A STUDY OF PHOTOPHOSPHORYLATION WITH OXYGEN-18

Mordhay Avron and Nathan Sharon

Section of Biochemistry and Department of Biophysics
Weizmann Institute of Science, Rehovoth, Israel

Received May 4, 1960

Recent studies have shown differences between the mechanism of adenosine triphosphate (ATP) formation in photophosphorylation by chloroplasts, and that occurring during oxidative phosphorylation (Avron and Jagendorf, 1959; Avron, 1960). It was found that the chloroplasts had no ATPase activity, and did not catalyse the exchange between inorganic phosphate and ATP, or between ADP and ATP.

In order to throw further light on this apparently irreversible formation of ATP during photophosphorylation, the following experiments were conducted, utilizing oxygen-18 labeled inorganic phosphate or water. The experiments were performed with swiss-chard chloroplasts (Avron, 1960). Phosphate labeled with oxygen-18 was prepared by equilibration with H_2O^{18} (Cohn and Drysdale, 1955).

The results are given in Table 1. The first two lines of the table show the results of reactions in which the water was initially labeled with 1.31 atom per cent excess of O^{18}. It is clear that only a very small proportion of the O^{18} in the water was incorporated into the inorganic phosphate of the medium. That amount was equivalent to 0.05 of one of the four atoms of inorganic phosphate becoming labeled. Although small, this incorporation was not within the experimental error. It is of interest that in an experiment not recorded here, approximately the same degree of incorporation was found in the dark.

The incorporation of the oxygen of water into ATP, on the other hand, was quite substantial. It was equivalent to approximately one of the four oxygen atoms of the terminal phosphate of ATP being completely replaced by an atom of oxygen from water. This incorporation from H_2O into ATP was completely absent when an identical reaction mixture containing ADP and ATP was incubated in the dark.

TABLE 1

Atom per cent excess O^{18}				Number of O^{18} atoms gained (+) or lost (-)	
Initial		Final			
H_2O	Pi	Pi	$\beta + \gamma$ -PO_4 of ATP	Pi	γ -PO_4 of ATP
1.31	0.00	0.017	0.162	+0.05	+1.0
1.31	0.00	0.018	0.190	+0.05	+1.1
0.00	6.92	6.72	1.65	-0.10	-2.1
0.00	6.92	6.86	1.74	-0.04	-2.0

Procedure: Each reaction mixture contained the following in micromoles: Tris, pH 7.8 - 300; NaCl - 400; $MgCl_2$ - 80; phosphate, pH 7.8 - 150 (containing 1.7 x 10^6 c.p.m. P^{32}); ADP, pH 7.8 - 50, phenazine methosulfate - 0.66; once washed chloroplasts containing 205 micrograms of chlorophyll, and water to a total volume of 20.0 ml. The reaction was carried out in the light (40,000 lux), at 15°C for 90 minutes (Avron, 1960) and was terminated by the addition of $HClO_4$ to a final concentration of 3%. After centrifugation, a sample was removed for ATP^{32} analysis, and acid washed charcoal was added twice to adsorb the ATP. The charcoal was washed four times and placed in 1 N HCl at 100°C for 10 minutes. The inorganic phosphate liberated (= $\beta + \gamma$ -PO_4 of ATP) and that of the medium were precipitated as $MgNH_4PO_4$ (Umbreit et al., 1957), washed, and dissolved in 0.1 N $HClO_4$. They were now precipitated twice as the silver salt at pH 6.5, dried in vacuo over P_2O_5, and analyzed for their O^{18} content (Anbar and Guttmann, 1959).

Assumptions made in calculation: The conversion of ADP to ATP was in all cases higher than 95%. It was, therefore, assumed that the phosphate obtained by the acid hydrolysis of the charcoal was composed of the $\beta + \gamma$ phosphates of the ATP formed, uncontaminated by the terminal phosphate of left-over ADP. For calculation of the loss or gain into the γ -PO_4 of ATP, the value of the atom per cent excess of the inorganic phosphate was taken as equal to the average between the initial and final values.

The two lower lines of the table illustrate the results obtained when the inorganic phosphate was initially labeled with O^{18}. It can be seen that at the end of the reaction period, the inorganic phosphate of the medium was found to contain almost the same percentage of O^{18} which it had initially. The small loss in its O^{18} content was equivalent to only 0.07 of one of the four atoms of oxygen being replaced by oxygen from water. This is in good agreement with the previously mentioned incorporation of 0.05 atoms of oxygen from H_2O^{18} into the inorganic phosphate of the medium. It can, therefore, be considered as indicating a small, but numerically significant, exchange reaction between inorganic phosphate and water.

In the same reaction mixture, the terminal phosphate of ATP was found to contain half of the O^{18} content of the medium inorganic phosphate. This is equivalent to two of its four atoms of oxygen being replaced by oxygen atoms from other sources. Since the first experiments demonstrated that only one atom of oxygen entered into ATP from water, it would seem most reasonable to conclude that during the esterification of ADP by inorganic phosphate, it is the phosphate, and not the ADP, which loses one of its oxygen atoms to form water. No O^{18} could be found in ADP and ATP incubated with O^{18} labeled inorganic phosphate under the same conditions in the dark.

In conclusion, three types of reaction were demonstrated:

a. An exchange reaction between water and inorganic phosphate. This exchange was extremely small, non-light dependent, and may be unrelated to the photophosphorylation reaction proper. It could for example, be a manifestation of the action of pyrophosphatase (Cohn, 1958), which was recently shown to be a component of chloroplasts (Bove et al., 1959).

b. A light-dependent incorporation of oxygen from water into ATP, equivalent to one atom per ATP molecule formed. This incorporation may reflect a total equilibration with water of one atom of oxygen of an intermediate or of the final product, in the path of ATP synthesis. It could, however, reflect an exchange which involves more than one of the oxygen atoms of the phosphate.

c. A light-dependent loss of two atoms of oxygen from inorganic phosphate during its conversion to ATP. On the assumption that one of the two is that which is exchanged with water (reaction b), it would seem simplest to conclude that the bond oxygen between the β and γ phosphates of the ATP formed is that which was initially on the ADP molecule.

We wish to thank the Department of Isotopes for supplying the H_2O^{18}, and for performing the mass-spectrometric analyses. We are indebted to Dr. B. Silver for his helpful suggestions.

References:

Anbar, M., and Guttmann,S., J. Appl. Rad. Isotopes, 5, 233 (1959).

Avron, M., Biochim. Biophys. Acta, in press, 1960.

Avron, M. and Jagendorf, A. T., J. Biol. Chem., 234, 967 (1959).

Bove, J., and Raacke, I. D., Arch. Biochem. Biophys., 85, 521 (1959).

Cohn, M., J. Biol. Chem., 230, 369 (1958).

Cohn, M. and Drysdale, G. R., J. Biol. Chem., 216, 831 (1955).

Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques, p. 276. Burgess Publishing Co., Minneapolis 15, Minn., 1957.