Dissociation of the succinoxidase systems of *Ascaris lumbricoides* and of rat kidney

The succinoxidase complex of mammalian tissues is considered to consist of a primary dehydrogenase, linked to an electron transporting chain of several constituents terminating with the cytochrome *c*-cytochrome oxidase system[1,2,3]. Methylene blue can act as an electron acceptor from some constituents of the succinoxidase complex, but not from a soluble beef heart succinic dehydrogenase[4]. However, the latter reacts with phenazine methosulfate[4,5].

The succinoxidase system of the parasitic worm, *Ascaris lumbricoides*, differs from that of mammalian tissues because transfer of electrons to atmospheric oxygen occurs without the participation of the cytochrome system[6]. Purification of the succinoxidase system of *Ascaris* muscle was carried out by extraction of the soluble proteins from the minced tissues, by homogenization of the residue in tris-(hydroxymethyl)-aminomethane (TRIS) buffer (0.04 molar; pH: 8.9) containing succinate (0.025 molar) and by differential centrifugation of the homogenate. The succinoxidase system was recovered in the supernatant fluid on centrifugation at 4000 r.p.m. for 30 minutes and in the residue after centrifugation at 70,000 × *g* for one hour. In contrast to the mammalian succinoxidase system, antimycin A[7] did not inhibit the oxidation of succinate by the preparation from *Ascaris* muscle. Its activity was stimulated by methylene blue and by phenazine methosulfate (Table I). When the purified succinoxidase preparation was treated with pancreatic lipase*, in the presence of calcium ions, and subsequently was centrifuged at 70,000 × *g* (one hour), succinic dehydrogenase activity in the absence of dye was markedly reduced; furthermore, a significant decrease in activity occurred when methylene blue was used as the electron carrier. On the other hand, dehydrogenase activity in the presence of the phenazine dye was not altered. After freezing and thawing of the lipase-treated preparation, a fraction soluble on centrifugation at 70,000 × *g* (one hour) had succinic dehydrogenase activity in the presence of phenazine methosulfate, but little or no activity was detected when methylene blue was used as the acceptor (Table I). The same preparation catalyzed the reduction of ferricyanide by succinate. Our observations indicate that, through the action of lipase, the succinic dehydrogenase of *Ascaris* can be dissociated from its electron transporting system.

TABLE I

ENZYMIC ACTIVITIES OF FRACTIONS OF *Ascaris* SUCCINOXIDASE

#	*Fraction*	*No dye*	*Methylene blue (0.05 mg per ml)*	*Phenazine methosulfate (0.5 mg per ml)*
1	Ascaris muscle homogenate	2.9	6.4	7.2
2	Purified succinoxidase	50	86	181
3	#2 after treatment with lipase	19	71	195
4	#3 after freezing and thawing followed by centrifugation at 70,000 × *g* (supernatant)	0	17	252

Conditions of assay: 0.025 *M* succinate, 0.04 *M* TRIS buffer (pH: 8.3), 0.001 *M* $MnCl_2$, 2 units catalase, 0.05 *M* ethanol; total volume: 1 ml. Temperature: 37°C. Activities are expressed as μl of oxygen taken up in 10 minutes per mg of protein.

Similar results were obtained with the succinic dehydrogenase system of rat kidney. With a histochemical method for localizing the system using tetrazolium salts[8], modified by the use of additional soluble oxidation-reduction dyes[9], it was found that incubation of frozen sections of rat kidney with lipase and $CaCl_2$ produced a dissociation of the succinic dehydrogenase system in such a manner that it could still react with blue tetrazolium via phenazine methosulfate while it no longer reduced blue tetrazolium alone or via methylene blue. These histochemical findings were confirmed by direct measurements of succinic dehydrogenase activities of particulate fractions obtained from rat kidney and from rat liver[10]. After incubation of these preparations with lipase their ability to reduce succinate in the presence of methylene blue was abolished while their enzymic acitvity in the presence of the phenazine dye remained unimpaired.

* This preparation had no demonstrable proteolytic activity. In order to insure the absence of the latter, soy bean and egg white trypsin inhibitors were added during incubation of the succinoxidase complex with the lipase preparation.

Evidence has been obtained which indicates the possibility that diphosphopyridine nucleotide (DPN) and flavine adenine dinucleotide (FAD) are constituents of the succinoxidase complex of *Ascaris*. This system was inactivated by incubation with a nucleotide pyrophosphatase*; following this treatment, reactivation of succinate oxidizing capacity could be observed when DPN and FAD were added. The addition of FAD alone generally did not produce any observable reactivation, while both FAD and DPN usually were required for maximal stimulation. Nicotinamide brought about inhibition of succinate oxidation in concentrations comparable to those found to inhibit some known DPN- and TPN-linked dehydrogenases [11,12]. Furthermore, incubation of the succinoxidase system with succinate and purified lactic dehydrogenase [13] resulted in a significant reduction of pyruvate to lactate.

So far, no metal requirement of the system has been detectable. Succinic dehydrogenase activity was stimulated by manganese as well as by ethylenediaminetetraacetate suggesting that manganese displaces an ion which has an inhibitory effect on the enzyme.

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Deuterium exchange between ribonuclease and water

As a link in a recent series of isotope-exchange studies carried out in the Carlsberg Laboratory[1-6] an investigation has been made of the exchange of deuterium between ribonuclease (RNase) and water. The results will be briefly reported below and compared with those found for insulin[2,4,6] to which protein RNase bears a rather close resemblence.

The sample used (Armour 38159) was presented to us by Dr. C. B. ANFINSEN. The method applied in the exchange experiments is described in[3,4,5]. 200 μl of a well-defined 1–2% aqueous solution of RNase are lyophilized and the water replaced by 200 μl 99.73% D_2O. After complete exchange the D_2O is removed by cryosublimation and the sample dried for 3 hours by heating to 60° against a trap at −60° (see [4]). The dry deuterium-loaded sample of protein is then dissolved in 200 μl H_2O for back-exchange and the exchange reaction followed by taking out 15 μl samples at suitable intervals, removing their water by cryosublimation and determining its concentration of deuterium by density determinations in the gradient tube.

According to analysis by HIRS, MOORE AND STEIN[7], RNase, Mw 13895, consists of 126 amino acids arranged in one peptide chain, with four intrachain S-S bridges. It has the all over composition: Asp_{16} Glu_{12} Gly_{13} Ala_{12} Val_9 Leu_2 $Ileu_3$ Ser_{15} Thr_{10} Cys_8 Met_4 Pro_5 Phe_3 Tyr_6 His_1 Lys_{10} Arg_4 $(CONH_2)_{17}$ and at the isoelectric point the molecule contains 238 theoretically exchangeable oxygen- and nitrogen-bound hydrogen atoms, out of which 120 belong to the –CO–NH–