gel filtration of the highly purified enzyme was due to an isolated catalytic subunit: (1) low molecular weight (as compared to the native enzyme), (2) linearity of double-reciprocal plots, and (3) reconstitution to the native enzyme. The latter criterion is also evidence that a subunit was present in the 22 000–26 000 molecular weight fractions. This subunit did not exhibit catalytic activity.

It was noted in the results of a previous report[1] that the presence of ampholine in the enzyme preparation from Step 6 prohibited further purification by chromatography on G-100 (in 0.05 M Tris–HCl buffer, pH 7.5). In this case it was assumed that the ampholine was capable of binding the different proteins together. This could also explain the results in which the enzyme in the presence of ampholine did not separate into sub-

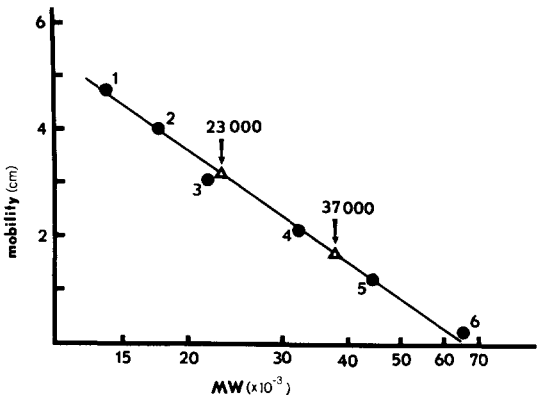


Fig. 3. Determination of molecular weights on sodium dodecyl sulfate–polyacrylamide disc gel electrophoresis. Gels and proteins were prepared as described in the text. Purified aldose reductase was treated with 1% sodium dodecyl sulfate and electrophoresed to detect the presence of subunits. Two subunits with molecular weights as indicated were observed. The following marker proteins were used:

| *Number* | *Marker protein* | *Molecular weight* | *(Ref.)* |
|---|---|---|---|
| 1 | Lysozyme | 14 000 | (6) |
| 2 | Myoglobin | 17 000 | (6) |
| 3 | α-Chymotrypsin | 21 500 | (6) |
| 4 | Pepsin | 35 500 | (7) |
| 5 | Peroxidase | 44 000 | (6) |
| 6 | Bovine serum albumin (monomer)★ | 66 000 | (8) |

★Bovine serum albumin was treated with 2-mercaptoethanol to dissociate the native hexamer completely into monomers[5].

units while the purified enzyme did. As for crude enzyme preparations, which gave a molecular weight of 61 000, it is possible that other proteins were present which mimicked the binding effect of ampholine. Another contributing factor could be that the native enzyme existed in equilibrium with its subunits, and upon dilution the equilibrium shifted to favor greater subunit formation.

A model for aldose from Rhodotorula may now be proposed. These results support a structure in which the native molecule (mol.wt. = 61 000) is in equilibrium with two subunits, one with a molecular weight of 38 000 and the other 23 000. $NADP^+$ appeared to stabilize the native dimer.

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REFERENCES

1 G.H. Sheys, W.J. Arnold, J.A. Watson, J.A. Hayashi and C.C. Doughty, *J. Biol. Chem.*, in the press.
2 D.E. Williams and R.A. Reisfeld, *Ann. N.Y. Acad. Sci.*, 121 (1964) 373.
3 A.K. Dunker and R.R. Rueckert, *J. Biol. Chem.*, 244 (1969) 5074.
4 P. Andrews, *Biochem. J.*, 91 (1964) 222.
5 A.K. Dunker and R.R. Rueckert, *J. Biol. Chem.*, 244 (1969) 5074.
6 H.A. Sober, *Handbook of Biochemistry*, The Chemical Rubber Co., Cleveland, Ohio, 1968, p. C-10.
7 H.A. Sober, *Handbook of Biochemistry*, The Chemical Rubber Co., Cleveland, Ohio, 1968, p. C-15.
8 H.A. Sober, *Handbook of Biochemistry*, The Chemical Rubber Co., Cleveland, Ohio, 1968, p. C-16.