PROTEINOID MICROSPHERES AND THE PROCESS OF PREBIOLOGICAL PHOTOPHOSPHORYLATION

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Abstract. A chemical model of prebiological photophosphorylation with participation of hemoproteinoid microspheres, mixed microspheres containing bonded riboflavin and microspheres obtained from glycine rich proteinoids was studied. The illumination of aqueous solutions containing microspheres, \( K_2HPO_4 \), ADP and electron acceptor leads to an increase of ATP concentration and to a decrease of concentration of inorganic phosphate. Initial photochemical reactions with participations of proteinoid microspheres could have evolved in the course of chemical evolution and led to the emergence of the photophosphorylation in its modern biochemical form.

1. Introduction

According to hypothesis of A. A. Krasnovsky, during the period of chemical (prebiological) evolution an important role was played by process of photochemical activation of substrates (Krasnovsky, 1974). Porphyrins and their complexes with proteins have been considered as primary photosensitizers because these compounds are sensitizers in some model reactions of photooxidation and photoreduction (Weber, 1970; Kolesnikov et al., 1979, 1981, 1984; Fox et al., 1978). Proteinoids containing bonded metallocorphyrins (hemoproteinoids) are suitable model compounds for studying of probable ways of prebiological evolution.

The purpose of our work was to study participation of hemoproteinoids, hemoproteinoid microspheres and mixed microspheres containing bonded riboflavin in the reaction of photophosphorylation of ADP to ATP. As shown earlier, illumination of solutions containing proteinoid, hemin dimethylester, AMP and inorganic phosphate (in dimethylacetamide) led to phosphorylation of AMP to ADP (Weber, 1970). Fox and his collaborators have also observed the photosynthetic formation of ADP and ATP in suspensions of proteinoid microspheres (Fox et al., 1978, 1980; Bahn and Fox, 1981), however satisfactory results were obtained only in organic solvents. In the context of chemical evolution on the Earth studies of photophosphorylation reaction in the water medium and at physiological-meaning values of pH are more relevant than these studies in organic solvents.

2. Methods

The following model system have been studied in our experiments:

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P_i + \text{ADP} + A + \text{proteinoids or microspheres} \xrightarrow{\text{light}} \text{ATP},
\]

A - electron acceptors:
  parabenzquinone or riboflavin

$P_i$ - inorganic phosphate ($K_2HPO_4$)

$ADP$ - disodium salt of adenosin-5'-diphosphoric acid, Reanal Co

To obtain the proteinoids dry mixtures of L-amino acids (Reanal Co) containing 1-3% hemin were heated at sealed evacuated ampules for 4-6 hr at 185-190 °C. Three initial mixtures of amino acids were used in our experiments

1) 4:4:1:3 (glutamic acid, aspartic acid, lysine and mixture of 9 amino acids in equimolar proportions: histidine, cysteine, glycine, alanine, proline, valine, leucine, isoleucine and phenylalanine).

2) 12 amino acids in equimolar proportions: (lysine, alanine, glycine, valine, proline, leucine, isoleucine, histidine, cysteine, phenylalanine, aspartic and glutamic acids)

3) 4:1:1 (lysine, histidine and 10 amino acids in equimolar proportions: cysteine, glycine, alanine, proline, valine, leucine, isoleucine, phenylalanine, glutamic and aspartic acids).

Histidine (or glycine) rich proteinoids were prepared from initial mixtures, containing 25% of these amino acids and 75% of 4:4:1:3 mixtures.

Reaction products were purified by acetonitrile (or methylene chloride) extraction and by subsequent gel filtration on Sephadexes G-25 and G-50 and in model experiment only the material with a molecular weight of over 5000 was used (Kolesnikov et al., 1979, 1984). To hydrolyze of imide linkages resultant proteinoids were treated with NH$_4$OH solution and evaporated (Fox and Nakashima, 1967).

Microspheres were obtained by the well-known method of Fox (Hsu and Fox, 1976) with small modification. Boiling solutions of hemoproteinoids (in water), containing a few drops of NH$_4$OH, cooled slowly to room temperature. Suspensions of microparticles were dialyzed against water (Kolesnikov and Maksudova, 1990).

Mixed microspheres were prepared by combination of hot solutions of two proteinoids (Hsu and Fox, 1976).

To obtain microspheres containing bonded riboflavin 100 ml of boiling solution of proteinoid was cooled to 80 °C and then 1.0 ml of warm riboflavin solution (80°C, 5×10^{-4} M) was added to this medium and the mixture was allowed to cool slowly to the room temperature. Microparticles obtained were then separated from solution by means of a glass filter (N4) and were washed by water.

Inorganic phosphate in solutions and iron content in hemoproteinoids were measured colorimetrically as described in the our preceding papers (Kolesnikov et al., 1979, 1984). Riboflavin content was estimated by fluorescence analysis.

To obtain the purified preparation of ADP it was twice passed through the DOWEX-1 column, after that the preparation contained only 0.15 - 0.5% ATP as a cotaminant. The experimental solutions were illuminated with incandescence lamp (500 W) through a two-lensed condenser and infrared filter (5% CuSO$_4$).