COMMUNICATIONS

Thiol Release during Adenosine Triphosphate Synthesis by Oxidative Phosphorylation

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The possible role of thiols in the coupling mechanism of oxidative phosphorylation has been investigated.

Two different methods were used to estimate free thiol groups in heart mitochondria, using *p*-chloromercuribenzoate (Boyer, 1954) or 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman, 1959). ATP synthesis was evaluated by the firefly luminescence method (Strehler & Totter, 1952) and the respiratory control ratio, in States 4 and 3 (Chance & Williams, 1956), by polarography.

In well-coupled mitochondria (State 4) the concentration of free thiol groups amounts to 43 nmol/mg of protein. Addition of ADP + P_i, which initiates State 3 of oxidation and ATP synthesis, provokes an increase in the concentration of free thiol groups (30% of State 4 values) that remains constant during ATP synthesis. If ADP or P_i is added alone there is no increase in the concentration of free thiol groups.

Uncoupling agents (e.g. carbonyl cyanide *m*-chlorophenylhydrazone) prevent the increase in the concentration of free thiol groups on the addition of ADP; moreover, the concentration of free thiol groups in mitochondria is 30% lower than in State 4, and the respiratory chain is stimulated.

The addition of *N*-ethylmaleimide to tightly coupled mitochondria blocks the free thiol groups measured in State 4 and strongly inhibits respiration in the presence of glutamate. On further addition of ADP the increase in the concentration of free thiol groups and stimulation of respiration (State 3) are totally suppressed as well as ATP synthesis.

Oligomycin does not affect the concentration of free thiol groups in State 4, which agrees with the fact that the respiration is not modified; but it completely suppresses the increase in the concentration of free thiol groups on the addition of ADP + P_i, the respiratory-chain stimulation (State 3) and ATP synthesis linked to respiratory-chain oxidations.

Hydroxylamine does not affect the concentration of free thiol groups in the absence of ADP (State 4), but suppresses the increase due to ADP and inhibits respiratory-chain stimulation and ATP synthesis only partially.

These results prove the direct participation of free thiol groups in the coupling mechanism of oxidative

phosphorylation. The increase in the concentration of free thiol groups on the addition of ADP + P_i cannot be related to changes in the redox state of the respiratory chain or the phosphate entry.

The effects of the inhibitors suggest the formation of a thiol ester precursor of ATP (probably not an acyl thiol) between the A~X (Slater, 1953) step and the discharge of the energy-rich bond on ADP. It could be of the -S~PO₃H₂ type. This conclusion should be compared with those concerning factor B (Sanadi, Lam & Rama-Kurup, 1968; Lam, 1968).

The results do not support directly Lambeth's & Lardy's (1969) model system.

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Characteristics of the Inhibition by Oligomycin of Reactions Involved in Oxidative Phosphorylation

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The inhibition by oligomycin of succinate oxidation, the 2,4-dinitrophenol-induced adenosine triphosphatase and the P_i-ATP exchange reaction in rat liver mitochondria has been studied.

When the rate of succinate oxidation at a given time-interval after the addition of oligomycin is plotted as a function of the amount of oligomycin, a sigmoidal curve is obtained (Lardy, Connelly & Johnson, 1964; Ter Welle, 1968). The inhibition by oligomycin is time-dependent, and the sigmoidicity of the curve increases as the time-interval between addition of oligomycin and measurement of respiration rate is increased. This time-dependence is observed even after prolonged preincubation with oligomycin. High P_i concentrations accelerate the onset of maximal inhibition. Maximal inhibition (85-90%) of State 3 respiration is obtained at 0.10-0.13 µg of oligomycin/mg of protein. With limiting