REACTIVATION OF SUCCINIC DEHYDROGENASE BY PHOSPHOLIPIDS

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Results from this and from other laboratories (Cerletti, Strom and Giordano, 1963; Singer and Kearney, 1963) have shown that succinic dehydrogenase (SD) solubilized with any of the available methods has a lower efficiency in catalizing succinate oxidation with artificial acceptors, than the enzyme bound to particulate preparations. The first major change in activity has been correlated with the extraction of the particulate preparation with lipid solvents (Cerletti, Strom and Giordano, 1963; Cerletti et al., 1964). Experiments reported here corroborated this finding and gave evidence that the decrease of activity is not only due to an inactivation during the extraction procedures, but depends on the loss of some specific component present in the original particulate preparation, and that appropriate additions may restore the full activity.

MATERIALS AND METHODS

The oxidation of succinate was followed spectrophotometrically at 25°C, in a medium containing 0.02 M succinate, 0.06 M phosphate buffer pH7.6, $1.2 \cdot 10^{-5}$ M pyridoxamine, $4 \cdot 10^{-5}$ M 2.6 dichlorophenolindophenol and phenazine methosulphate (PMS). The reaction was started by adding the enzyme preparation. Succinoxidase activity was inhibited with 0.8 mM KCN.

Comparison of SD activity in different preparations was made on bound flavin basis, peptide bound flavins being determined as described previously (Cerletti, Strom and Giordano, 1963; Cerletti et al., 1964). Turnover numbers (T.N.s) represent enzyme activity as moles succinate oxidized/min/mole flavin at 25°C. The reaction rate at 25°C is, for heart SD, 50% of that measured at 38°C. Under the conditions adopted values of T.N. obtained with mitochondrial preparations measure what, at the present level of research (Cerletti,

Strom and Giordano, I963; Singer, I964), appears to be the full oxidation potential of external succinate with PMS as an acceptor, by the enzyme in the mitochondrion. Reference to them is made as to a provisional measure of the activity in the assay system used, of SD bound to the respiratory assembly.

Heart mitochondria were prepared according to Bernath and Singer(I962). Before use they were disrupted by freezing and thawing in hypotonic saline. Acetone extraction was performed in a refrigerated bath at -10° to -15°C, by treating mitochondria suspended in 0.06 M phosphate buffer pH 7.6 (with or without 5 mM succinate) with 0.1 vol t amyl alcohol and 10 vol.acetone precooled at -15°C. The sedimenting fraction was dried with acetone and ether. To obtain soluble SD, the acetone powder was suspended in 0.06 M phosphate buffer pH 7.6 containing 40 mM succinate and kept 15 min. at pH 9, 0°C; then neutralized and the sediment at 140.000 x g discarded.

RESULTS AND DISCUSSION

Lipid in heart mitochondria is predominantly phospholipid (Fleischer, Klouwen and Brierley, I96I) most of which is extracted by acetone under the conditions used in the present work (Lester and Fleischer, I96I). This is accompanied by a considerable drop of T.N. for SD. A further decrease occurs in the subsequent solubilization step, when the lipid depleted preparation is treated with alkali. As shown in table 1, the presence of succinate prevents to a considerable extent both these losses of activity. However, the addition of succinate under our conditions, never preserved the original T.N. which the enzyme had in unextracted preparations.

A full reactivation is instead obtained when mitochondrial lipids extracted during the acetone treatment are added. Commercial lecithin can be substituted, with somewhat lower efficiency, to the lipids extracted from the mitochondrion. The effect of micellar phospholipid is not duplicated by mitochondrial extracts dispersed by homogenizing and cholate addition.

As shown in Fig. 1, the T.N. of soluble SD, which is about 4,000 for the untreated enzyme, passes to 6,000 and to 14,000 respectively, by adding increasing amounts of lipids.

The effect of lipids is not restricted to the soluble enzyme, but, as shown in Table I, is observed on the acetone powder as well. It is evident also in

préparations (e.g. first line in Table I) not treated with succinate at any stage before the assay.

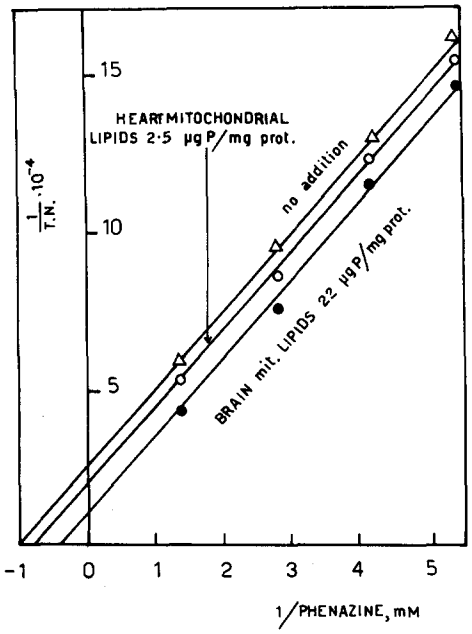

Fig. 1: Double reciprocal plot of PMS concentration versus T.N. of soluble SD with different additions of lipids. The enzyme was obtained from an acetone powder which had been prepared in the absence of succinate. Lipid "solubilized" according to Fleischer and Klouwen (1961) was equilibrated 1 hour at 0°C under N_2 with the enzyme preparation before the assay. Phosphate was determined by the method of Chen, Toribara and Warner (1956), protein by microkjehldahl.

TABLE 1

EFFECT OF LECITHIN ON SD ACTIVITY OF ACETONE-EXTRACTED MITOCHONDRIA

Addition to mitoch. suspension before acetone treatment	No lipids added ---	No lipids added +40 mM succ.	Lecithin added 40 µg P/mg prot.
none	22.0[a]	25.1	49.6
5 mM succ.	25.4[b]	48.4	58.1

Activity after treatment with alkali in the absence of succinate (15 min. at pH 9, 0°C); a: 10.6 b: 25.9.

Activities of acetone powders suspended in 0.06 M phosphate buffer pH 7,6 and equilibrated with or without lecithin before the assay, are given as µmoles succinate oxidized min/g protein, at fixed (0.3 mM) PMS concentration. Other conditions same as fig. 1.

Table 2 summarizes the effect of lipids at different stages of solubilization. When lipids are added, no effect is seen on the enzyme in the unextracted mitochondrion, while all other preparations, in which the T.N. was decreased, recover the original activity. The values for the acetone powder and the

TABLE 2

TURNOVER NUMBERS OF SD BEFORE AND AFTER SOLUBILIZATION

		Lipids added
Mitochondria	10,200	10,300[a]
Acetone powder	4,830	10,550[b]
" " prepared in 5 mM succ.	7,960	14,500[c]
Soluble enzyme	4,265	14,120[d]

a - 60 μg P/mg protein b - 1 μg P/mg protein
c - 22 μg P/mg protein d - 24 μg P/mg protein

T.N.s are calculated at infinite PMS concentration. For other experimental details, see fig. 1 and text.

soluble enzyme exceed, after addition of lipids, the T.N. value of the unextracted mitochondrion by more than the experimental fluctuation. This may indicate, though further investigation is required, that the enzyme disconnected from the context of the respiratory chain may display in the assay system used, a higher catalytic activity.

Data in the literature (Wang, Tsou and Wang, 1958; King, 1962) and the present results, suggest that in the presence of succinate the enzyme assumes a form which less easily undergoes modifications during the extractive procedures. Such modifications are very probably not to be identified with those connected with the activation process.

On the other hand phospholipids appear to have a specific effect in restoring full catalytic efficiency of the enzyme: they seem to be necessary not only for the activity of the succinoxidase system and of various fragments of the respiratory chain (Brierley, Merola and Fleischer, 1962; Hafkenscheid, Links and Slater, 1963) but for that of the succinic flavoprotein as well. It seems reasonable to suppose that as with the reactivation of β-hydroxybutyric dehydrogenase (Jurtschuck, Sekuzu and Green, 1963) by phospholipids, the effect

of phospholipid on SD occurs at the level of the enzymic molecule either by producing a favorable medium for the reaction or by modifying the protein.

The present results are relevant only to the phenazine-reductase activity of SD. Undoubtedly the study of other activities strictly related to the succinic flavoprotein molecule, such as, for instance, reconstitution experiments or fumarate reduction may yield further interesting data. It seems, however, that this first group of experiments opens some stimulating insight on the modification of the SD molecule during its disconnection from the respiratory assembly.

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REFERENCES

Bernath, P., Singer, T.P. (1962). In "Methods in Enzymology" (S.P. Colowick and N.O. Kaplan, eds.) vol. V, p. 597, Academic Press, New York.
Brierley, G.P., Merola, A.J., Fleischer, S. (1962). Biochim. Biophys. Acta, 64, 218.
Cerletti, P., Strom, R., Giordano, M.G., Balestrero, F., Giovenco, M.A. (1964). Biochem. Biophys. Res. Comm., 14, 408.
Chen, P.S., Toribara, T.Y., Warner, M. (1956). Anal. Chem. 28, 1756.
Fleischer, S., Klouwen, H. (1961). Biochem. Biophys. Res. Comm. 5, 378.
Fleischer, S., Klouwen, H., Brierley, G.P. (1961). J. Biol. Chem. 236, 2936.
Hafkenscheid, J.M., Links, J., Slater, E.C. (1963). Biochim. Biophys. Acta 64, 202.
Jurtschuck, P., jr., Sekuzu, I., Green, D.E. (1963). J. Biol. Chem. 238, 3595.
King, T.E. (1962). Biochim. Biophys. Acta, 59, 492.
Lester, R.L., Fleischer, S. (1961). Biochim. Biophys. Acta, 47, 558.
Singer, T.P. (1964). In "Comprehensive Biochemistry" (M. Florkin and E.M. Stotz eds.) Vol. 14, chapt. III, Elsevier Publ. Co, Amsterdam, in press.
Singer, T.P., Kearney, E.B. (1963). In "The Enzymes" 2nd ed. (P.D. Boyer, H. Lardy, K. Myrbäck, eds.) vol. 7, p. 416, Academic Press, New York.
Wang, T.Y., Tsou, C.L., Wang, Y.L. (1958). Scientia Sinica (Peking), 7, 65.