FUNCTION OF THE TWO CYTOCHROME COMPONENTS IN CHLOROPLASTS: A WORKING HYPOTHESIS

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THE conversion of light into a form of chemically available energy by chloroplasts *in vitro* can be observed in two ways: by the reduction of a hydrogen acceptor with the production of a stoichiometric equivalent of molecular oxygen[1] or by the formation of adenosine triphosphate from inorganic phosphate in the presence of adenosine diphosphate[2].

In the green plant, coenzyme II can be regarded as the hydrogen acceptor for the complete photochemical system of the chloroplast. The reduction of coenzyme II is mediated by a soluble protein[3], which can be separated from the green insoluble or particulate system containing both chlorophyll and cytochrome components. The phosphorylation reaction is a property of the insoluble fraction, since the coenzyme and soluble protein can be replaced by other hydrogen acceptors. This is shown diagrammatically in Fig. 1, the rectangle representing the green insoluble part of the chloroplast. The four equivalents of hydrogen can be regarded as being derived from water (they might possibly arise from the water removed when phosphorylation of adenosine diphosphate takes place). Removal of the hydrogen by an acceptor system liberates molecular oxygen and, for each molecule liberated, two molecules of inorganic phosphate can be esterified.

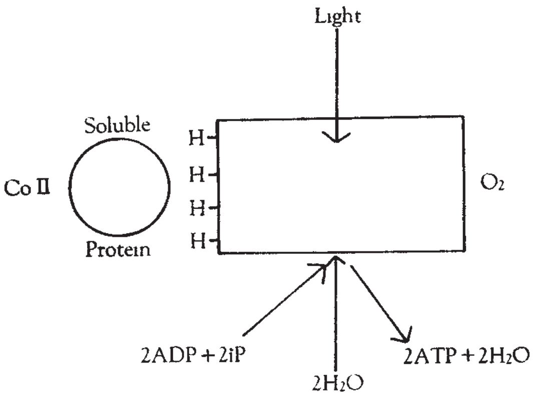

Fig. 1. ADP = adenosine diphosphate; ATP = adenosine triphosphate; iP = inorganic phosphate

The reduction process can be symbolized by the transfer of hydrogen (or electrons) from water to the hydrogen acceptor, the overall reactions per equivalent of hydrogen transferred being usually represented as follows:

$$H_2O + X + Y \rightarrow XH + YOH$$
$$XH \rightarrow X + (H)$$
$$YOH \rightarrow Y + \tfrac{1}{2}H_2O + \tfrac{1}{4}O_2$$
$$\tfrac{1}{2}H_2O \rightarrow (H) + \tfrac{1}{4}O_2 \qquad (1)$$

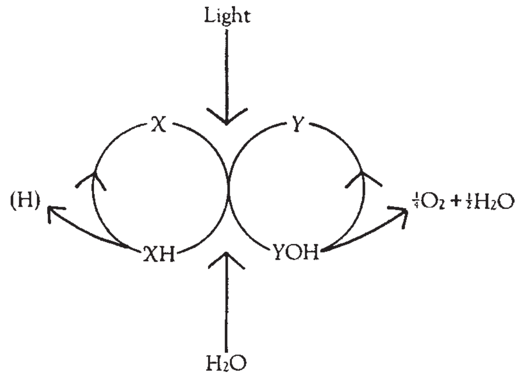

Fig. 2. *X* and *Y* are the hypothetical first acceptors of the products of the photolysis of water, forming respectively the reduced product *XH* and the oxidized product *YOH*

It can also be represented as a diagram as shown in Fig. 2. The symbols *X* and *Y* represent a postulated mechanism which separates the oxidized from the reduced part, (H) represents a source of active hydrogen transfer in a thermochemical sense. This representation corresponds with the reversal of a 'terminal oxidase' in respiration, the reaction characteristic of a mitochondrion or its fragments. In the mitochondrion, the transfer of hydrogen is from a hydrogen donor to molecular oxygen to give water. This can be represented by reversing all the arrows in (1) or in Fig. 2, and this generally will represent a thermochemical reaction taking place in the dark.

From this representation we are led to generalize that light absorbed by the pigments can transfer hydrogen against the thermochemical gradient.

In the respiratory chain mechanism of mitochondria the transfer of hydrogen (or electrons) is mediated in a stepwise way by the flavoprotein and cytochrome system. The transfer of the hydrogen is found to be coupled with phosphorylation reactions which appear to correspond with the stepwise transfer of the hydrogen (or electrons). Part of the energy at each step derived from a thermochemical oxidation process is conserved in the form of adenosine triphosphate.

In the chloroplast we find two cytochromes, *f* and b_6, which appear to be similar to the cytochromes *c* and *b* of the mitochondrial system. But instead of the terminal oxidase (associated with cytochrome *a* components), there is a relatively massive amount of chlorophylls which absorb the light[5]. To explain the formation of adenosine triphosphate from inorganic phosphate and adenosine diphosphate by the illuminated chloroplast, the very simple hypothesis would be that *XH* and *YOH* react through the two cytochromes to give water again, concurrently with the phosphorylation reaction.

However, this simple hypothesis, involving one 'light-driven reaction', does not at present seem to

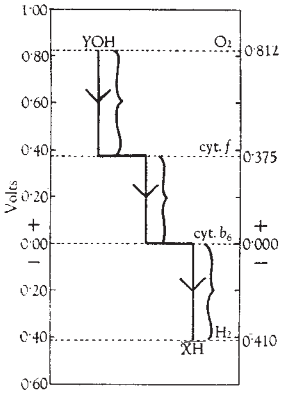

Fig. 3. Symbols as in Fig. 2

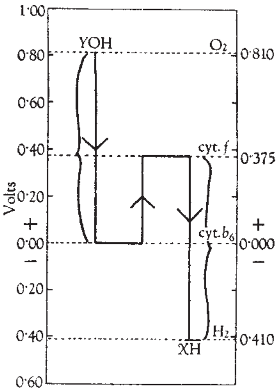

Fig. 4. Symbols as in Fig. 2

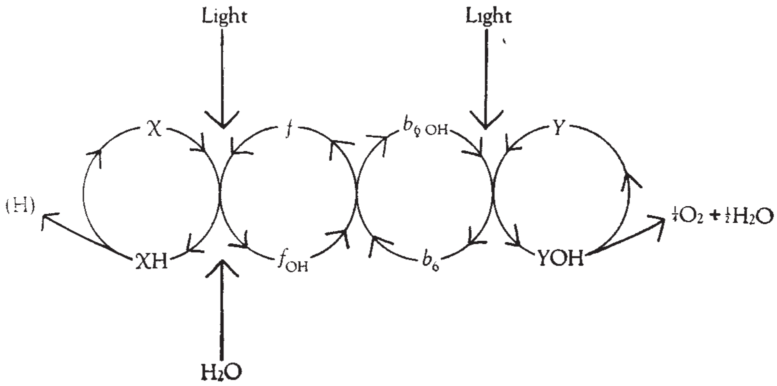

Fig. 5. X, Y, XH and YOH as in Fig. 2. f and b_6 represent ferrous and f_{OH} and b_{6OH} ferric or oxidized cytochromes

fit in with experiment. If the hydrogen transfer, represented as recombination of hydrogen and hydroxyl to give water again, is coupled with the phosphorylation, we should expect the addition of phosphate acceptor (adenosine diphosphate) and inorganic phosphate to diminish the observable rate of reduction of an added hydrogen acceptor and also of the corresponding observable rate of oxygen production. If the coupling was not obligate, then at the best we should expect no observable effect. But, on the contrary, Arnon's group[6] discovered that, in the presence of adenosine diphosphate, inorganic phosphate and magnesium ions, the rate of reduction of ferricyanide and the rate of production of oxygen were significantly increased and even doubled.

Thus the chloroplast system would appear to act by reversing a stepwise hydrogen transfer, as suggested by Davenport and Hill[7]. The phosphorylation reaction in light would then be regarded as 'reductive'[8], for the hydrogen (or electron) transfer is against the thermochemical gradient. This, if it is supposed that cytochromes b_6 and f are concerned, would involve three separate light-driven reactions. This scheme is shown as a potential diagram in Fig. 3. The downward arrows show hydrogen transfer against the thermochemical gradient and the brackets show the effect of each of three light-driven reactions.

The postulation of two light-driven steps, rather than three, would be in better accord with present experimental results. In this case oxidized cytochrome b_6 would have to be reduced by Y to give YOH and cytochrome f would have to be oxidized by X to give XH. The reaction between cytochromes f and b_6 would then be a thermochemical process and quite analogous with a hydrogen transfer step characteristic of the mitochondrion. This is shown as a potential diagram in Fig. 4, and by the diagram in Fig. 5 to correspond with Fig. 2. In Fig. 5, f and b_6 represent ferrous and f_{OH} and b_{6OH} ferric or oxidized cytochromes. If the reaction between f and b_6 could be inhibited, we should expect from this hypothesis that f would become oxidized and that b_6 would tend to be reduced in light. In bacterial chromatophores it might be possible to test this hypothesis relating c_2 (corresponding with f) with the cytochrome b_6 component[9]. In the green plant there is no evidence so far as we know at present which would indicate a phosphorylation step between f and b_6.

In the scheme put forward by Arnon[10], the proposed properties of the cytochromes do not correspond with the observed properties of cytochromes f and b_6. They would have to have oxidation-reduction potentials more positive than + 0·81 V. at pH 7. The present hypotheses are consistent with the data regarding the cytochrome components, but the nature of X and Y is still obscure. We might perhaps assume for the moment that both X and Y are forms of a part of chlorophyll. But the present experimental evidence taken as a whole could not consistently support such an assumption, particularly in the case of Y. It must therefore be emphasized that Fig. 5 is only a hypothesis in barest outline; for example, the reaction between the two cytochrome components is not likely to be a direct process, as shown.

The reconstituted chloroplast system of Vishniac[11] would correspond with the left-hand half of the system in Fig. 5, being active while the right-hand half, responsible for oxygen production, is inactive. With the reduction of coenzyme by the partially inactive system, a corresponding amount of some hydrogen donor would have to be oxidized.

In summary, it can be stated that if the cytochrome in chloroplasts is directly concerned in hydrogen (or electron) transfer, the system would require more than one light-driven reaction to act in opposition to the thermochemical gradient.

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