PHOSPHORYLATION COUPLED TO ELECTRON TRANSPORT INITIATED BY SUBSTITUTED PHENYLENEDIAMINES

Earl E. Jacobs[1]

Department of Biochemistry, Dartmouth Medical School, Hanover, N. H.

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A characteristic lability of phosphorylation coupled to electron transport initiated by high potential electron donors has been described by Jacobs and Sanadi (1960) and Imamoto (1959). The former authors found that rat liver mitochondria were unable to sustain phosphorylation coupled to the antimycin-insensitive electron transport initiated by silicomolybdate, ferrocyanide or cytochrome c unless the reaction medium was supplemented with certain auxiliary ions which were not required to obtain maximal P/O ratios for succinate oxidation. Imamoto noted that mitochondria exposed to low concentrations of cholate completely lost their potential capacity to couple phosphorylation to ferrocytochrome c oxidation even though they still gave maximal P/O ratios with succinate as substrate. Jacobs and Sanadi postulated that in order to initiate electron transport at the potential level of endogenous cytochrome c electron donors must permeate some critical structure of the mitochondrion which normally maintains the terminal coupled phosphorylation but which is subject to disruption by the electron donors while in transit unless stabilized by the auxiliary ions. It is conceivable that this situation might obtain if the elements comprising the structure are maintained in stable equilibrium by electrostatic interaction forces between their charged groups. Since the high potential electron donors used previously are all polyvale

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ions, their consequent interaction with these charged groups could upset a delicate balance of forces which characterizes the condition of structural stability. Accordingly it was reasoned that non-ionic high potential electron donors should be inherently capable of initiating coupled electron transport in a medium lacking auxiliary ions. Experiments similar to those previously described by Jacobs and Sanadi were therefore carried out with a series of methyl-substituted phenylenediamines, these compounds being almost electrically neutral and having appropriate oxidation-reduction potentials. For example the E_o' of N,N,N',N'-tetramethylphenylenediamine is about +0.26 and the pK of its amine groups about 6.3 (Michaelis and Hall, 1933). At pH 7.0 about 80% of the reduced (diamine) molecules will therefore be electrically neutral while all of the oxidized (diimine) molecules will carry a net charge of +2.

Keilin and Hartree (1938, 1940), Belitzer and Tsibakowa (1939), Borei and Renvall (1949) and Borei and Bjorklund (1953) had previously observed that various phenylenediamines could be directly oxidized by the cytochrome chain. Borei and Bjorklund pointed out, however, that some oxidation products were eventually toxic to the reaction. Maley and Lardy (1954) and Lehninger et al. (1954) did not obtain any phosphorylation coupled to electron transport initiated by external cytochrome c which was maintained reduced with phenylenediamine and suggested that the oxidation products of the latter acted as uncoupling agents. Nevertheless, it seemed entirely possible that catalytic levels of substituted phenylenediamines which were maintained reduced with ascorbate might be able to initiate coupled electron transport where the higher concentrations proved toxic. Representative results obtained with N,N,N',N'-tetramethyl p-phenylenediamine (TMPD) are summarized in Table I.

The data of Table I clearly show that catalytic levels of TMPD can very effectively initiate antimycin-insensitive electron transport to which phosphorylation is coupled with 100% of theoretical efficiency in the presence of auxiliary ions (7.5×10^{-3} M Mg^{++}) and about 50% in their absence. Similar results were obtained with the dimethyl derivative except that relatively higher concentrations were required to saturate electron transport. The N-methyl and unsubsti-

Table I

Coupled Electron Transport Initiated by TMPD

pH	Mg^{++} (mM)	TMPD (mM)	Oxygen (μA 15')	P/O
7.0	1.5	None	0.0	-
"	7.5	None	0.5	-
"	1.5	0.3	17.5	0.49
"	7.5	0.3	16.5	0.96
"	1.5	0.3*	17.9	0.53
"	7.5	0.3*	17.0	0.99
6.5	1.5	0.3	12.6	0.52
"	7.5	0.3	10.9	1.05
7.5	1.5	0.3	17.1	0.73
"	7.5	0.3	16.0	1.03
7.0	1.5	Succinate Control	16.9	1.60
"	7.5	"	15.9	1.73

Complete reaction mixture contained potassium ascorbate (1.5×10^{-2} M), potassium phosphate (1.1×10^{-2} M), ATP (1.5×10^{-3} M), glucose (6×10^{-2} M), sucrose (0.25 M hexokinase (0.25 mg protein), rat liver mitochondria (8 mg protein) and additions of $MgCl_2$ and TMPD as indicated in Table. Reaction mixture adjusted to indicated pH with all components except mitochondria present. TMPD originally added to reaction mixture as unneutralized dihydrochloride.

* plus 1γ antimycin A.

tuted compounds were essentially inactive in this reaction system. The greater relative effectiveness with which the tetramethyl derivative initiates electron transport is probably a combined result of its higher lipid solubility and lower potential.

Considerable importance can be attached to the finding that P/O ratios for electron transport initiated by substituted phenylenediamines in the absence of auxiliary ions are as high as 0.5 since P/O ratios obtained with the more strongly charged donors, cytochrome c, silicomolybdate, and ferrocyanide were always

vanishingly small under such reaction conditions (Jacobs and Sanadi, 1960). The data of Table I may therefore be considered as evidence in support of the aforementioned hypothesis that some structure of the mitochondrion which normally maintains coupled phosphorylation in the antimycin-insensitive segment of the electron transport chain is poised by electrostatic interaction forces.

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