TRANSFER OF H FROM SUCCINATE TO DPN CATALYZED BY THE SLATER-KEILIN-HARTREE PREPARATION

Oscar Gawron, Andrew J. Glaid, III, Sidney Nobel and Minerva Gan

Department of Chemistry, Duquesne University
Pittsburgh, Pennsylvania 15219

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Studies on the mechanism of the succinic dehydrogenase catalyzed reaction demonstrating stereospecificity (1, 2) and trans-stereoselectivity (2, 3), exchange of one hydrogen between substrate and water (4, 5) and transfer of H from succinate to fumarate (6) have led to the concepts (4, 7) that succinate oxidation occurs via removal of a hydride ion and this hydride ion may be transferred to DPN^+ in the respiratory chain. The hypothesis of H transfer from succinate to DPN^+, also implicit in the reduction of mitochondrial DPN^+ by succinate plus ATP (8, 9), and the reduction of acetoacetate (10, 11) and of α-ketoglutarate plus ammonia (12) by succinate and respiring mitochondria, has now been tested in a preliminary fashion and found to occur when succinate-H^3 and DPN^+ are incubated with the Slater-Keilin-Hartree (13, 14) preparation from horse heart (2). The Slater-Keilin-Hartree preparation in addition to its known succinic oxidase and DPNH oxidase activities is able to utilize ATP for the anaerobic reduction of DPN^+ by succinate as well as fumarate and chlorofumarate for the anaerobic oxidation of DPNH (15). Fumarate plus DPN^+ was not oxidized by the preparation (15).

From Table I it is seen that aerobic incubation of succinate H^3 with DPN^+ in the presence of the Slater-Keilin-Hartree preparation results in

Table I

Transfer[a] of H^3 from Succinate-H^3 to DPN^+

mins.	oxygen uptake μatoms	Nicotinamide-H^3 cpm/μmole[c]	H_2O-H^3 cpm/μmole[d]
0[b]	19.4	nil	(49)
15	7.8	1.9×10^5	---
30	9.6	3.2×10^5	53
60	11.3	3.2×10^5	70
120	12.9	3.4×10^5	73

a. Reactions run at 32° in Warburg vessels with 0.2 ml. 2N potassium hydroxide in central well. Reaction mixtures consisted of 60 μmoles phosphate, pH 7.3, 225 μmoles sucrose, 9 μmoles tris chloride, pH 7.0, 12 μmoles magnesium chloride, 30 μmoles succinate-H^3 (41 x 10^5 cpm per μmole), 0.30 ml. of Slater-Keilin-Hartree preparation, (40 mg. protein per ml.; 0.029 μmoles DPNH per min. per mg. protein oxidized at 28°) and 3 μmoles of DPN^+ in a total volume of 3.0 ml., DPN^+ being tipped in from the side arm at zero time.

b. Incubated for 140 mins. and then placed in boiling water bath for 2 mins. prior to addition of DPN^+. A blank was also run with enzyme previously inactivated by heating at 100° for 2 minutes. No oxygen uptake was noted over 30 mins. and incorporation of H^3 into nicotinamide was nil.

c. Isolated by the method of Krakow, Udaka and Vennesland, Biochem., 1, 254 (1962), after the addition of 50 mg. of nicotinamide. Isolated material diluted 3 x with nicotinamide prior to lyophilization from 2 ml. of water and recrystallization (3x) from benzene. Counted in a system similar to that used by Bray, Anal. Biochem., 1, 279 (1960) and calculated on the basis of 3 μmoles DPN^+ present in the reaction mixture.

d. The reaction mixture, after extraction of nicotinamide with ether, was distilled to yield a sample for counting. The zero time value is anomalous.

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the transfer of H^3 to DPN^+. This transfer of H^3 from succinate-H^3 to DPN^+ cannot proceed via water since at best 369 cpm per μmole can be incorporated from succinate into water ($30 \times 41 \times 10^5 \div 2 \times 3000/18$) while over 30 to 120 mins. some 3.2×10^5 cpm per μmole have been incorporated into the DPN^+ present in the initial reaction mixture. On the basis that only one of the four hydrogens of succinate-H^3 may be transferred to DPN^+, and disregarding rate effects, some 10×10^5 cpm per μmole DPN^+ would be

expected for complete transfer. Accordingly, it would seem some 32% transfer has been effected.

From Table II it is to be noted that in an one-hour aerobic period transfer varies, albeit not to a great extent, with the amount of Slater-Keilin-Hartree preparation employed. Again, some 26% to 35% transfer was effected.

Table II

Effect of Amount of Preparation on Transfer[a] of H^3 from Succinate-H^3 to DPN^+

ml. Preparation[b]	μatoms oxygen uptake	cpm per μmole Nicotinamide[c]
0.1	11.8	2.3×10^5
0.3	20.0	2.7×10^5
0.6	28.8	3.1×10^5

a. Experimental conditions employed are the same as those of Table I, total volumes being kept constant by varying the phosphate concentration. The succinate-H^3 used had 3.6×10^5 cpm per μmole. Reactions were run for one hour.

b. Forty-eight mg. protein per ml., 0.037 μmoles DPNH per min. per mg. protein oxidized at 28°.

c. Recrystallized two times.

Table III presents results of transfer experiments carried out under anaerobic conditions. It is to be noted that transfer is considerably diminished, 1.02×10^5 cpm per μmole at 30 min. and 1.16×10^5 cpm per μmole at 60 min. being incorporated into DPN^+ as compared to (Table I) 3.2×10^5 cpm per μmole at 30 min. and 60 min. under aerobic conditions.

Table III

Transfer[a] of H^3 from Succinate - H^3 to DPN^+ under Anaerobic Conditions

min.	Nicotinamide cpm per μmole
0	0.04×10^5 [b]
30	1.02×10^5 [c]
60	1.16×10^5 [c]

a. Reaction mixtures similar to those of Table I were made up in Thunberg tubes with DPN^+ in the side arm. After evacuation for 30 mins. at the water aspirator, tube contents were mixed. The starting succinate showed 41×10^5 cpm per umole. Preparation used contained 65 mg. protein per ml. and at 28° oxidized 0.031 μmoles DPNH per min. per mg. protein.
b. Recrystallized three times.
c. Recrystallized four times.

Seemingly, although this point has not as yet been studied, ATP should augment this anaerobic transfer.

It is clear from the above results that, at least, a portion of the H of succinate goes through DPN^+ on oxidation. It would also seem that this fraction of H from succinate does not go through a reduced form which readily exchanges with the protons of water prior to reaching DPN^+ and, seemingly, ubiquinone is, for this fraction, thereby not to be considered as intermediate between succinic dehydrogenase and DPNH dehydrogenase. The question of a pathway for H of succinate other than through DPN^+ might be answered by finding experimental conditions under which all or none of the H from succinate is found in DPN^+. It is of interest, in this connection, to note that DPNH dehydrogenase, if not DPN^+, has been considered (16) to

be intermediate between succinate and oxygen and it is also of interest to note that the observed (17) inhibition of the rate of succinate oxidation by DPN^+ might be due to intervention of DPN^+ between succinate and oxygen.

It is assumed, of course, albeit not as yet demonstrated that H is transferred from succinate to one side of the pyridine ring of DPN^+ at carbon 4 and subsequently hydrogen from the other side of the ring at carbon 4 is removed by DPNH dehydrogenase. Of further interest, of course, is experimental description of the stereochemistry of the DPNH dehydrogenase system and the possibility of a cyclic transfer of H from succinate to DPN^+ (perhaps mediated by transfer of H from DPNH to DPN^+ (18)) since transfer occurs (Table III) under presumed anaerobic conditions. It is also clear that effects of inhibitors, amytal, for example, and uncouplers on the transfer are of interest as well as the possibility of tracing, via H^3 labelling, of intermediates arising from succinate and DPN^+ during the reduction.

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