Relationship between the 43 kDa chlorophyll-protein of PS II and the rapidly metabolized 32 kDa Q_B protein

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The chlorophyll-proteins of L-[^{35}S]methionine-labelled thylakoid membranes isolated from cycloheximide-treated *Vicia faba* plants were electrophoretically separated and correlated to the rapidly metabolized Q_B protein. Additionally, the chlorophyll *a*-protein band with the highest specific radioactivity was excised and subjected to denaturing re-electrophoresis. Based on these experiments it is presumed that the native Q_B protein is associated with pigments and represents a protein moiety of the 43 kDa chlorophyll *a*-protein (Chl a-P3) of PS II. This is reconcilable with the hypothesis that the Q_B protein carries the reaction center of PS II as deduced from its homology to the L and M subunits of the bacterial reaction center complex.

Chlorophyll-protein *Q_B protein* *Reaction center* *Photosystem II* (Vicia faba)

1. INTRODUCTION

The so-called 32 kDa protein which binds the secondary quinone electron acceptor (Q_B) is the site of action for several herbicide families [1]. It is synthesized at rates greater than the most abundant thylakoid membrane proteins and characterized by high metabolic activity [2,3]. According to recent experiments the 32 kDa Q_B protein shows sequence homology to the subunits L and M of the bacterial photosynthetic reaction center complex [4,5] as well as to a 34 kDa polypeptide encoded by the so-called D-2 gene [6]. Based on these findings it was speculated that the 32 kDa Q_B protein (coded by D-1 gene) and the D-2 polypeptide of higher plant chloroplasts play the same role as the L and M subunits of photosynthetic bacteria [7,8].

Isolated reaction center complexes from photosynthetic bacteria generally contain quinones, nonheme Fe, and pigments [9]. The Q_B protein and the D-2 polypeptide, in spite of their sequence homology to bacterial reaction center proteins, have not yet been found to carry chlorophyll [7]; this could be the result of their having a lower stability in the presence of detergents. With this in mind, L-[^{35}S]methionine-labelled thylakoid membranes isolated from cycloheximide-treated *Vicia faba* plants were dissociated and electrophoresed under mild conditions to keep chlorophyll and protein associated. Electrophoretic separation of the chlorophyll-proteins was performed for a 10–32 h period and their positions were correlated to the radioactivity distribution of the corresponding autoradiograms. The chlorophyll-protein band with the highest specific radioactivity was excised and subjected to denaturing re-electrophoresis and autoradiography.

2. MATERIALS AND METHODS

Vicia faba plants were cultivated in a growth chamber for 18 days at 20°C under light (10000 lux; 16 h) and 15°C in darkness. They were adapted to L-[^{35}S]methionine incubation by growing them in a N-deficient Hoagland solution of one-tenth strength under darkness for 32 h. Immediately prior to being incubated, the shoots were cut 10 cm below the growing point and all leaves

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were removed except for the two fully expanded youngest leaves and the plumula. The excised shoots were transferred to small beakers and pre-incubated in darkness at 20°C with cycloheximide (200 μg/ml) dissolved in N-deficient Hoagland solution. After 30 min of pre-incubation, eight shoots were transferred to 5 ml of the same medium additionally containing 5 mCi L-[^{35}S]methionine with a specific radioactivity of 1100 Ci/mmol. The volume taken up during incubation time was supplemented by methionine-free N-deficient solution with 20 μg/ml cycloheximide. The experiments were terminated after 8 h of incubation under light (10000 lux).

Thylakoid membranes were isolated and stored according to [10]. Membrane dissociation was performed in an ice bath with *n*-octyl-glucoside and SDS to a final ratio of 20:1:1 (octylglucoside/SDS/chlorophyll). Chlorophyll-proteins were separated under 'non-denaturing' conditions at 4°C using a 7.5–20% polyacrylamide gel gradient in combination with buffer system IV [11]. The completely dissociated polypeptide pattern was obtained by heating the samples at 60°C for 5 min in presence of 5% DTT and denaturing electrophoresis using a 10–20% gel gradient containing 6 M urea. ORWO HS 11 X-ray films were used for autoradiography.

3. RESULTS

Thylakoid membranes from *Vicia faba* plants dissociated as described and subjected to non-denaturing electrophoresis yielded six chlorophyll-protein bands besides free pigments (fig.1). According to the nomenclature published in [11] the bands are termed chlorophyll *a*-proteins and chlorophyll *a/b*-proteins, respectively. Concerning their function, band 1 represents the structural equivalent of PS I and band 2 the corresponding reaction center chlorophyll-protein. Bands no.3 and 6 are constituents of light-harvesting complex II and represent oligomeric and monomeric chlorophyll *a/b*-proteins. The two chlorophyll *a*-protein bands forming a doublet (bands no.4 and 5) are known as reaction center and antenna of PS II.

Incubation of excised shoots with 200 μg/ml cycloheximide completely inhibited incorporation of L-[^{35}S]methionine into thylakoid membrane polypeptides synthesized in the cytoplasm. Of the fractionated chlorophyll-proteins only the bands no.4 and 5 were found to be labelled under these conditions. In contrast to previous results [12] no incorporation of labelled methionine into chlorophyll-*a* protein 1 (Chl *a*-P1) could be detected in the present experiments. The labelled band of the upper gel region marked X corresponds to neither Chl *a*-P1* nor Chl *a*-P1 (figs 1 and 2). Both chlorophyll-protein bands are not labelled demonstrating inhibition by cycloheximide. The discrepancy between previous and recent results concerning the inhibitory effect of cycloheximide can be explained as the result of the procedure applied for electrophoresis. If 7.5–20% gradient gels not containing urea are used, band X is then separate from Chl *a*-P1 indicating complete inhibition of PS I chlorophyll *a*-protein synthesis by cycloheximide (figs 1 and 2). With 10–20% gradient gels containing 6 M urea, however, X was superimposed on Chl *a*-P1 stimulating non-inhibition by cycloheximide (fig.3). The chemical nature of polypeptide X is unknown. It shows no stainable equivalent in fig.2a.

Inhibition of synthesis on cytoplasmic ribosomes reduced the number of labelled bands and enabled better identification of chloroplast synthesized polypeptides. This mainly concerns the rapidly metabolized 32 kDa protein (RMP) which is both of chloroplast origin and characterized by high specific radioactivity. This characteristic feature was used in the present experiments to find out whether a correlation exists to one of the known chlorophyll-proteins. For this purpose a series of electrophoretic experiments was performed to identify which chlorophyll-proteins had a high specific activity and to observe their transition from the native state into the denatured state. Of the eleven experiments of this series the characteristic results of four are shown in fig.1 and those of two in fig.2.

Under the conditions of the 'short-time electrophoresis' (up to 10 h at 5 mA and max. 100 V) the intensively labelled band characteristic for RMP (marked by arrows in fig.1) corresponds exactly in its shape and position to that of chlorophyll *a*-protein 3 (Chl *a*-P3). Prolonged separation time primarily dissociates Chl *a*-protein 2 (Chl *a*-P2) (band no.4) which disappeared completely after approx. 18 h. At this stage Chl *a*-P3

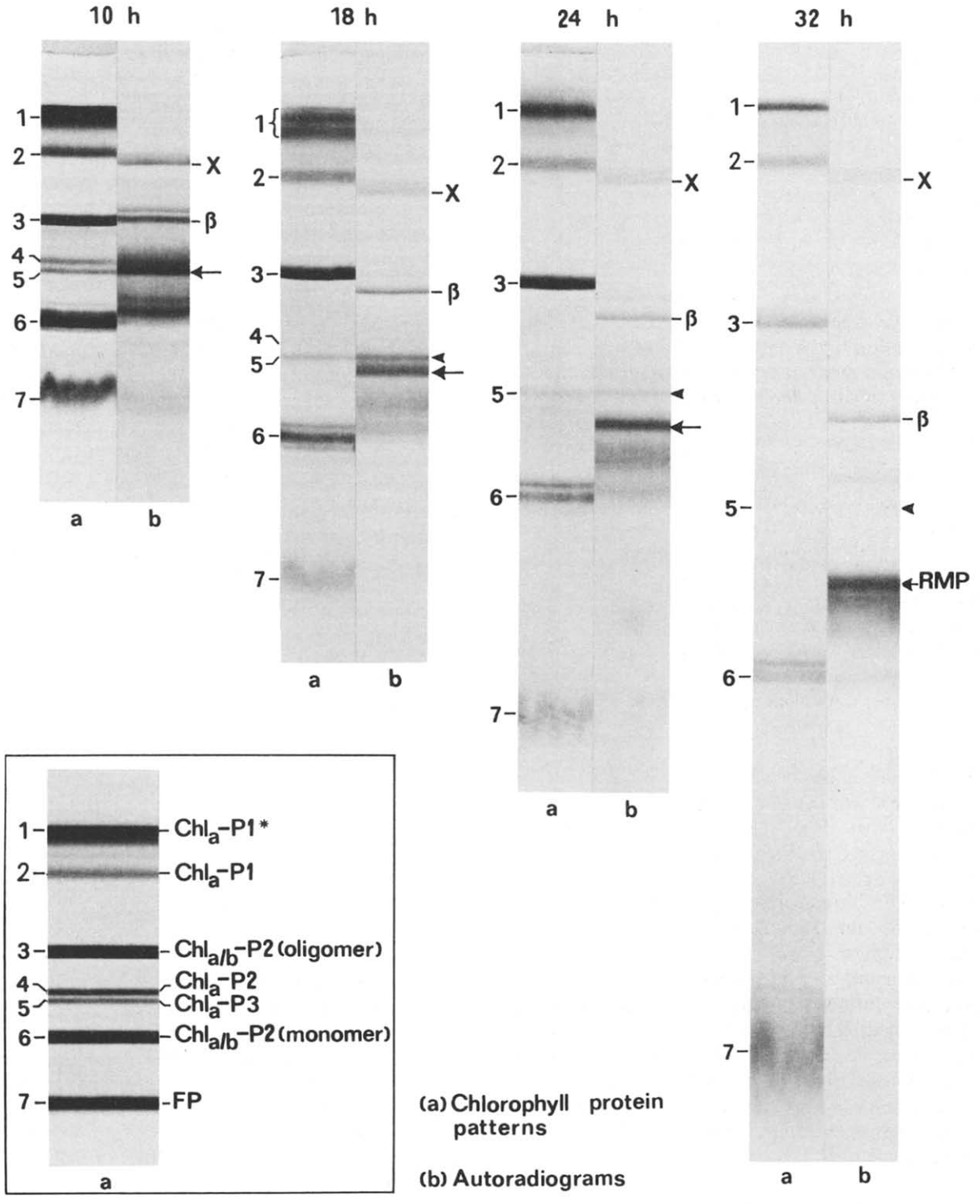

Fig.1. Correlation of Chl *a*-P3 with the rapidly metabolized protein analysed with non-denaturing gels. Increasing dissociation of Chl *a*-P3 is connected with the shift of the radioactivity to the position of RMP.

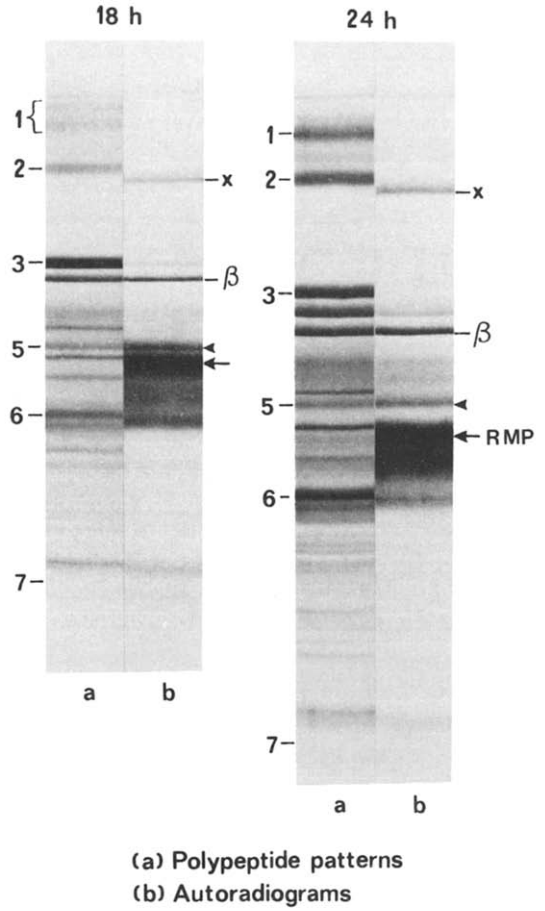

Fig.2. Polypeptide patterns obtained by Coomassie staining with gels from the 18 h and 24 h experiments as shown in fig.1. Slots (a) show no stainable protein in the position of the labelled band X.

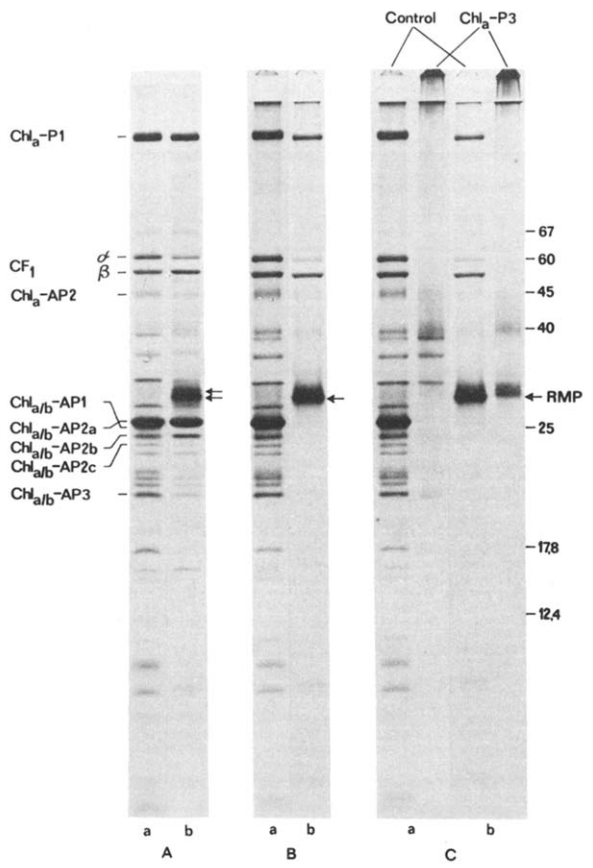

Fig.3. Polypeptide patterns (a) and autoradiograms (b) of L-[^{35}S]methionine-labelled thylakoid membranes obtained with denaturing gels. A, inhibition with 20 μg/ml cycloheximide; B, inhibition with 200 μg/ml cycloheximide; C, re-electrophoresis of Chl *a*-P3 isolated as shown in fig.1.

was still visible as a green band but was reduced in intensity and radioactivity indicating partial dissociation (marked by arrowhead). This effect is accompanied by the appearance of a new band which, according to its high specific radioactivity, must be attributed to RMP and very probably represents its denatured or partially denatured state. Further dissociation of Chl *a*-P3 resulted in a decrease of both chlorophyll content and radioactivity, and an increase in the intensity of the RMP band.

The experiments point to a structural relationship between Chl *a*-P3 and RMP. It is presumed that under the conditions of short time electrophoresis applied in the present experiments the native conformation of RMP is preserved. In this state it corresponds exactly to Chl *a*-P3. Dissociation of Chl *a*-P3 releases the corresponding apoprotein which empirically is now of increased electrophoretic mobility. Since Chl *a*-P3 dissociation is accompanied by a shift of the radioactivity to the position of RMP, it seems reasonable to conclude that RMP represents a constituent of Chl *a*-P3.

The relationship between Chl *a*-P3 and RMP was supported by re-electrophoresis experiments with isolated Chl *a*-P3 performed under denaturing conditions. The polypeptide pattern and radioactivity distribution in thylakoid membranes of *Vicia faba* plants treated with 20 μg/ml (A) and

200 μg/ml (B) cycloheximide, respectively, are shown in fig.3 indicating complete inhibition of cytoplasmic protein synthesis in B. The results obtained by re-electrophoresis of Chl *a*-P3 are shown in fig.3C. The corresponding autoradiogram (b) demonstrates that most of the radioactivity associated with the native Chl *a*-P3 band was found in the 30 kDa region of the gel when the sample was subjected to denaturing electrophoresis.

The results of both approaches, mild electrophoresis of complete thylakoid membranes and denaturing electrophoresis of isolated Chl *a*-P3, are indicative of the native rapidly metabolized Q_B protein being a constituent of PS II Chl *a*-P3.

4. DISCUSSION

In the experiments described in this paper the specific radioactivity of L-[^{35}S]methionine-labelled chlorophyll-proteins and their controlled dissociation was used to find out whether the native state of the rapidly metabolized protein is associated with pigments. Based on the conclusions drawn by another group [13] it is accepted that the rapidly metabolized protein is identical with the so-called 32 kDa protein which binds the secondary electron acceptor Q_B and herbicides of several families. Although the molecular mass of the RMP analysed in [12] and here was found to be 28–30 kDa, the designation 32 kDa protein was applied as this terminology has been introduced into the literature. According to the results obtained the RMP represents a (the) protein moiety of the 43 kDa chlorophyll *a*-protein (Chl *a*-P3) of PS II. This presumption is reconcilable with the hypothesis that the Q_B protein carries the reaction center of PS II as deduced from its sequence homology to the subunits L and M of the bacterial reaction center complex [7,8]. Consequently, Chl *a*-P3 must represent the reaction center chlorophyll *a*-protein of PS II. This, however, contradicts the generally agreed concept of the 47 kDa chlorophyll *a*-protein (Chl *a*-P2) as the site of charge separation which is based on numerous experiments [14–17].

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