NONIDENTICAL SUBUNITS OF RIBULOSE DIPHOSPHATE CARBOXYLASE*

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Received July 7, 1967

Ribulose diphosphate carboxylase, RuDPCase (E.C. 4.1.1.39, 3-phospho-D-glycerate carboxy-lyase (dimerizing)), catalyzes the carbon dioxide fixation of the Calvin photosynthetic cycle. The native carboxylase from spinach leaf, as prepared in our laboratory (Paulsen and Lane, 1966) is homogeneous by sedimentation analysis, has a molecular weight of 557,000, and is free of nucleic acid, carbohydrate, and contaminating enzyme activities usually associated with "Fraction I" leaf protein (Wildman and Bonner, 1947). A preliminary report (Hazelkorn et al., 1966) suggested that cabbage leaf "Fraction I" protein, purportedly RuDPCase, is composed of 24 identical subunits. Ridley et al., (1967) have calculated a minimal molecular weight of 24,400 for the spinach beet protein from its amino acid composition. During investigations in our laboratory on the subunit structure of spinach leaf RuDPCase, it became evident that this protein is composed of two distinct kinds of non-covalently linked polypeptide chains (subunits) which differ in molecular weight and amino acid composition.

Spinach leaf RuDPCase purified by a procedure slightly modified from that of Paulsen and Lane (1966) was homogeneous by sedimentation velocity analysis at high protein concentration (10-12 mg per ml) and by polyacrylamide gel

*This investigation was supported by Research Grant AM-09117 from the National Institutes of Health, U.S. Public Health Service.

**Career Development Awardee of the U.S. Public Health Service, Research Career Program Award, K3-AM-18487.

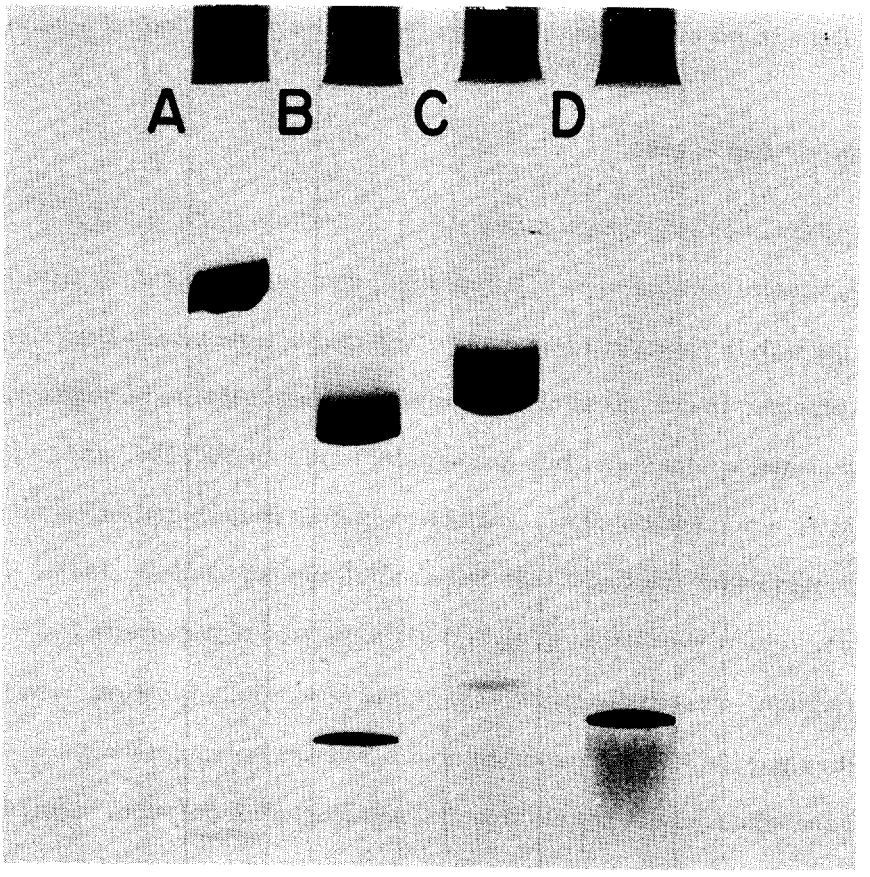

Fig. 1. Polyacrylamide gel electrophoresis patterns. The general procedure of Davis (1964) was followed. Samples were applied to gel columns (5 mm diameter) in 0.1 ml of dilute Tris buffer (pH 8.5) containing 25% sucrose and 0.1 M 2-mercaptoethanol. The cathode buffer contained 10 mM mercaptoethanol and in the case of gels B-D, 0.03% SDS. A current of 3 ma per gel was applied at room temperature until the tracking dye (bromphenol blue) was approximately 1 cm from the bottom of the gel. A 5% acrylamide-0.2% N, N'-methylenebisacrylamide (Bis) gel was employed for A and 10% acrylamide-0.2% Bis gels for B-D. The patterns shown are: A, native carboxylase (200 μg); B, native carboxylase (35 μg) in 0.03% SDS; C and D, aliquots of pooled peaks 1 and 2, respectively, from aminoethylated RuDPCase resolved by gel filtration on a Sephadex G-100 column in 0.5% SDS (see text and Fig. 2.).

electrophoresis (Davis, 1964) at pH 9.3 (see A, Fig. 1.). Sodium dodecyl sulfate (SDS) dissociates RuDPCase into subunits of about 2 S (Trown, 1965). In our hands, 1 mM SDS instantly inactivates the carboxylase, and following dialysis against buffered 1 mM SDS, a single nearly symmetrical sedimenting boundary is obtained in the analytical ultracentrifuge which has an s_{20} of 3.0 S. However, molecular weight determinations on similar preparations by sedimentation equilibrium (Yphantis, 1964) indicated a high degree of weight heterogeneity not attributable to interference by the detergent.

In order to verify the presence of more than one weight class of polypeptide, RuDPCase was analyzed by gel electrophoresis in the presence of SDS. Two distinct bands were evident as illustrated in B, Fig. 1. Since electrophoretic separation of protein-SDS complexes in acrylamide gels is primarily a function of the molecular size of the complex and not the charge of the original polypeptide (Viñuela et al., 1967), an attempt was made to resolve the two polypeptide species on a preparative scale by gel filtration on Sephadex G-100 in the presence of 0.5% SDS.

Carboxylase was prepared for gel filtration by dissociation in SDS and reduction, followed by the blocking of -SH groups by aminoethylation with ethylenimine (Raftery and Cole, 1963). Ninety milligrams of homogeneous enzyme, in 0.5% SDS, 0.14 M 2-mercaptoethanol, buffered at pH 8.6, were incubated for 1 hour at 37° under N_2. Excess ethylenimine was then added and the incubation continued for another hour. Unreacted ethylenimine was decomposed with additional 2-mercaptoethanol and the reaction mixture passed through a Sephadex G-25 column equilibrated with 0.5% SDS to remove excess reactants. The aminoethylated protein was then subjected to gel filtration on a Sephadex G-100 column equilibrated with buffered 0.5% SDS. As illustrated in Fig. 2.,

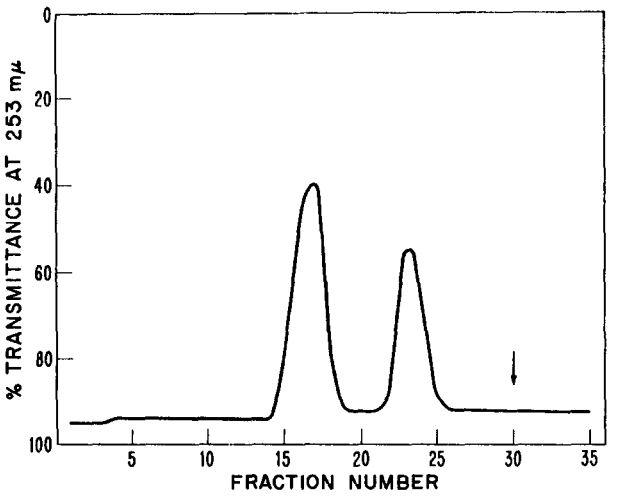

Fig. 2. Gel filtration of aminoethylated RuDPCase on Sephadex G-100 in the presence of SDS. Aminoethylated RuDPCase (60 mg in 15 ml) after prior gel filtration with Sephadex G-25 was applied to a Sephadex G-100 column (2.5 x 90 cm) equilibrated with 50 mM Tris (Cl^-), 0.1 mM EDTA, and 0.5% SDS at pH 8.6. Elution was accomplished at room temperature using the same buffer. Nine milliliter fractions were collected and the effluent was monitored at 253 mμ using an LKB Uvicord Absorptiometer. Peak 1 was eluted with the excluded volume; the included volume is indicated by the small arrow.

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two well-resolved peaks (Peaks 1 and 2) were obtained. The ratio of the total absorbancies at 280 mμ of Peak 1 to Peak 2 was 1.9. Electrophoretic analysis of the pooled peak fractions on polyacrylamide in the presence of SDS revealed their correspondence to the two polypeptide species obtained from SDS-dissociated native enzyme (B, Fig. 1.). The first peak (C, Fig. 1.) consisted primarily of the slow-moving polypeptide observed in the SDS-treated native enzyme (B, Fig. 1.), which was slightly contaminated with the fast-moving polypeptide. The second peak (D, Fig. 1.), however, was the nearly homogeneous fast-moving polypeptide.

After recovery of the protein from the pooled fractions by acetone precipitation (50%, v/v), samples from each peak were subjected to amino acid analysis

to ascertain whether the peaks were in fact different polypeptides or merely aggregates of the same basic unit. It is clear from the large differences in their amino acid compositions shown in Table I that the two peaks constitute different polypeptides. Most striking are the differences in their relative tyrosine, phenylalanine, and glycine contents. The large polypeptide fraction has a phenylalanine:tyrosine:glycine ratio of 1 : 0.9 : 2, while the mole ratio of these residues in the small subunit is 1 : 1.6 : 1.1. From the data in Table I, it is further evident that there are other large compositional differences between the two subunits.

Another indication of subunit heterogeneity and most likely weight dissimilarity is the difference between their sedimentation coefficients in SDS. Elec-

TABLE I. Amino Acid Composition of Native Carboxylase and Subunit Fractions Resolved by Gel Filtration in the Presence of SDS.

Amino acid	Mole ratio[a] (relative to phenylalanine)		
	Native carboxylase	Subunit Peak 1	Subunit Peak 2
Phenylalanine	1.00	1.00	1.00
Tyrosine	1.07	0.92	1.57
Glycine	2.05	2.10	1.15
Lysine	1.15	1.18	1.21
Histidine	0.67	0.67	0.45
Arginine	1.34	1.44	0.99
Aspartic	2.01	2.18	2.14
Threonine	1.37	1.75	1.19
Serine	0.68	0.81	0.77
Glutamic	2.18	2.20	2.26
Proline	1.24	1.13	1.56
Alanine	1.89	2.16	0.86
Methionine	0.45	0.42	0.46
Isoleucine	0.84	0.88	0.56
Leucine	2.00	2.09	1.63
1/2 Cystine[b]	0.46	c	c
Tryptophan[d]	0.64	-	-

[a]Analyses performed on duplicate samples hydrolyzed for 48 hr in 6 N HCl at 110°. [b]Determined as cysteic acid (Moore, 1963). [c]Present as aminoethylcysteine but not quantitated. [d]Determined spectrophotometrically (Goodwin and Morton, 1946).

trophoretically homogeneous (polyacrylamide in SDS) samples of the two polypeptide species were obtained by gel filtration of a "light-load" (7 mg) of aminoethylated RuDPCase on a Sephadex G-200 column (1.5 x 80 cm) in buffered 0.5% SDS. The two widely resolved polypeptides obtained were subjected to sedimentation analysis in the analytical ultracentrifuge using U.V. absorption scanning optics (Schachman and Edelstein, 1966). The large polypeptide (Peak I, Fig. 2.) exhibited an s_{20}=3.0 S and the small polypeptide (Peak 2, Fig. 2.) an s_{20}=1.8 S.

Summary. Homogeneous preparations of spinach leaf ribulose diphosphate carboxylase have been dissociated into subunits with sodium dodecyl sulfate. The dissociated aminoethylated protein was resolved into two distinct polypeptides by gell filtration on Sephadex G-100 and -200 columns in the presence of 0.5% SDS. It has been demonstrated that the two polypeptides have grossly different electrophoretic mobilities, sedimentation velocities, and amino acid compositions. These results clearly indicate that spinach leaf ribulose diphosphate carboxylase is composed of two distinctly different kinds of subunits. Work is in progress to determine the catalytic or possible regulatory role of each subunit species.

We gratefully acknowledge the technical assistance of Miss Dietlinde Wolter in this work.

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