NON-SYNCHRONOUS INCORPORATION OF $C^{14}O_2$ INTO AMINO ACIDS OF THE TWO SUBUNITS OF FRACTION I PROTEIN

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Received October 30, 1969

Summary. Fraction I protein (RuDP carboxylase) consists of two subunits which differ in molecular weight and amino acid composition. Tobacco leaves were supplied $C^{14}O_2$ during 15 min of photosynthesis resulting in 8 of the amino acids of Fraction I protein becoming radioactive. The amount of C^{14} incorporation into the larger subunit was greater than into the smaller subunit. Resolution of the amino acids showed the specific radioactivity of each of the 8 amino acids was greater in the larger subunit compared to the smaller. Evidently, a large pool of smaller subunits exist or synthesis of Fraction I protein involves two ribosome sites and two DNA cistrons.

As first detected by Rutner and Lane (1), spinach Fraction I protein (RuDP carboxylase) is composed of two distinct polypeptides which differ in molecular weight and amino acid composition. This result has been confirmed and extended to tobacco (2) and spinach beet (3) Fraction I proteins. There is a remarkable similarity in the total amino acid composition of the larger

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subunit of the three species of Fraction I protein. In contrast, the amino acid compositions of the smaller subunit are markedly different. The similarity in composition of the larger subunit and variability in the smaller is consistent with immunological analyses which have shown that Fraction I proteins from many different plants possess shared and unshared antigenic structures (4). In this communication, experiments were performed to ascertain whether in vivo synthesis of the two subunits and assembly into Fraction I protein was a synchronized or non-synchronized process.

Five of the largest leaves (75 g) from about 2 month old plants (Nicotiana tabacum, Bright Yellow) were detached and enclosed in a plastic chamber while protecting the leaves from wilting by wrapping the cut end with wet absorbent cotton. The detached leaves were then allowed to photosynthesize for 15 min at a saturating light intensity (ca. 4×10^4 lux) in an enclosed atmosphere containing 50 μC $C^{14}O_2$ in 300-400 ppm CO_2 with constant circulation of the atmosphere. The leaves were then chilled on ice. In 15 min, 70% of the $C^{14}O_2$ was absorbed by the leaves.

Fraction I protein was prepared from the leaves by ammonium sulfate fractionation, Sephadex G-200 gel filtration and DEAE-cellulose column chromatography according to a previously described method (2). The purified sample was homogeneous by polyacrylamide gel electrophoresis and immunological double diffusion analysis.

Purified Fraction I protein (47 mg), which contained 1.5×10^5 cpm was dissociated by 0.5% sodium dodecylsulfate in 0.14 M mercaptoethanol buffered at pH 8.6 under N_2 gas, and then sulfhydryl groups were aminoethylated by ethyleneimine. After removing the unreacted reagents by passage of the mixture through a Sephadex G-25 column, the protein fraction was subjected to

gel filtration on a Sephadex G-100 column to separate the dissociated subunits, using procedures exactly the same as previously reported (2).

As shown in Fig. 1, two, well-resolved peptide peaks were eluted. The larger and smaller peptides are tentatively called subunit A and B, respectively. The recoveries of protein and radioactivity after the gel filtration are shown in Table 1.

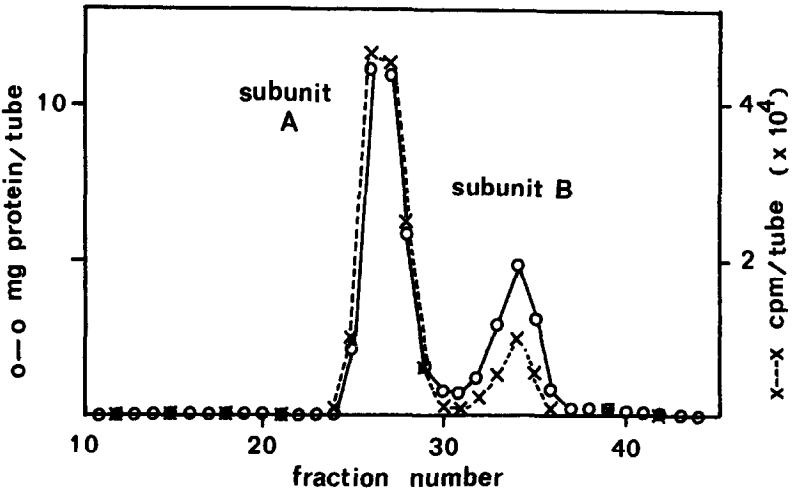

Fig. 1. Distribution of radioactivity and protein after elution of SDS dissociated Fraction I protein from Sephadex G-100.

TABLE I. Recovery of Protein and Radioactivity after Dissociation of Fraction I Protein and Separation of Two Subunits by Sephadex G-100 Column Chromatography.

	Protein* (mg)	Radioactivity (cpm)	Specific activity (cpm/mg)
Native protein	47.0	152,360	3240
Recovered			
Subunit A	29.57	121,824	4130
Subunit B	10.52	20,880	1980
Recovery (%)	87.0	93.5	

*Protein amounts were measured by Folin's method.

The ratio of subunit A:subunit B was about 75:25 as based on Folin analysis, whereas the ratio was 68:32 based on OD_{280}. These numbers were almost the same as previous results (2). On the basis of the Folin analysis, the radioactivity/mg protein for subunit A was about double that for subunit B, suggesting that subunit A and B might have been synthesized independently. However, the differences in amino acid composition between the two subunits, as well as uncertainty in the protein determination because of possible SDS complexing with the subunits might have contributed to the differences. To eliminate such uncertainties, the specific radioactivity of individual amino acids was determined.

Four mg of subunit A and 8 mg of subunit B were each precipitate by 50% acetone, washed several times, and hydrolyzed by 6 N HCl at 110° for 20 hours under reduced pressure. The hydrolysates were analyzed by a Beckman Amino Acid Analyzer equipped with a Packard Liquid Scintillation Counter. The results of the two analyses are shown in Table II. Eight out of the 18 amino acids constituting the primary structure of Fraction I protein were found to have radioactivity in both subunit A and B. As expected,

TABLE II. Comparison of Specific Radioactivity of Amino Acids in the Two Subunits of Fraction I Protein.

	Subunit A			Subunit B		
	Amount (μmole)	(cpm)	Sp.Act. (cpm/μmole)	Amount (μmole)	(cpm)	Sp.Act. (cpm/μmole)
Asp.	1.78	710	399	2.16	600	230
Ser.	0.69	1590	2304	1.43	2040	1420
Gly.	1.98	2992	1511	2.40	2580	1075
Ala.	1.80	2040	1333	1.91	1585	830
Val.	1.03	430	417	1.46	440	301
Met.	0.28	1078	3850	0.54	1163	2154
Tyr.	0.68	1025	1505	2.36	2600	1016
Phe.	1.03	1265	1622	1.06	1355	1075

the amino acids made radioactive from $C^{14}O_2$ were equivalent to those appearing as the primary products of photosynthesis with the probability that methionine, tyrosine and phenylalanine were derived from an "active C_1 unit" also produced very rapidly from CO_2 during photosynthesis. In every case, the specific activity of an amino acid in subunit A was higher than in subunit B. The greatest difference was for methionine whose specific radioactivity in subunit A was 1.8 times larger than in B. The smallest difference was for glycine which was 1.4 times larger in subunit A than in B. The experiment was repeated in its entirety with closely similar results.

From in vivo experiments using antibiotics, Smillie et al. (5) have indicated the probability that Fraction I protein is synthesized by 70S chloroplast ribosomes. If this is the case, then the results of the present experiments may indicate that a pool of non-radioactive B subunits is present. It would seem necessary for the pool to be quite large to have prevented equalization of the specific activities, i.e., large enough to offer a significant dilution factor throughout the 15 min period when synthesis of radioactive subunit B occurred. Otherwise, the specific activity of each amino acid should have been the same in the two subunits. An alternative explanation would be that the two subunits are synthesized on ribosomes separated from each other and would furthermore imply that the m-RNA's are derived from different chloroplast DNA cistrons. The antibiotic experiments would not rule out the further possibility that one of the subunits is synthesized by chloroplast ribosomes and the other by ribosomes outside of chloroplasts with the implication that m-RNA's are derived from different sources of DNA. A choice between the pool vs. the two ribosome interpretation could come from success

in obtaining antibodies specific to the subunit B.

Acknowledgement. The author wishes to record his sincere thanks to Prof. S.G.Wildman for his stimulating discussions and for help in preparing the manuscript. He is also grateful to Misses Y. Sugisaki and M. Yokoyama for their technical assistance.

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