THE ISOLATION AND PROPERTIES OF A SOLUBLE SUCCINIC COENZYME Q REDUCTASE FROM BEEF HEART MITOCHONDRIA*

Daniel M. Ziegler and K. A. Doeg**

Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin

Received December 11, 1959

In a previous communication of this series (Ziegler and Doeg, 1959) it was shown that the enzymatic reduction of coenzyme Q (CoQ) by succinate does not involve the cytochromes; whereas the antimycin A sensitive oxidation of reduced CoQ is mediated by the cytochrome system (Hatefi, 1959; Pumphrey and Redfearn, 1959; and Green et al, 1959). These data demonstrated that CoQ, known to be an essential component in the antimycin sensitive oxidation of succinate (Lester and Fleischer, 1959) is required to link the succinic dehydrogenase to the cytochrome system. Succinic dehydrogenase in the form isolated by Singer et al (1956) does not catalyze the reduction of CoQ by succinate. However, a succinic dehydrogenase can be isolated in a soluble form which will catalyze the rapid reduction of CoQ by succinate. The flavin and non-heme iron contents of the purified form of such a reductase are essentially the same as those of the Singer-Kearney flavoprotein. In addition CoQ reductase contains lipid and heme in an amount equivalent to the flavin.

Protein, flavin, heme and non-heme iron were determined by methods described previously (Järnefelt et al, 1958). Succinic phenazine methosulfate activity was measured either manometrically by the method described by Singer

* This work was supported in part by National Institutes of Health (USPHS), Division of Research Grants, Graduate Training Grant 2G-88 and research grant RG-5873 and National Heart Institute Grant H-458; National Science Foundation Grant G-3227; AEC Contract AT(11-1)-64, Project 4. Meat by-products were generously furnished by Oscar Mayer and Co., Madison. The authors are indebted to Dr. D. E. Green for his advice during the course of this work and to Mrs. Sally Krueger for her valuable technical assistance.

** Post-doctoral trainee of the Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin.

and Kearney (1957) or spectrophotometrically by measuring the reduction of cytochrome c with phenazine as mediator in the manner described by Hauge (1956). When the rates are expressed as μmoles succinate oxidized per min per mg enzyme the two methods of assay are in close agreement. In the manometric assay 2 μatoms of oxygen are consumed for each μmole succinate oxidized since hydrogen peroxide and not water is the final product formed by the oxidation of reduced phenazine by oxygen. The methods used to measure succinic CoQ reductase activity will be described in a separate report.

A suspension of beef heart mitochondria in 0.3 M phosphate, pH 7.4 is treated with cholate (0.6 mg/mg protein) and warmed to 38°C for 10 minutes. It is then rapidly cooled to 5° and fractionated with ammonium sulfate. The fraction that precipitates between 30-40% saturation is collected by centrifugation and dissolved in a minimal volume of 0.25 M sucrose. After the solution has been dialyzed against 0.25 M sucrose to remove excess ammonium sulfate, potassium deoxycholate (0.75 mg/mg protein) and ethanol (final concentration 30%) are added. The precipitate that forms is removed by centrifugation and discarded. The supernatant fluid is emulsified with an equal volume of cold cyclohexane and centrifuged at 40,000 rpm for 20 min. in the Spinco Model L ultracentrifuge. Succinic CoQ reductase quantitatively precipitates as a translucent red pellet. The pellet is resuspended in a small volume of 0.25 M sucrose and centrifuged at 40,000 rpm for 30 min. The succinic CoQ reductase activity remains in solution. Between 10-15% of the mitochondrial succinic flavin is recovered in this final extract of purified CoQ reductase. The enzyme solution can be frozen in 0.25 M sucrose for 2 to 3 days without appreciable loss of activity, but repeated freezing and thawing leads to a progressive inactivation of the enzyme.

Succinic CoQ reductase at the highest purity level contains 4.5-5.0 mμmoles flavin (extractable by acid only after tryptic digestion), 18-20 mμmoles non-heme iron and 4.8-5.2 mμmoles heme per mg protein. All of the heme is extractable by acetone from the lipid-free protein. Eight to 10% of the dry weight of the enzyme is soluble in both ethanol and petroleum ether, and this moiety

has been assumed to be lipid.

Table I summarizes the dehydrogenating activities of the enzyme with each of three electron acceptors. The enzyme reacts rapidly with CoQ only when supplemented with small amounts of the Q lipide extract (Basford and Green, 1959) and a non-ionic detergent such as Triton X-100. The detergent solubilizes CoQ and accessibility of the coenzyme to the enzymes in an aqueous medium may depend upon this solubilization. The function of the lipide extract is not known. The CoQ reductase reacts more rapidly with CoQ than with phenazine or ferricyanide in contrast to the Singer-Kearney flavoprotein which reacts only with phenazine or ferricyanide.

The difference spectrum (oxidized minus reduced) of the enzyme is shown in Figure 1. The flavin, but not the heme component, is reduced by succinate. The Q lipoprotein with or without Triton X-100 does not promote the reduction of the heme by succinate.

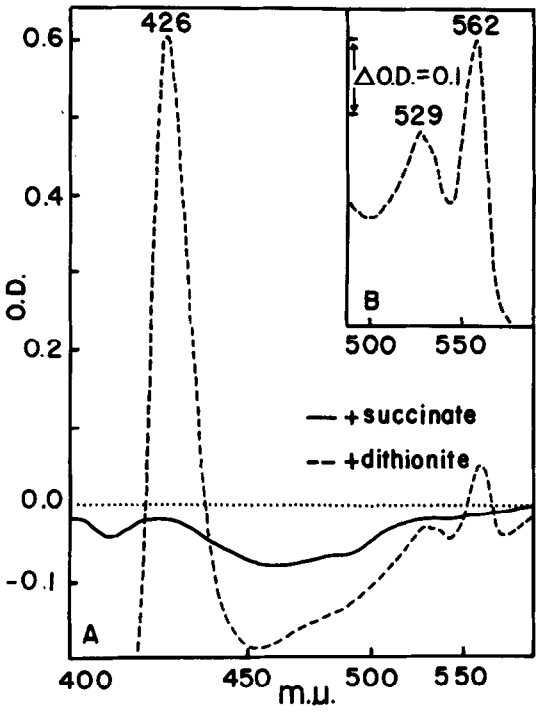

Fig. 1. Difference spectra of solutions of succinic CoQ reductase recorded with a Beckman DK-2 spectrophotometer. The enzyme was dissolved in 0.1 M phosphate buffer pH 7.4 at a final concentration of 1.16 mg protein per ml.

Table I

The Interaction of Succinic CoQ Reductase and the Succinic Dehydrogenase with Electron Acceptors

Form of the Dehydrogenase	Electron Acceptors		
	Coenzyme Q[1]	Phenazine[2]	Ferricyanide
Succinic CoQ Reductase	2	29	9
" " " + Q lipid extract and triton X-100	36	29	15
Singer-Kearney flavoprotein	0	11	9
" " " + Q lipid extract and triton X-100	0	11	9

1 The basic assay mixture contained 0.12 μmole CoQ, 50 μmoles phosphate buffer pH 7.0, 40 μmoles succinate, and 1-2 μgrams enzyme per ml. For optimum reaction rates this medium was supplemented with 50 μgrams triton and 1-2 μgrams of the Q lipid extract.

2 The phenazine rates are not corrected for optimum substrate and dye concentration. All of the rates are expressed as μmoles succinate oxidized per min per mg protein at 38°C.

While the heme group does not participate as an electron carrier in the reduction of CoQ, it may be required to preserve some structural arrangement within the enzyme necessary for CoQ reduction. All attempts to prepare a CoQ reductase free of heme have been unsuccessful. It would appear that the CoQ reductase is not a simple mixture of a hemoprotein and a flavoprotein, since the ratio of protoporphyrin to succinic flavin remains constant in the final stages of the purification and the two components cannot be separated by methods ordinarily used to fractionate proteins.

A heme free flavoprotein, similar to the Singer-Kearney flavoprotein can be prepared by converting the CoQ reductase to an acetone powder in the presence of 1% tertiary amyl alcohol in the manner described by Basford et al, (1957). The heme group is extracted by acetone but the flavin to protein ratio of the acetone-extracted material is the same as that of the untreated flavohemoprotein. CoQ reductase activity is irreversibly destroyed by this treatment, but the resulting heme-free flavoprotein can still carry out the succinate-phenazine or succinate-ferricyanide reactions. Since Singer et al (1956) have reported that a heme-free succinic dehydrogenase, with an identical flavin content, was a homogeneous protein, it would appear that the succinic CoQ reductase enzyme is a single protein with two prosthetic groups, and only one of these (the flavin) is involved in the reduction of CoQ by succinate.

In many respects the succinic CoQ reductase is analogous to the yeast lactic dehydrogenase (Appleby and Morton, 1954; Nygaard, 1959; and Horio et al, 1959). Both enzymes can be isolated as flavohemoproteins and in each case the heme component is not an oxido-reduction intermediate in the reaction between the flavoprotein and external cytochrome c.

References

Appleby, C. A. and Morton, R. K., Nature, 173 (1954) 749.

Basford, R. E. and Green, D. E., Biochim. Biophys. Acta, 33 (1959) 193.

Basford, R. E., Tisdale, H. D. and Green, D. E., Biochim. Biophys. Acta, 24, (1957) 294.

Green, D. E., Ziegler, D. M., Doeg, K. A., Arch. Biochem. Biophys. (1959).

Hatefi, Y., Biochim. Biophys. Acta, 34 (1959) 183.

Hauge, Jens. G., J. Am. Chem. Soc., 78 (1956) 5266.

Horio, L., Yamashita, J. and Okunuki, K., Biochim. Biophys. Acta, 32 (1959) 593.

Järnefelt, J., Basford, R. E., Tisdale, H. D. and Green, D. E., Biochim. Biophys. Acta, 29 (1958) 123.

Lester, R. L. and Fleischer, S., Arch. Biochem. Biophys., 80-2 (1959) 470.

Nygaard, A. P., Biochim. Biophys. Acta, 33 (1959) 517.

Pumphrey, E. and Redfearn, E. R., Biochem. J., 72 (1959) 2P.

Singer, T. P. and Kearney, E. B., in D. Glick Methods of Biochemical Analysis Vol. IV (1957) 312.

Singer, T. P., Kearney, E. B. and Bernath, P., J. Biol. Chem., 223 (1956) 599.

Ziegler, D. M. and Doeg, K. A., Arch. Biochem. Biophys. (1959), in press.