Inorganic Pyrophosphate and the Evolution of Biological Energy Transformation

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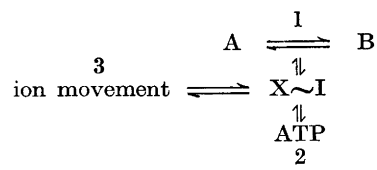
Inorganic pyrophosphate (PP_i) may, according to rather recent evidence presented by Miller and Parris [1] and by Lipmann,[2] be regarded as perhaps the most likely primary chemical energy donor in prebiological systems and/or very early forms of living organisms. Formation of this energy-rich compound from inorganic orthophosphate (P_i) has earlier been obtained in various chemical model systems by coupling to redox reactions, at the expense of potential energy liberated during the transfer of reducing equivalents from reductant to oxidant.[3-5] Already in 1963 Calvin [4] suggested that the formation of PP_i from P_i in such model systems may well have its energy-conserving biological counterpart only remaining to be discovered.

The above propositions may now be strongly considered in the light of recent experimental evidence. The demonstration by H. Baltscheffsky *et al.*[6] that PP_i is formed during illumination of chromatophores from the photosynthetic bacterium *Rhodospirillum rubrum* showed that there exists a biological energy conservation process leading to formation of PP_i in an electron transport coupled phosphorylation system. This phosphorylation of P_i to PP_i occurs at the pre-adenosine phosphate level, *i.e.* the energy transfer reactions involved in light-induced formation of PP_i from P_i are situated on the electron transport side of the final (phosphorylation) reactions involved in light-induced formation of ATP from P_i and ADP, as was shown by H. Baltscheffsky and von Stedingk [7] in experiments with the inhibitor oligomycin. More recently, M. Baltscheffsky *et al.*[8] reported that PP_i can function in chromatophores as donor of energy for an energy-requiring reaction of endogenous cytochrome. This capacity of PP_i to function as a donor of biologically useful chemical energy was confirmed in independent experiments of Keister,[9] also working with chromatophores but following another energy-requiring reaction, the transhydrogenase reaction first described by Danielson and Ernster.[10] As has now been shown by M. Baltscheffsky,[11] PP_i may act as energy donor not only in such primitive photosynthetic systems as bacterial chromatophores but also in respiring mitochondria from both lower and higher organisms. In experiments with oligomycin she was able to demonstrate that, in agreement with the formation of PP_i, also the utilization of PP_i as energy donor occurs at the pre-adenosine phosphate level, this being valid both in bacterial chromatophores [8,12] and in animal mitochondria.[11] The possible implications of this close proximity between electron transport and energy transfer to and from PP_i for any current concepts of the evolution of biological energy transformation appear to be rather far-reaching and will be considered in this presentation.

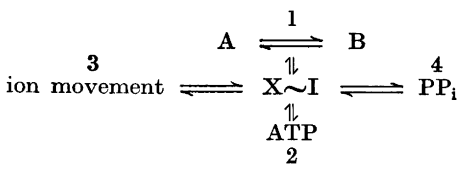
It has been known for some time that subcellular structures involved in energy transfer of photophosphorylation or oxidative phosphorylation contain the enzymic apparatus necessary for performing reactions of electron transport, energy and phosphate transfer, and ion movement according to the following scheme (minimum scheme, chemical hypothesis with A and B representing electron carriers and X~I representing the hypothetical non-phosphorylated intermediate):



Scheme 1

In the given scheme, the reaction pathway between any two of the three component parts 1, 2, and 3 may, theoretically, have evolved first.

The experimental results from this laboratory showing formation of PP_i and its utilization as energy donor, both the reactions occurring at the pre-adenosine phosphate level of biological electron transport coupled energy transfer systems, lead to the following extension of the above scheme:



Scheme 2

Scheme 2 shows that, in principal agreement with the argument presented in connection with Scheme 1, PP_i has emerged as a fourth component part to be included in any consideration of the bioevolution of energy transformation in the systems under discussion. The simplicity of the structure of PP_i as compared to that of ATP, as well as the possible occurrence of PP_i containing minerals on the primitive Earth,[1] when taken together with the recently demonstrated metabolic position of PP_i, as shown in Scheme 2, would seem to us to provide sufficient background for a strong focussing of attention on the possibility that PP_i was an early participant in the sequences of energy transforming reactions evolving with time to the metabolically advanced level given in Scheme 2. Thus, for example, very early reactions of energy and phosphate metabolism may well have been those between PP_i and electron transport or between PP_i and ion movement.

In conclusion, while it would appear to be an open question, whether the main direction of early energy transfer between an energy-rich phosphate compound and, for example, electron transport was from electron transport to energy-rich phosphate or the reverse, it seems quite likely that the demonstrated electron transport coupled formation and utilization of PP_i in subcellular energy transfer systems may constitute experimental background for insight into early metabolic processes of major energetic significance for primitive forms of life, *i.e.* that PP_i indeed preceded ATP during the evolution of biological energy transformation.

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Thin-layer Chromatography of Bile Acids on Lipophilic Sephadex

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In a previous paper the preparation of a methylated Sephadex derivative was described.[1] As thin-layer gel filtration is now an established technique,[2,3] the possibility of using methylated Sephadex in thin-layer chromatography (TLC) of lipids has been briefly investigated in this laboratory. Because of the volatility of most organic solvents, a closed developing chamber had to be used, in which the thin-layer plate also was kept during the sample application.

Experimental. Thin-layer plates are prepared on glass-plates, 5 × 20 cm, in the following way: 5 g of Sephadex G-25 superfine (Pharmacia, Uppsala, Sweden), methylated as described previously,[1] is mixed with 25 ml of chloroform or dioxane. The slurry is poured on the glass plate and spread into a thin layer with a glass rod. A proper thickness of the layer is obtained by having two stainless steel wires, 0.5 mm diameter, placed across the plate, parallel to and near the two long sides. The glass rod is pressed against the wires while the slurry is spread with a single movement. The plate is then allowed to dry for a few seconds until the gel surface seems dry but still swollen. The stainless steel wires are removed and the plate