Preliminary Notes

Succinic dehydrogenase of the ergot fungus and mussel

Succinic dehydrogenase has been successfully solubilized and purified from several sources mainly by SINGER, WANG and their co-workers[1,2]. In this communication we wish to report briefly our studies of the solubilization and some properties of succinic dehydrogenase isolated from the mycelium of the ergot fungus, *Claviceps purpurea*, and from the adductor muscle of the bay mussel, *Mytilus edulis*. These organisms are divergent in morphology and in evolutionary development. The dehydrogenase derived from these two sources possesses dissimilarities as expected and also some striking resemblances not only to each other but to the enzyme isolated from other sources.

The *Claviceps* succinic dehydrogenase can be solubilized from its mycelium with great ease, *e.g.* by extraction with acetone, sonic disintegration or merely homogenizing with alumina in a Waring blender for 2 min. In each operation, the activity recovered is more than 75 %. In contrast to the *Claviceps* enzyme, the succinic dehydrogenase of mussel resists solubilization. Sonic disintegration, digestion with snake venom, incubation with wheat germ, microbial or pancreatic lipase, or treatment with lipid solvents either failed to extract or completely inactivated the enzyme. The dehydrogenase is evidently tightly bound to the particulate matter. The particles can be purified through rather drastic treatments. In fact, the purified particles were made by tryptic digestion (1 mg crystalline trypsin to 150 mg protein for 10 min at 35°) and then isolated by differential centrifugation. Only from the acetone powder of the purified particles could the dehydrogenase be brought into solution, with 0.06 *M* tris(hydroxymethyl)aminomethane buffer at pH 8.9. This method resembles the one originally used by SINGER *et al.*[1] for heart-muscle particles. However, butanol, which has been used effectively for the liberation of succinic dehydrogenase from the Keilin–Hartree heart preparation[2], has no solubilizing effect whatsoever on the particles derived from the mussel. The enzyme prepared from *Claviceps* or mussel is in true

TABLE I

THE ACTIVITY OF SUCCINIC DEHYDROGENASE FROM *Claviceps* AND MUSSEL*

	Claviceps	*Mussel*
Mycelium or adductor muscle	7.5	8.2
Particles, purified	—	150
Soluble enzyme, crude	60	270
Soluble enzyme, purified**	300	—

* The activity is expressed in μl O_2 consumed/mg dry weight/h in the assay system with phenazine methosulfate as the electron acceptor.

** The partially purified preparation was made by treatment of the crude soluble extract with protamine sulfate and then by fractionation with ammonium sulfate.

solution and is not sedimented at 140,000 × *g*. Table I summarizes the two enzymic activities. Succinic dehydrogenase from either *Claviceps* or mussel catalyzes the oxidation of succinate by phenazine methosulfate only. Tetrazolium chloride, methylene blue, ferricyanide and oxygen do not react. The inactivity of ferricyanide is genuine because the assay system which was the same as used by other authors[1,2] responded as usual when the heart preparation was used. This selectivity of electron acceptor is striking; succinic dehydrogenase from all other sources may use ferricyanide with the activity ranging from 10 to 40 % of the rate with phenazine methosulfate.

The soluble succinic dehydrogenase from *Claviceps* is extremely stable in contrast to the enzyme from animal heart or from mussel. The *Claviceps* enzyme, in the partially purified form, can be stored in the frozen condition for 4 weeks without measurable loss in activity. Its half-life in a crude extract is longer than one week at 0°, and about 40 h at 20°. The succinic dehydrogenase activity of the particles from mussel in the form of a suspension is as stable as the Keilin–Hartree preparation. Once the dehydrogenase emerges from the particles it is then very unstable with a half-life less than 24 h at 0°.

The enzyme prepared from either source, reminiscent of the heart enzyme, is not inactivated by the reaction with cyanide performed under the conditions described by KEILIN AND KING[3] when phenazine methosulfate was used as the electron acceptor. However, more rigid tests by reconstitution[4] have not been done.

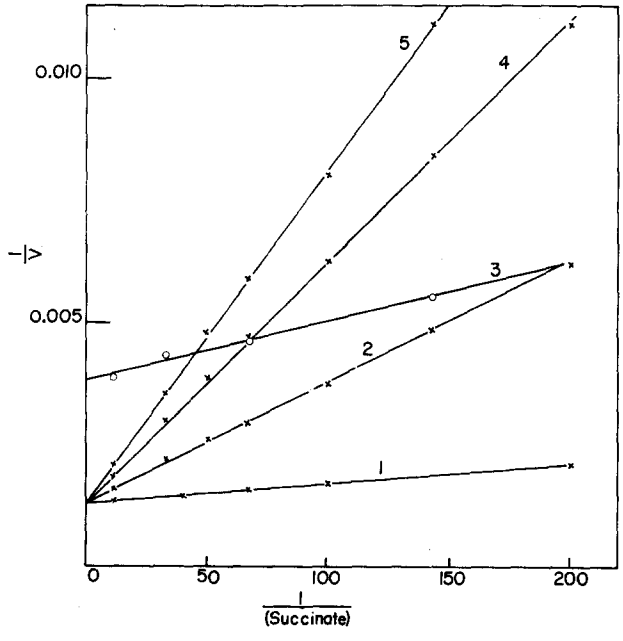

Fig. 1. The inhibition of succinate oxidation catalyzed by the *Claviceps* succinic dehydrogenase. The system contained 0.5 ml 0.3 *M* phosphate buffer, pH 7.7; 0.2 ml 0.15 *M* KCN; 0.2 ml 2 % phenazine methosulfate, 3.2 mg protein; final volume 3.0 ml, temperature 35°. The rate was taken from the initial slope of the oxygen consumption *vs.* time which was linear up to 8 min. Curve 1, with no inhibitor; curve 2, with $5 \cdot 10^{-3}$ *M* fumarate; curve 3, with $3.0 \cdot 10^{-5}$ *M* *o*-phenanthroline; curve 4, with $5 \cdot 10^{-3}$ *M* pyrophosphate; and curve 5, with $5 \cdot 10^{-4}$ *M* malonate.

The dehydrogenase solubilized from *Claviceps* shows a kinetic behavior similar to that from heart particles. Fumarate, malonate and pyrophosphate competitively inhibits the oxidation of succinate with inhibition constants of $9.3 \cdot 10^{-4}$ *M*, $3.0 \cdot 10^{-5}$ *M* and $4.2 \cdot 10^{-4}$ *M*, respectively (Fig. 1). *o*-Phenathrolin partially inhibits the oxidation in an apparently non-competitive manner whereas α,α'-dipyridyl even at $3 \cdot 10^{-3}$ *M* does not show inhibition. The Michaelis constant for succinate is $3 \cdot 10^{-3}$ *M*, which is somewhat higher than that for the heart enzyme but lower than that for the *Micrococcus* succinic dehydrogenase ($5.3 \cdot 10^{-3}$ *M*)[5].

Cultivation of *C. purpurea* was described previously[6]. The adductor muscle was dissected from mussel which was collected from the bays on the Oregon Coast.

This work was supported by grants from National Science Foundation (G-8966), the U.S. Public Health Service (H-4852 and A-1080) and Office of Naval Research (Nonr-1286).

Department of Chemistry and Science Research Institute, Oregon State College, Corvallis, Oreg. (U.S.A.)

TSOO E. KING
CLARENCE A. RYAN
VERNON H. CHELDELIN
J. KEN MCDONALD

[1] T. P. SINGER, E. B. KEARNEY AND V. MASSEY, *Advanc. Enzymol.*, 18 (1957) 65.
T. P. SINGER, *Proc. Intern. Symposium on Enzyme Chemistry*, 1958, Maruzen Co., Tokyo, p. 330.
[2] T. Y. WANG, C. L. TSOU AND Y. L. WANG, *Sci. Sinica (Peking)*, 5 (1956) 73.
[3] D. KEILIN AND T. E. KING, *Proc. Roy. Soc. (London)*, *B* 152 (1960) 163.
[4] D. KEILIN AND T. E. KING, *Nature*, 181 (1958) 1520.
[5] M. G. P. J. WARRINGA AND A. GIUDITTA, *J. Biol. Chem.*, 230 (1958) 1110.
[6] J. K. MCDONALD, V. H. CHELDELIN AND T. E. KING, *J. Bacteriol.*, 80 (1960) 61.

Received October 18th, 1960

Nature of the two forms of malic dehydrogenase from rat liver

Malic dehydrogenase activity may be found in both the mitochondrial and supernatant fractions obtained by differential centrifugation of rat-liver homogenates[1,2]. Investigation of the nature of the two forms was pertinent to the purification studies of MDH reported earlier from this laboratory[3,4]. The chromatographic and electrophoretic behavior of the two forms (obtained by procedure C, below) is in general agreement with other recent studies[2,5]. The two preparative procedures A and B, outlined below, also yield fractions of MDH which have chromatographic and electrophoretic characteristics similar to either the "mitochondrial" or the "supernatant" forms. However, the experiments to be described in this communication demonstrate that the "supernatant" form can be converted to the "mitochondrial" one on treatment with *n*-butanol.

The three procedures are as follows. *Procedure A*.[3] 1, Homogenization of rat liver with aq. EDTA-NaCl; 2, heat treatment; 3, alcohol fractionation; 4, chromatography on Amberlite XE-64, which yields two fractions. About 30–40 % of the enzyme is not retained and passes through the column along with a large amount of colored proteins

Abbreviations: MDH, malic dehydrogenase; DEAE-, diethylaminoethyl-; EDTA, ethylenediaminetetraacetic acid.