BIOGENESIS OF THE CHLOROPLAST PHOSPHATE TRANSLOCATOR

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1. Introduction

The phosphate translocator located in the inner membrane of the chloroplast envelope facilitates the export of fixed carbon in the form of triosephosphate and 3-phosphoglycerate in exchange with phosphate from the chloroplast stroma into the cytosol [1]. Therefore it is an important step in CO_2 fixation. The membrane constituent catalyzing this transport is a major envelope protein with app. M_r 29 000, as determined by SDS gel electrophoresis [2]. This protein has been isolated and reconstituted into liposomes [3]. This paper investigates whether nuclear or chloroplast genes code for this translocator.

2. Methods

Eight spinach leaves (*Spinacea oleracea*, US hybrid 424 from Ferry-Morse Seed Co., Mountain View CA) 4–6 weeks old, grown in water culture as in [4] were illuminated and supplied with water through the cut ends with or without the presence of inhibitors. The inhibitors used were: cycloheximide (Sigma), 20 μg/ml; chloramphenicol (Sigma) and lincomycin (Sigma), 100–200 μg/ml. After 30 min 0.7–1.2 mCi [^{35}S]sulfate (carrier free, New England Nuclear) were added and the leaves illuminated for a further 3 h. Intact chloroplasts were isolated [5,6], from which the envelope membranes were prepared and purified by discontinous sucrose density gradient centrifugation [7]. In this centrifugation, the stromal proteins stay at the top of the gradient whereas the envelope membranes appear as a yellow interface band. The stromal and the envelope membrane proteins were analyzed by SDS–polyacrylamide gel electrophoresis [8]. Gels with 12.5% acrylamide and 0.33% *N,N'*-methylene bisacrylamide were used. The staining procedure and the analysis for radioactivity have been described in [9]. Protein was measured as in [10] using bovine serum albumin as standard.

3. Results and discussion

Protein synthesis in spinach leaves was followed by incorporation of [^{35}S]sulfate into proteins in the presence or absence of compartment-specific antimetabolites of protein synthesis. Cytoplasmic protein biosynthesis was selectively inhibited by cycloheximide, whereas chloroplast protein synthesis was inhibited by chloramphenicol or lincomycin [11]. It was then determined under which conditions the phosphate translocator was no longer synthesized. The validity of these experiments depends absolutely on the specificity of the inhibitors employed [12]. Therefore it should be established that under the experimental conditions the ribosomal inhibitors act specifically at the different cell sites. Thus, the action of cycloheximide, chloramphenicol and lincomycin on the synthesis of ribulose bisphosphate carboxylase was checked. The constituent subunits of this protein are synthesized in different cell sites: the large subunit on the 70 S chloroplast ribosomes and the small subunit as a higher M_r precursor on the 80 S cytoplasmic ribosomes, respectively [13–18].

The radioactive label in the stromal fraction is mostly incorporated into the large and the small subunit of the ribulose bisphosphate carboxylase (fig.1). ^{35}S in the large and small subunit is ~4:1, which corresponds to the M_r ratio of the large and small subunit. In the presence of cycloheximide, however, the synthesis of the small subunit was totally depressed whereas in the presence of chloramphenicol or lincomycin the synthesis of the large subunit was reduced to a large extent (table 1). These results demonstrated

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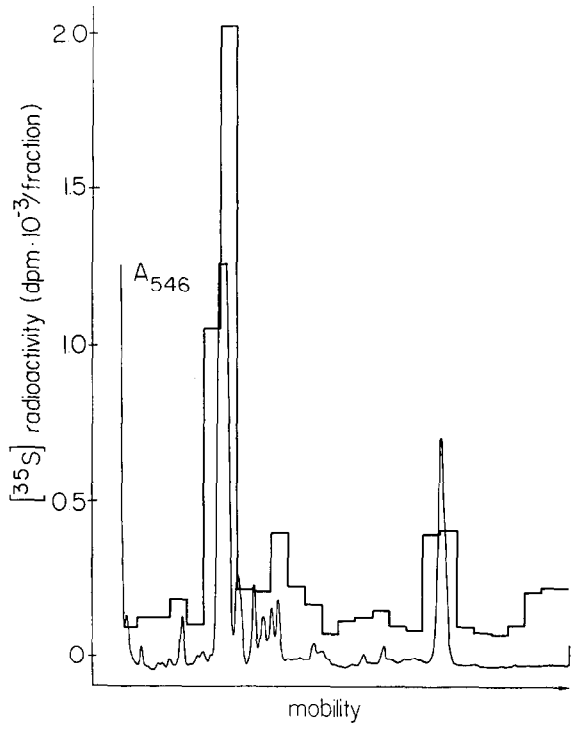

Table 1
Incorporation of [^{35}S]sulfate into the large and small subunit of ribulose bisphosphate carboxylase

	^{35}S Radioactivity incorporated (dpm)		
	Large subunit	Small subunit	Ratio
Control	3000	700	4.3
Cycloheximide	2300	0	≫
Chloramphenicol	400	650	0.6

Aliquots (equiv. 50 μg protein) of the stromal fractions were applied to SDS–polyacrylamide gel electrophoresis and analyzed as in [9]

Fig.1. SDS gel electrophoresis of the stromal proteins. The incorporation of [^{35}S]sulfate, the isolation of intact chloroplasts and the subsequent preparation and purification of the stromal proteins were done as in section 2. Stromal proteins, equivalent to 50 μg protein, were applied to gel electrophoresis. Separate gels were used for the absorbance scan (continuous curve) and for radioactivity scan (discrete lines).

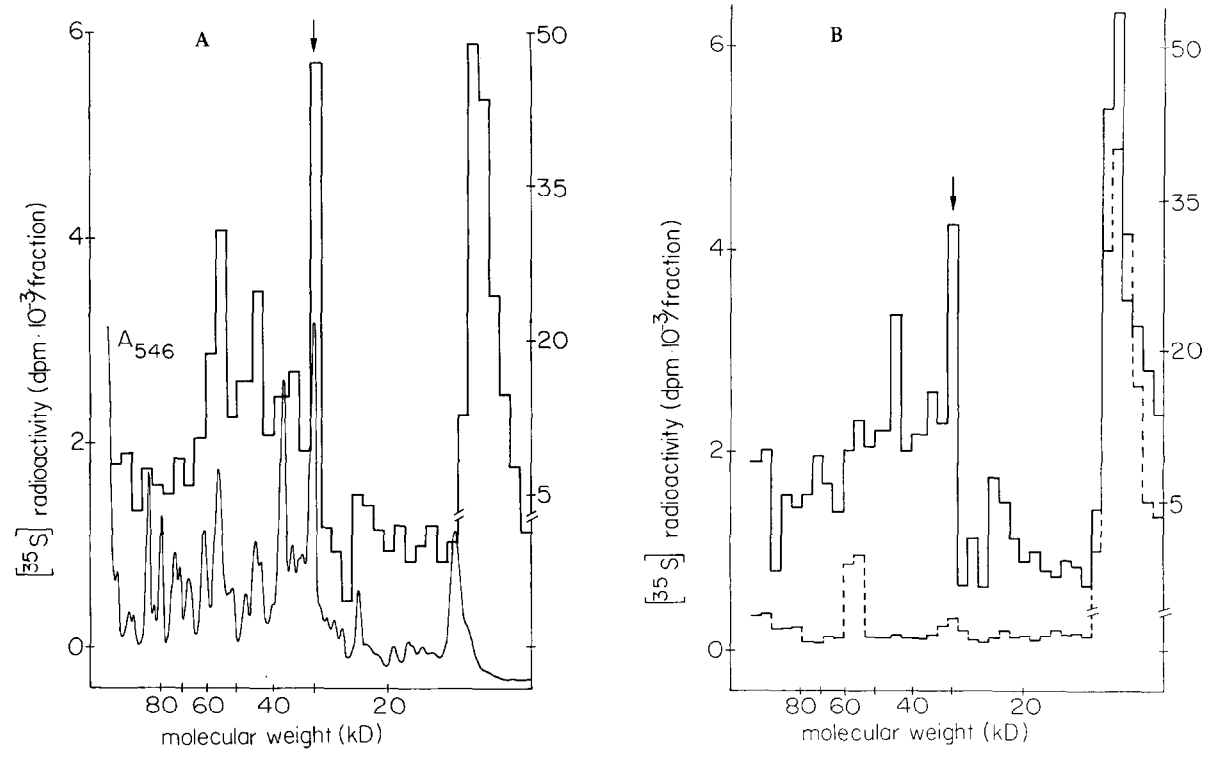

Fig.2. SDS gel electrophoresis of envelope membrane proteins. For details see section 2 and fig.1: (A) Absorbance scan (continuous curve) and radioactivity scan without any inhibitor (discrete lines); (B) radioactivity scans in the presence of chloramphenicol (discrete lines) and cycloheximide (dotted lines). The position of the phosphate translocator is indicated by an arrow.

that the inhibitors employed act specifically, cycloheximide inhibiting the accumulation of the small subunit synthesized on cytoplasmic ribosomes and chloramphenicol or lincomycin blocking the synthesis of the large subunit coded on chloroplast genes.

The conclusions drawn from the results obtained during the inhibitor studies with the phosphate translocator were based on the specificity of these inhibitors. In the absence of any inhibitor (fig.2A) the ^{35}S-label is incorporated into most of the envelope polypeptides. The phosphate translocator, one of the major membrane polypeptides with M_r 29 000, shows the highest incorporation of radioactivity.

When the incubation is carried out in the presence of chloramphenicol (fig.2B) the incorporation of the radioactive label into the membrane polypeptides, especially into the phosphate translocator, is only slightly affected. The same results were obtained with lincomycin. However, in the presence of cycloheximide labelling of the membrane proteins including the phosphate translocator was drastically reduced (fig.2B). This implies that the site for the synthesis of the phosphate translocator is in the cytosol. In studies with chloroplasts from pea seedlings, it was found that only two chloroplast envelope polypeptides with M_r 32 000 and 65 000 are synthesized on chloroplast ribosomes [19]. Many proteins localized within the chloroplast stroma and the thylakoids are synthesized on cytoplasmic ribosomes [20]. These data, from spinach leaves, show that not only the phosphate translocator but also most of the envelope membrane proteins are made in the cytosol. Only one of them, with $M_r \sim 55\,000$–$60\,000$, seems to be synthesized inside the organelle. However, this protein might represent the large subunit of the ribulose bisphosphate carboxylase which, although it is a stromal protein, sticks very tightly to the envelope membrane during its isolation. Conceivably, labelling of plastid-produced components may escape detection if their synthesis is dependent upon cytosolic protein synthesis. Also, with increasingly sensitive methods, more genes for thylakoid polypeptides have been localized on the plastid chromosome (R. Herrmann, personal communication) and hence the intracellular origin of envelope proteins deserves further study.

The fact that the phosphate translocator, which is an hydrophobic, integral inner envelope membrane protein, is coded on nuclear genes implies that it is synthesized in the cytosol in a soluble (precursor) form, in which its hydrophobic domains are directed towards its interior. After synthesis it must be transported post-translationally through the outer envelope membrane and to be inserted correctly into the inner envelope membrane. There is experimental evidence, that the mitochondrial ATP/ADP carrier, an integral inner membrane protein, which is made in the cytosol, exists in a soluble oligomeric precursor form. Afterwards it is integrated into the inner membrane, whereby it undergoes a conformational change [21]. So far, nothing is known in which form and how hydrophobic proteins of the chloroplast envelope reach their specific membrane.

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