Vol. 92, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 12, 1980 Pages 775-780

ISOLATION AND CHARACTERIZATION OF A PROTEIN HAVING LIPOIC ACID AS PROSTHETIC GROUP FROM ASCARIS MUSCLE MICROSOMES

Sadayuki MATUDA and Fujio OBO

Department of Biochemistry, Faculty of Medicine,
Kagoshima University, Kagoshima 890, JAPAN

Received December 12, 1979

SUMMARY

A protein which had lipoic acid as a prosthetic group was found in Ascaris muscle microsomes. The protein was solubilized with 0.1 % trypsin, and purified by heat-treatment and column chromatographys, and showed a single band on sodium dodecyl sulfate(SDS)-polyacrylamide gel electrophoresis, having a molecular weight of about 14,000. The protein was heat stable and capable of oxidizing reduced microsomal lipoamide dehydrogenase in Ascaris muscle.

INTRODUCTION

Ascaris lumbricoides var. suum, the parasitic roundworm, resides in the small intestine of swine. We purified a lipoamide dehydrogenase from Ascaris muscle microsomes and investigated some properties of it[1,2].

In addition we obtained in a homogenous state a protein(A_1) which had lipoic acid as a prosthetic group from Ascaris muscle microsomes. The protein having the lipoic acid as the prosthetic group is known to function in the oxidative decarboxylations of α-ketoacids and glycine[3-5]. However, Ascaris microsomes did not show such as functions.

In the present study, we report the purification of the protein having lipoic acid as the prosthetic group from Ascaris muscle microsomes and some properties of it.

MATERIALS AND METHODS

Ascaris muscle microsomes. Adults of Ascaris suum were obtained at a public slaughterhouse. Ascaris muscle microsomes were prepared as described previously[6].

0006-291X/80/030775-06$01.00/0

Ascaris muscle microsomal lipoamide dehydrogenase. Ascaris muscle microsomal lipoamide dehydrogenase was purified as follows; Ascaris muscle microsomes were treated with 0.1 % trypsin for 20 min at 37°C, and then centrifuged at 105,000 x g for 1 hour. The fractions containing lipoamide dehydrogenase activity were pooled and placed on DEAE-23 cellulose column and chromatographed using a linear gradient of sodium chloride. Again the fractions containing lipoamide dehydrogenase activity was pooled, concentrated and gel-filtrated by Sephadex G-200. The purified lipoamide dehydrogenase showed a single band on SDS-polyacrylamide gel[1].

Assay of enzyme activities. All spectrophotometric measurements were conducted in a Hitachi 124 recording spectrophotometer at room temperature. Lipoamide dehydrogenase activity was measured by the method of Massey[7]. The activity of the protein(A_1) having lipoic acid as prosthetic group is measured by following the increase of absorbance(at 412 nm) of reduced 5,5'-dithiobis-(2-nitrobenzoic acid) in the presence of lipoamide dehydrogenase [5].

Other methods. The molecular weight was determined by gel-filtration on Sephadex G-50(8] and by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate(SDS)[9].

Isolation of lipoic acid from the purified A_1 was carried out as follows; the protein was hydrolyzed with 6 N HCl for 4 hours at 100°C. The prosthetic group was extracted with $CHCl_3$ from the hydrolysate, dried in vacuo and dissolved in a minimum volume of 80 % ethanol(v/v).

Paper chromatography was carried out using n-butanol:acetic acid:NH_3(35 %) = 7:3:2 as a solvent system and the spots were visualized under ultraviolet light[10].

Protein was determined by the method of Lowry et al[11] using bovine serum albumin as a standard.

Reagents and Chemicals. Sephadex and DEAE-23 cellulose were obtained from Pharmacia Fine Chemicals, Uppsala, and Whatman Biochemicals, Ltd, respectively. NADH was from Boehringer Mannheim GmbH. Trypsin(type III) was obtained from Sigma. DL-α-lipoic acid and 5,5'-dithiobis-(2-nitrobenzoic acid) were purchased from Nakarai Chemicals, Ltd., and other chemicals used were commercial products of reagent grade.

RESULTS AND DISCUSSION

Solubilization and purification of A_1 protein from Ascaris muscle microsomes. All the column chromatographys were carried out in a cold room at 4°C.

Step 1. Solubilization of A_1. The microsomes suspended in 50 mM Tris-HCl buffer(pH 8.0) were digested with 0.1 % trypsin for 20 min at 37°C and centrifuged at 105,000 x g for 60 min. The supernatant was concentrated on Amicon PM 10 filter.

Step 2. Gel-filtration on Sephadex G-200. The concentrated protein was applied to a column of Sephadex G-200(2.6 x 50 cm) equilibrated with 50 mM Tris-HCl buffer. The fractions with A_1 activity were combined(Fig.1).

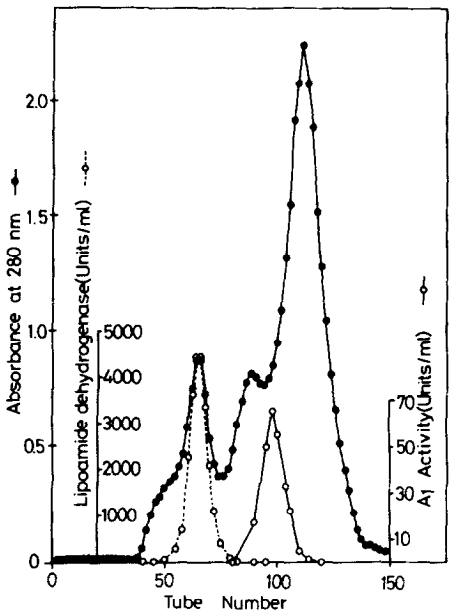


Figure 1. 1st Gel-filtration on a Sephadex G-200 column. Experimental procedures are described in the text. Fractions of 3.2 ml were collected. One unit is defined as an absorbance change of 0.1 per min.

Step 3. DEAE-23 cellulose column chromatography. Step 2 enzyme was applied to a DEAE-23 cellulose column(1 x 20 cm) equilibrated with 50 mM Tris-HCl buffer, pH 8.0(T-buffer). After the column was washed with T-buffer and again with one containing 0.1 M NaCl, A_1 was eluted with T-buffer containing 0.2 M NaCl. The fractions with A_1 activity were combined and concentrated.

Step 4. Gel-filtration on Sephadex G-100. The concentrated A_1 was applied to a column of Sephadex G-100(1.5 x 50 cm) equilibrated with 50 mM T-buffer. The fractions with A_1 activity were combined and concentrated.

Step 5. Heat treatment. The concentrated A_1 was heated for 5 min in boiling water. The precipitate was discarded by centrifugation.

Step 6. Gel-filtration on Sephadex G-50. The supernatant was applied to a column of Sephadex G-50(Fine) equilibrated with 50 mM T-buffer. The results of purification of the A_1 protein are summarized in Table I.

Properties of the purified A_1 protein.—Purity and molecular weight. As demonstrated in Fig.2, SDS-polyacrylamide gel electrophoresis of the

Table I. Summary of the purification of A_1

Steps	Protein (mg)	Specific Activity (Units/mg)	Total Activity (Units)	Yield (%)
Microsomes	1040	1.3	1352	100
Supernatant	432	2	864	64
Sephadex G-200	86	6.5	559	41
DE-23 cellulose	6.7	67	449	33
Sephadex G-100	3	82	246	18
Heat treatment	2.8	84	235	17
Sephadex G-50	1.5	94	141	10

The specific activity was expressed as units per mg of protein and one unit was defined as an absorbance change of 0.1 per min.

purified A_1 showed a single band. By elution from a Sephadex G-50 column (A) and by it's relative migration in SDS-polyacrylamide gel(B), the molecular weight of the A_1 was estimated to be about 14,000(Fig.3).

Ability of A_1 to couple to lipoamide dehydrogenase in the transfer of electrons from NADH to DTNB. Fig.4 shows the ability of A_1 in transferring

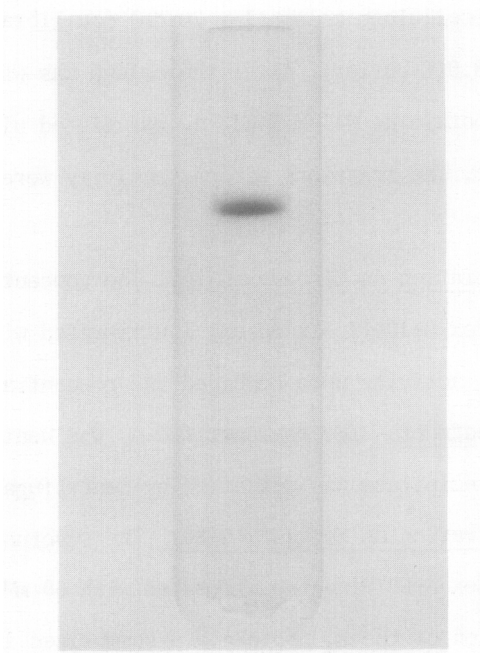
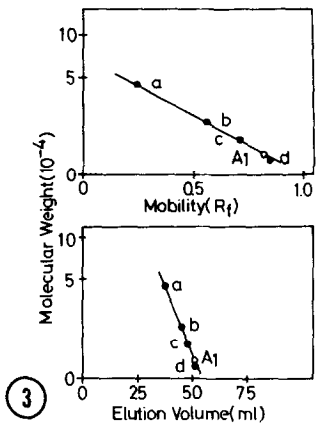


Figure 2. SDS-gel electrophoresis of the purified A_1 in 15 % polyacrylamide gel containing 0.1 % sodium dodecyl sulfate.



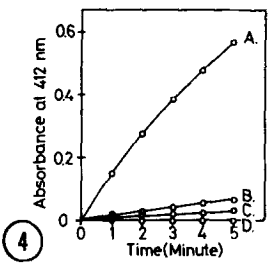
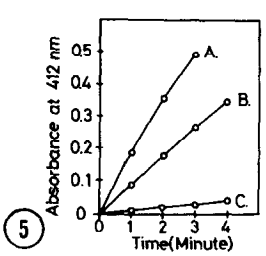


Figure 3. Estimation of the molecular weight of purified A_1 protein. A; Gel-filtration on Sephadex G-50(Medium). B; SDS-polyacrylamide gel electrophoresis. Polyacrylamide gels were prepared with 15 % polyacrylamide. a; ovalbumin, b; trypsin, c; myoglobin, d; cytochrome c.

Figure 4. Ability of A_1 in transferring electrons from NADH through lipoamide dehydrogenase to DTNB. 0.1 M phosphate buffer(pH 7.2, 1.5 ml), 3 mM DTNB (0.1 ml) and 2 mM NADH(0.1 ml) were used. The amount of A_1 and lipoamide dehydrogenase were 1.9 x 10^{-5} M and 1.5 x 10^{-6} M, respectively. A; Complete system, B; Complete minus A_1, C; Complete minus lipoamide dehydrogenase, D; Complete minus NADH, or Complete minus(A_1 + lipoamide dehydrogenase).

electrons from NADH and lipoamide dehydrogenase to DTNB(A). When A_1 or lipoamide dehydrogenase was eliminated from complete system, the ability in transferring electrons from NADH to DTNB was very low(B,C). As shown in Fig. 5, lipoic acid and A_1 were effective in transferring electrons, but cystine and oxidized glutathione were not substituted for A_1.



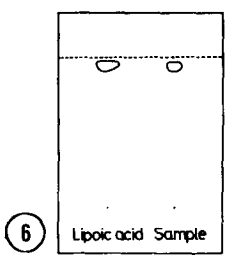


Figure 5. Comparative activities of several disulfide compounds substituted for A_1 in transferring electrons from NADH through lipoamide dehydrogenase to DTNB. The conditions of assay were as described in Fig.4. The concentration of lipoic acid, cystine and oxidized glutathione were all 2 x 10^{-4} M. The amount of A_1 and lipoamide dehydrogenase were 1.3 x 10^{-5} and 10^{-6} M, respectively. A; Lipoic acid, B; A_1, C; cystine, or glutathione.

Figure 6. Identification of the prosthetic group by paper chromatography. Paper chromatography was run as described in the text.

Lipoic acid as a component of A_1. To establish the presence of lipoic acid in A_1 experiment was carried out using paper chromatography. As shown in Fig.6, the prosthetic group extracted with $CHCl_3$ from hydrolysate of A_1 exhibited R_f value of 0.98 which was identical with that for authentic lipoic acid, and was able to transfer electrons from NADH through lipoamide dehydrogenase to DTNB. These results suggested that lipoic acid was the coenzyme of A_1.

The lipoic acid generally plays a role in the oxidative decarboxylations of α-ketoacids and glycine. In heat stability and molecular weight, A_1 has properties similar to P_2 which functions in oxidative decarboxylations of glycine in Peptococcus glycinophilus[5]. However, Ascaris microsomes did not show the oxidative decarboxylations of glycine and α-ketoacids[1].

REFERENCES

1. Matuda,S. and Obo,F. Submitted for publication.
2. Matuda,S. and Obo,F.(1979) Biochem. Biophys. Res. Commun. in press.
3. Tanaka,N., Koike,K., Hamada,M., Ogasahara,K. and Koike,M.(1974) J. Biol. Chem. 249, 191-198.
4. Koike,M., Reed,L.J. and Canoll,W.R.(1963) J. Biol. Chem. 238, 30-39.
5. Robinson,J.R., Klein,S.M. and Sagers,R.D.(1973) J. Biol. Chem. 248, 5319-5323.
6. Matuda,S.(1979) J. Biochem(Tokyo). 85, 343-350.
7. Massey,V.(1960) Biochim. Biophys. Acta. 37, 314-322.
8. Andrews,P.(1965) Biochem. J. 96, 595-606.
9. Weber,K. and Osborn,M.(1969) J. Biol. Chem. 244, 4406-4412.
10. Silver,M.(1978) in "Methods in Enzymology"(McCormick,D.B. and Wright, L.D., eds) V.62, part D, pp.135-137.
11. Lowry,O.H.,Rosebrough,N.J., Farr,A.L. and Randall,R.J.(1951) J. Biol. Chem. 193, 265-275.