ATP-DEPENDENT EFFECTS ON REDOX REGULATION OF CHLOROPLAST PROTEIN SYNTHESIS

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1. Introduction

The chloroplast is a subcellular organelle that contains an extra-nuclear genetic system that codes for a limited but relatively constant subset of proteins that are essential for photosynthesis. It is known that synthesis of specific proteins in the chloroplast is enhanced up to 100-times by illumination of plants (1-2). In order to test whether the light-regulation occurs through the electron transport chain, and to test if the chloroplast protein synthesis is under redox control, protein synthesis in isolated chloroplasts was examined in the presence of the electron transport inhibitors DCMU and DBMIB, and in the presence of the redox reagents ferricyanide, ascorbate, dithiothreitol and dithionite.

2. Procedure

Chloroplasts were isolated from shoots of 8-day-old pea (*Pisum sativum* L.) plants. Protein synthesis assays were based on the method of Mullet et al. (3) with modification as described in Allen et al. (4). Redox reagents used are as in (4). After 20 min pulse-labelling, the chloroplasts were broken by osmotic lysis, and thylakoids and stoma were separated by centrifugation. Samples were solubilised and applied to SDS-polyacrylamide gels containing 4 M urea. Proteins were electrophoretically transferred to nitrocellulose, and the radioactive protein bands were detected by autoradiography. To identify the newly synthesised psbA and petA, immunoprecipitation was performed as described in (5).

3. Results and Discussion

3.1 Redox effects

It was observed recently that different subsets of chloroplast proteins are selected for synthesis in the presence of different electron transport inhibitors in the light, and in the presence of different external redox reagents in darkness (4). The results presented here are consistent with this observation (Fig. 1a & b). In addition, it is also found that some proteins, mostly in stromal fractions, are synthesised to the same extent under these different conditions (Fig. 1b). It seems that some of the proteins are synthesised under the control of a redox regulatory mechanism, whereas some are not.

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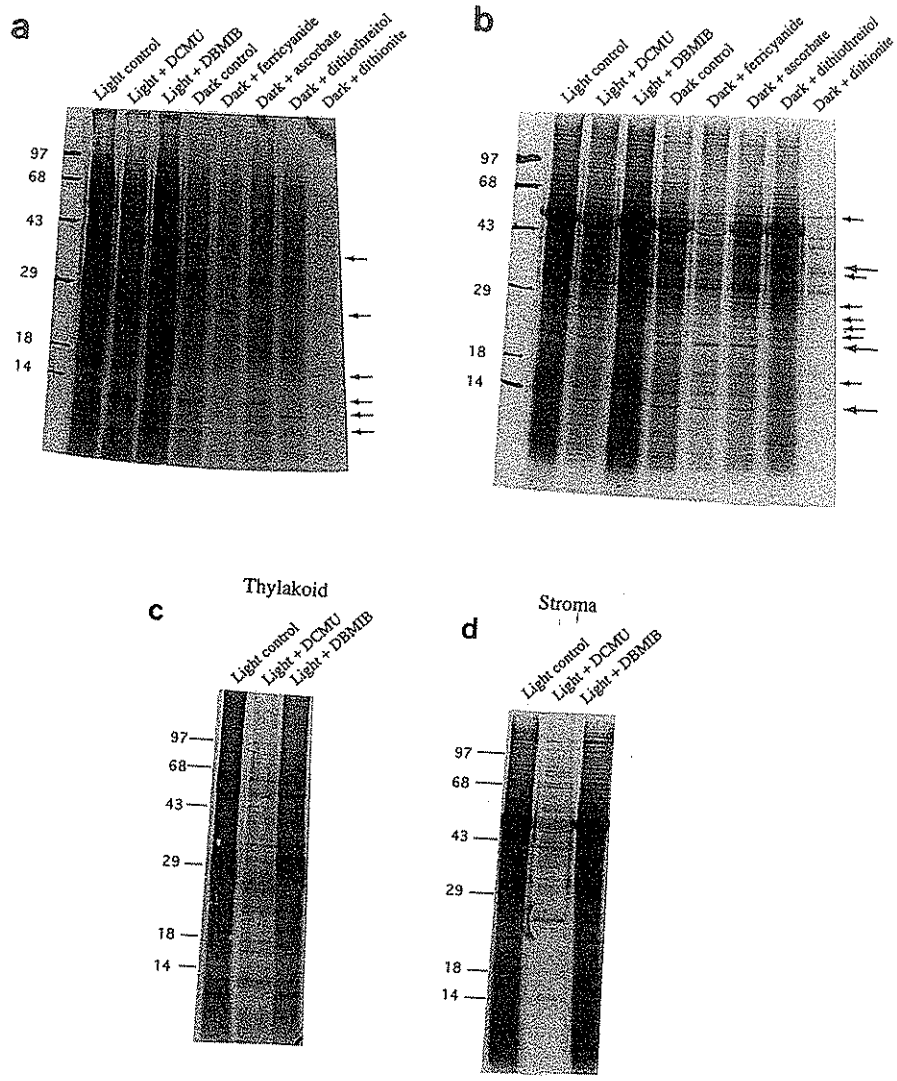

Figure 1. Autoradiograph of ^{35}S-labelled SDS-PAGE of protein synthesis in isolated pea chloroplasts. In the presence of 1 mM ATP (a. thylakoid fraction; b. stroma fraction) and absence of ATP (c & d). The positions of some specific protein bands with changed intensity (small arrows) or with the same intensity (large arrows) compared to the light control are indicated.

The protein synthesis patterns of psbA and petA obtained by immunoprecipitation using antisera of psbA and petA under different redox conditions are shown in Fig. 2. It is seen that the electron transport inhibitor DCMU, which block plastquinone (PQ) reduction, at

1 mM ATP concentration strongly inhibits synthesis of both psbA and petA in the light, while the inhibitor DBMIB, which at 2 μM concentration blocks plastoquinol oxidation, keeping the PQ pool reduced, does not inhibit the synthesis of the two proteins. Synthesis of psbA and petA is inhibited in darkness, that is, when the PQ pool is oxidised. However, in contrast to reducing the PQ pool by DBMIB addition of reducing agents in dark treated chloroplast did not restore protein synthesis. Sodium ascorbate is a mild reducing agent and a PS I electron donor, but since the electron transport mediator DCPIP was not present, electrons could not pass to PS I, thus the PQ pool remains oxidised in the presence of ascorbate. Inhibition of protein synthesis was not recovered in the presence of ascorbate. The reducing agents DTT and sodium dithionite showed similar effects as ascorbate. It is not clear why these external reductants cannot recover the dark inhibition of psbA and petA synthesis. It is possible that these external reductants are not able to reduce some components that lie after PS I in the electron transport chain. Thioredoxin has been recently suggested to be the key mediator of regulating protein synthesis by modulating the binding of activator protein to mRNA (6).

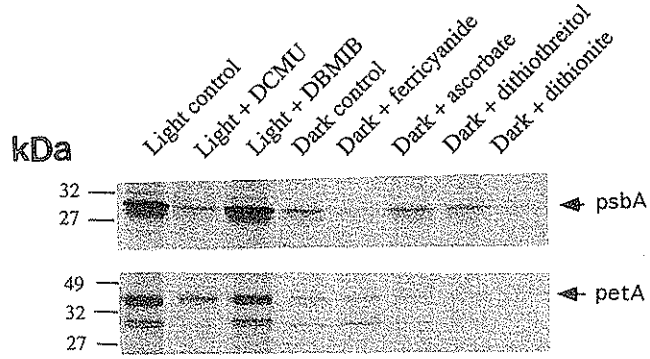

Figure 2. Immunoprecipitation of thylakoid membrane proteins ^{35}S-labelled in isolated pea chloroplasts with antiserum of psbA or petA in the presence of 1 mM ATP.

3.2 ATP dependency

The inhibitory effect of DCMU on the protein synthesis in the light is much stronger in the absence of ATP (Fig. 1c & d) than in the presence of 1 mM ATP (Fig. 1a & b). Moreover the inhibitory effect of DCMU in the light could be recovered to some extent by increasing the ATP concentration to 10 mM (Fig. 3). The similar recovery of protein synthesis by 10 mM ATP in both the dark incubation sample and the samples with reducing agents present was also observed (Fig. 3), except for the sample with the oxidising agent ferricyanide present. ATP-driven protein synthesis in darkness by isolated pea chloroplasts was reported previously (7). It is interesting to know that ATP also stimulated protein synthesis in the light with DCMU present. It was proposed that light can be partially replaced as an energy source by added ATP (8). Alternatively, when there is a high concentration of exogenous ATP present, the ATP synthase complex may build a proton motive force which could cause the chloroplasts to remain in reduced condition by photosynthetic control. Since the ferricyanide is a strong oxidising agent, it may maintain oxidising condition in the chloroplast even with 10 mM ATP present.

3.3 Conclusions

1) The light-regulation of the synthesis of some thylakoid proteins, including psbA and petA, is coupled to the electron transport chain; 2) Some chloroplast proteins are synthesised under the control of a redox regulatory mechanism, whereas some are not; 3) Since high ATP concentration (10 mM) maintains protein synthesis in darkness, but protein synthesis remains sensitive to inhibition by the oxidising agent ferricyanide, ATP may serve not only as an energy source, but also as a redox buffer. The level of gene expression at which redox control is exerted remains to be determined (9), but these experiments show that isolated, intact chloroplasts incubated with electron transport inhibitors and redox reagents are an effective model system and can be used to elucidate redox control of gene expression.

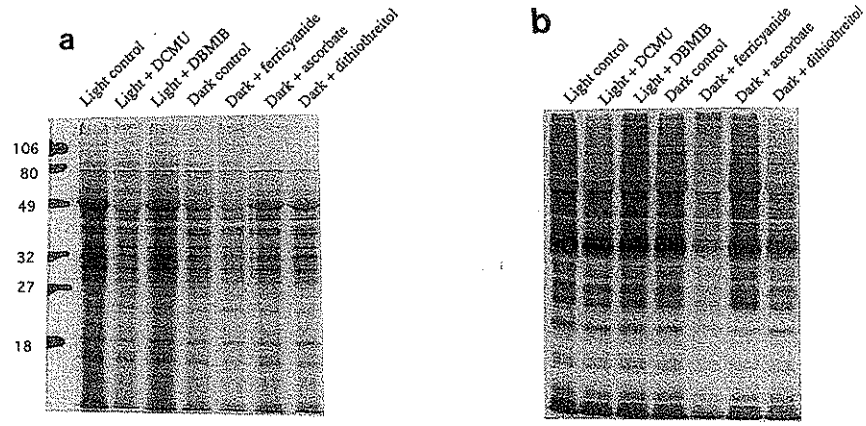

Figure 3. Autoradiograph of ^{35}S-labelled SDS-PAGE of chloroplast protein synthesis pattern in the presence of 1 mM ATP (a) and 10 mM ATP (b).

Acknowledgements

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