REVERSIBILITY OF THE ACTIVATION OF SUCCINATE DEHYDROGENASE*

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The activation of succinate dehydrogenase (SD), discovered in 1955 (Kearney et al., 1955; Kearney, 1957) is an intramolecular change in the SD molecule initiated by its combination with succinate or any substance capable of binding at the active center, and resulting in a greatly increased turnover number in subsequent assay. The activation process is relatively slow; it has a large activation energy, and it appears to be accompanied by characteristic spectral changes. These studies have been confirmed and extended by Thorn (1962). Although seldom considered in this context, the phenomenon is of great practical importance in activity measurements, particularly of initial rates at or below room temperature, and in EPR studies of SD, since without provision for preactivation only a minor fraction of the activity may be measured and since, as shown below, it appears quite likely that only those enzyme molecules which already contain bound substrate or competitive inhibitor possess catalytic activity.

In earlier studies (Kearney, 1957) dialysis was the only practical method for testing the reversibility of the activation. Prolonged contact with succinate inactivated SD; hence dialysis of the malonate activated enzyme was used to test the reversibility. Under these conditions neither the increased activity nor the spectral shift was reversed on dialysis. Hence the activation appeared to be irreversible. With the advent of rapid separation methods (e.g., Sephadex filtration) it has been possible to re-examine this point.

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As shown in Table I, the activation of both respiratory chain-bound and soluble, purified SD by succinate is extensively reversed by brief passage through Sephadex G-25 columns. With ETP, the fully activated enzyme is 99% deactivated by this treatment; with the

TABLE I

Reversible Activation of Succinate Dehydrogenase

Preparation	Sample	SD activity at 15°	
		Activated before assay	Not activated before assay
		μmoles/min./mg	μmoles/min./mg
Keilin-Hartree	Untreated	0.458	0.172
" "	Succinate activated	0.446	0.421
" "	Same after Sephadex	0.454	0.208
ETP	Untreated	0.941	0.052
"	Succinate activated	0.968	0.968
"	Same after Sephadex	1.01	0.010
"	Malonate activated	0.952	0.606
"	Same after Sephadex	1.00	0.548
Soluble enzyme (Bernath and Singer, 1962)	Untreated	5.16	1.89
	Succinate activated	4.66	4.66
"	Same after Sephadex	2.72	0.10
"	Malonate activated	5.12	5.12
"	Same after Sephadex	3.22	2.42
Soluble enzyme (Wang *et al.*, 1956)	Untreated	5.81	0.95
	Malonate activated	5.16	5.16
"	Same after Sephadex	5.79	1.95

All enzymes were derived from beef heart; ETP and Keilin-Hartree preparations as previously described (Ringler *et al.*, 1963). Spectrophotometric phenazine assays (Arrigoni and Singer, 1962). Each sample was assayed with and without preactivation. In the former case the complete reaction mixture including succinate but without dyes was incubated for 7 mins. at 38°, then cooled to 15°, and the reaction was started by adding DCPIP and phenazine methosulfate. In the latter case preincubation of the enzyme and succinate was omitted; the reactants were separately equilibrated at 15°, then mixed, and the reaction was started by adding the dyes. Activation of enzyme preparations (2nd column) was performed under N_2 for 10 to 15 mins. at 38° in the case of particles, 20 to 30 mins. at 25° with soluble SD, with 80 mM succinate or 2 to 10 mM malonate. Deactivation was performed by slow passage through Sephadex G-25 columns, usually at 0°. Soluble preparations suffered a slight loss of activity on prolonged incubation with succinate; the Bernath-Singer preparation is known to be partly inactivated on gel filtration (Bernath and Singer, 1962).

Bernath-Singer preparation 96% deactivation occurs. When malonate is used for activation, however, only moderate or slight deactivation occurs. The failure of malonate to dissociate sufficiently under the conditions of Sephadex treatment even when coupled with repeated washings in the case of particle preparations, is in accord with Kearney's previous results on the apparent irreversibility of malonate-activation on dialysis.

Similar reversible activation has been observed with all other preparations of heart muscle SD, including CoQ reductase (Ziegler and Doeg, 1962) and the Singer et al. preparation (1956). Contentions that certain SD preparations are activable and others are not are incorrect. The failure of Wang et al. (1958) to find activation of their preparation by phosphate, while confirming the activation by succinate, was due to the fact that these authors failed to take into account the different rates of activation by phosphate and succinate. At V_{max} with respect to activator, all activators yield the same degree of activation (Kearney, 1957). The report of Dervartanian and Veeger (1962) that the Wang et al. preparation is fully activated may have been due to their use of a crude sample before the succinate added in the extraction is removed; at later stages this preparation behaves identically with all other SD samples (Table I).

The type of reversal demonstrated here differs from the small degree of deactivation observed by Thorn (1962) on cooling of Keilin-Hartree preparations previously "activated" by warming in Tris-acetate buffer in the absence of added substrate or competitive inhibitors. Preparations activated with added succinate or malonate do not lose their activity on cooling but are rapidly deactivated by Sephadex passage without lowering the temperature. Possibly, in Thorn's experiments the "spontaneous" activation was due to the presence of metabolites still attached to the active center (cf. Table II and below), although the deactivation by cooling remains to be explained.

Spectral effects - The changes in the absorption spectrum of SD accompanying activation by malonate, observed by Kearney (1957, 1958), were not reversed on dialysis. In the present study it has been found that gel filtration of a malonate-activated preparation does not reverse the absorption changes accompanying activation, although extensive deactivation occurs under these conditions (Table I). On the basis of the observation that an SD preparation, presumably ac-

TABLE II

Deactivation of Succinate Dehydrogenase in ETP Preparations

Sample	SD Activity at 15° (μmoles/min./mg)		Ratio
	Activated before assay	Not activated before assay	Act./Unact.
Untreated beef heart ETP	0.763	0.057	13
Same after two washings	0.760	0.037	21
Same after Sephadex G-25	0.761	0.027	28

Assays and activation as in Table I. The ETP preparation was washed by resuspension in 0.05 M imidazole buffer, pH 7.6, and ultracentrifugation. The last column refers to the ratio of activities at 15° in samples with and without preactivation with succinate. Similar results were obtained with Keilin-Hartree preparations.

tivated by succinate, undergoes spectral shifts on the addition of competitive inhibitors similar to those previously reported for the unactivated enzyme, Dervartanian and Veeger (1962) have suggested that these absorption changes are not due to activation but to the formation of enzyme-inhibitor complexes of a charge-transfer type. Failure of the spectral changes elicited by malonate to be reversed under conditions of deactivation indeed suggests that the spectral shift attending activation cannot be entirely attributed to the intramolecular rearrangement which results in increased catalytic activity. Nevertheless, the two phenomena appear to be intimately associated as judged by the following observations. (1) Activation of an unactivated preparation by malonate and the increase in absorbancy in the 503 to 508 mμ region proceed at comparable rates at 20°. (2) Both processes have a very high temperature coefficient and hence neither is detectable at 2°. Possibly activation is a prerequisite for the formation of the complex which results in altered absorption spectrum.

Biological significance - Kearney (1958) has emphasized that the activation does not appear to be the removal of an inhibitor but an intramolecular rearrangement of SD resulting in increased catalytic activity. The following data agree with this hypothesis. Successive washing and gel filtration of respiratory chain prepara-

tions cause progressive deactivation, although brief incubation with succinate re-establishes the original catalytic activity (Table II). This suggests that an activator of metabolic origin is attached to the enzyme and may be gradually dissociated on successive centrifugations. That this activator is more apt to be a tightly bound competitive inhibitor than succinate is suggested by the more extensive deactivation of succinate-activated ETP (Table I) than of untreated ETP (Table II) on Sephadex passage. It is quite possible that on complete removal of the bound activator SD would be completely inactive in catalytic tests performed without prior activation. Considering the rapidity of deactivation even at low temperatures and the much slower rate of activation, a process which shows a high activation energy, one may wonder whether the level of succinate and other metabolites capable of activating SD might not serve a regulatory function in controlling the rate of succinate oxidation in intact mitochondria.

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REFERENCES

1. Arrigoni, O., and Singer, T. P., Nature, 193, 1256 (1962).
2. Bernath, P., and Singer, T. P., in "Methods in Enzymology", Colowick, S. P., and Kaplan, N. O., eds., Vol. V, 597, Academic Press, New York, 1962.
3. Dervartanian, D. V., and Veeger, C., Biochem. J., 84, 65P (1962).
4. Kearney, E. B., J. Biol. Chem., 229, 363 (1957).
5. Kearney, E. B., in "Proceedings of the International Symposium on Enzyme Chemistry, Tokyo-Kyoto, 1957", p. 340, Maruzen, Tokyo, 1958.
6. Kearney, E. B., Singer, T. P., and Zastrow, N., Arch. Biochem. Biophys., 55, 579 (1955).
7. Ringler, R. L., Minakami, S., and Singer, T. P., J. Biol. Chem., 238, 801 (1963).
8. Singer, T. P., Kearney, E. B., and Bernath, P., J. Biol. Chem., 223, 599 (1956).
9. Thorn, M. B., Biochem. J., 85, 116 (1962).
10. Wang, T. Y., Tsou, C. L., and Wang, Y. L., Scientia Sinica, 5, 73 (1956).
11. Wang, T. Y., Tsou, C. L., and Wang, Y. L., Scientia Sinica, 7, 65 (1958).
12. Ziegler, D. M., and Doeg, K. A., Arch. Biochem. Biophys., 97, 41 (1962).