Note

Purification of NAD-dependent
1,2-Propanediol Dehydrogenating
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In the previous paper,¹⁾ we reported the presence of NAD-dependent 1,2-propanediol dehydrogenating enzyme in the cells of *Microcycus eburneus* grown on 1,2-propanediol under aerobic conditions, and also reported that the enzyme activity was separated into two fractions, namely enzyme I and II on DEAE-Sephadex A-25 column chromatography.

This paper deals with further purification of the enzyme II by affinity chromatography, and estimation of molecular weights of whole enzyme II and its subunit by gel filtration and disc electrophoresis.

Microcycus eburneus ATCC 21373 was cultivated by the same procedures as those previously mentioned under aerobic conditions.²⁾ Preparation of cell-free extract and assay of the enzyme activity were done in the same way as previously mentioned.¹⁾ Protein was estimated by the method of Lowry *et al.*³⁾ Disc electrophoresis on polyacrylamide gels was carried out by the methods of Ornstein *et*

*al.*⁴⁾ and Davis *et al.*⁵⁾ Molecular weight of enzyme was estimated according to the method of Andrews⁶⁾ using gel filtration on Sephadex G-200. Molecular weight was also estimated according to the methods of Schwartz and Rorder⁷⁾ using electrophoresis on SDS polyacrylamide gels. The standard enzymes used were catalase (mol wt: 190,000), RNA polymerases (mol wt: 165,000; 155,000; 90,000, 40,000), γ -globulins (160,000; 75,000; 50,000; 22,000), bovine serum albumins (130,000; 65,000), chicken egg albumin (45,000), pepsin (36,000) and lysozyme (14,400).

An affinity chromatography on blue dextran-polyacrylamide gels was carried out by the method of Kopperschläger *et al.*⁸⁾ using blue dextran fixed with 10% polyacrylamide gel.

The enzyme II was purified according to the procedures as follows: Enzyme II was separated from enzyme I by the same procedure as mentioned previously (protamine treatment, ammonium sulfate fractionation, DEAE-Sephadex A-25 column chromatography). The fraction of enzyme II was passed through hydroxylapatite column. The effluent was applied to a blue dextran polyacrylamide gel column (2.5 $\times$ 12 cm) preliminarily equilibrated with 0.01 M sodium phosphate buffer (pH 7.3) and eluted with 0.01 M sodium phosphate buffer (pH 7.3) containing 1 mM NAD. The active fractions were collected, concentrated by collodion bag and dialyzed against 0.01 M sodium phosphate buffer (pH 7.3). The purification processes are summarized in Table I. The enzyme II was purified about 330-fold at the step of affinity chromatography on blue dextran polyacrylamide gels.

The purified enzyme II was electrophoretically homogeneous as shown in Fig. 1.

The molecular weight of this enzyme was estimated to be 155,000 by gel filtration using Sephadex G-200 column as shown in Fig. 2. The molecular weight also estimated by SDS-polyacrylamide gel electrophoresis was 38,000 as shown in Fig. 3. Consequently, NAD-dependent 1,2-propanediol dehydrogenase II seems to consist of four identical subunits of molecular weight of 38,000.

TABLE I. PURIFICATION OF 1,2-PROPANEDIOL DEHYDROGENASE II FROM THE CELLS OF *Microcycus eburneus* GROWN ON 1,2-PROPANEDIOL AEROBICALLY

Purification step	Specific ^a activity	Purification	Total ^b activity	Yield (%)	Protein (mg)
Crude extract	110	1.0	79,000	100	718
Protamine sulfate	150	1.4	66,000	83	440
Ammonium sulfate	190	1.7	58,200	73	306
DEAE-Sephadex A-25	2,000	19	8,400	10.6	4.2
Hydroxylapatite	9,500	86	6,700	8.5	0.7
Blue dextran polyacrylamide	36,600	333	3,660	4.6	0.1

^a Specific activity is expressed as nmol of NADH formed per min per mg protein.

^b Total activity is expressed as nmol of NADH formed per min.

FIG. 1. Polyacrylamide Gel Electrophoresis of 1,2-Propanediol Dehydrogenase II Preparation.

The electrophoresis was carried out in 7.5% gel at pH 8.3 at 2 mA. The gels were stained with 0.25% Coomassie Brilliant Blue R. BPB, bromophenol blue.

FIG. 2. Molecular Weight Estimation of 1,2-Propanediol Dehydrogenase II by Gel Filtration on Sephadex G-200.

Standard proteins: 1, catalase; 2, γ -globulin; 3, bovine serum albumin, dimer; 4, bovine serum albumin, monomer; 5, chicken egg albumin. K_D : Distribution coefficient.

A column of 1.5×90 cm was used.

For elution, sodium phosphate buffer (pH 7.3) was used. Elution was made at 5 ml/hr.

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FIG. 3. Molecular Weight Estimation of 1,2-Propanediol Dehydrogenase II by SDS-Polyacrylamide Gel Electrophoresis.

A solution containing 0.125 M Tris-HCl buffer (pH 6.8), 6% SDS, 20% glycerol, 10% mercaptoethanol, 0.01% bromophenol blue and a sample (ca. 10 μ g of protein) were applied onto 12% polyacrylamide gels containing 0.1% SDS and then subjected to the electrophoresis at pH 8.3 of Tris-glycine buffer containing 0.1% SDS at 2 mA. After then, the gels were stained with 0.25% Coomassie Brilliant Blue R.

Standard proteins: 1, *E. coli* RNA polymerase; 2, γ -globulin; 3, *E. coli* RNA polymerase; 4, bovine serum albumin, dimer; 5, *E. coli* RNA polymerase; 6, γ -globulin; 7, bovine serum albumin, monomer; 8, γ -globulin; 9, *E. coli* RNA polymerase; 10, pepsin; 11, γ -globulin; 12, lysozyme.

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