Estimation of the Molecular Weight of Ribulose Diphosphate Carboxylase Sub-units*

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Summary: The molecular weights of the subunits of spinach ribulose diphosphate carboxylase have been estimated as 55, 800 and 12, 000 by SDS-acrylamide gel electrophoresis. It is suggested that the native enzyme has 8 heavy catalytic subunits and 8-10 light structural or regulatory subunits.

Introduction

Ribulose diphosphate carboxylase (3-phospho-D-glycerate carboxy-lyase (dimerizing), E.C. 4.1.1.39) RuDP-case, from spinach leaves, has been shown to be composed of two classes of subunits which differ in their size and amino acid compositions (1). Recently, these results have been confirmed and extended to the enzyme from tobacco (2). Moon and Thompson (3) have likewise shown the RuDP-case from spinach beet chloroplasts to be composed of two types of sub-units. The molecular weights of the reduced and carboxymethylated subunits were estimated to be 54, 000 and 16, 000 by elution position on Sephadex G-200 in urea and each subunit seemed to give one band on electrophoresis in urea at pH 9.5 (3). In contrast to the situation in higher plants, Kuehn and McFadden (4) reported only one electrophoretically homogeneous subunit for the enzyme from two species of Hydrogenomonas, of molecular weight 38, 000 to 40, 700. The native spinach enzyme has been found to have 8 RuDP binding sites per molecule (5, 6).

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In this communication, I describe the results of the determination of the molecular weight of the subunits from the spinach enzyme by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, SDS (7, 8).

Materials and Methods

Proteins used as standards are listed below with their molecular weights (as cited in ref. 8) in parentheses. Bovine serum albumin (fraction V, 68, 000) was obtained from Sigma Chemical Company; cytochrome c (11, 700, dimer 23, 400), pyruvic kinase (57, 000), LDH (36, 000, dimer 72, 000) and lysozyme (14, 300) from Boehringer; catalase (60, 000), ovalbumin (43, 000, dimer 86, 000), trypsin (23, 300) and aldolase (40, 000) from Worthington; chymotrypsinogen from Nutritional Biochemicals and myoglobin (17, 200, dimer 34, 400) from Mann. Homogeneous RuDP-case was prepared as before (1, 9).

The methods of sample preparation and electrophoresis were essentially those of Weber and Osborn (8). Acrylamide and N, N'-methylene bis acrylamide (Eastman) were recrystallized from chloroform and acetone, respectively (10). SDS (Sigma) was recrystallized from absolute ethanol (11). At the end of the run, the portion of the gel distal to the leading edge of the dye marker was cut off, and the protein stained with amido black. The electrophoretic mobility of the protein was calculated from the equation

$$\text{Mobility} = \frac{\text{distance of protein migration}}{\text{length of gel after staining (distance of leading band)}}$$

In duplicate experiments, this technique gave the same results as the more involved measuring method of Weber and Osborn (8).

Results and Discussion

Figure I shows the electrophoretic patterns obtained from a mixture of standards, enzyme alone, and standard proteins plus enzyme. It can be seen that the large subunit of RuDP-case moves faster than pyruvic kinase (Mol. wt.

57,000). The small subunit seems to overlap the position of lysozyme (Mol. wt. 14,200). When the electrophoretic mobilities were plotted against the logarithm

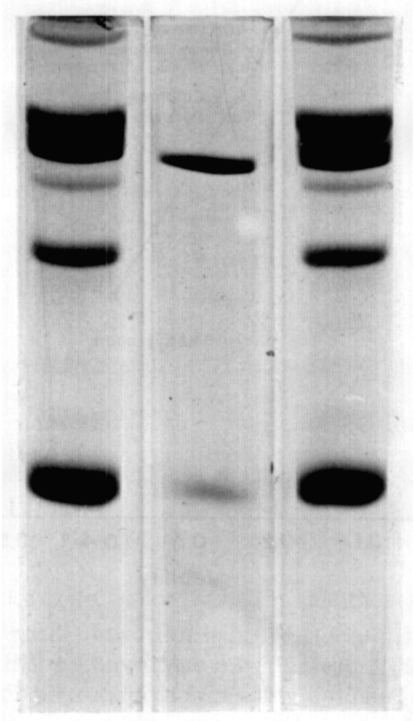

Fig. 1. Electrophoretic mobility of protein standards and native carboxylase. A 10% acrylamide-0.20% bis gel in 0.1% SDS was employed; direction of migration was from top to bottom. The gel on the left contains (reading down) serum albumin, pyruvic kinase, LDH, and lysozyme; the center gel contains the heavy chains (upper band) and light chains (lower band) of RuDP-case. The gel on the right shows the pattern obtained when the standards and enzyme are coelectrophoresed; the heavy chain runs just ahead of pyruvic kinase, while light chain is obscured by lysozyme.

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of the molecular weight, a linear relationship was obtained (Figs. 2 and 3). From triplicate analyses of dissociated native enzyme run on several gel formulations, the subunit molecular weights were found to be 55,800 ± 2,300 and 12,100 ± 1,600. Not shown in Fig. 3 is the observation that lysozyme consistently ran at about the same position as the RuDP-case "light chain" (mobility = 0.672 for lysozyme, 0.681 for light chain; in another experiment). A similar anomaly is reported for lysozyme when it is chromatographed in guanidine HCl on

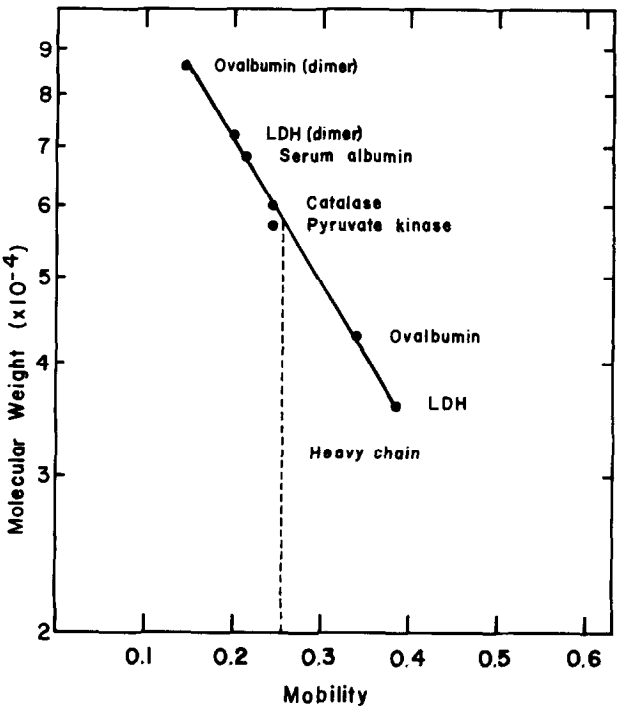

Fig. 2. Electrophoretic mobility of protein standards and native carboxylase. A 10% acrylamide-0.27% bis gel was employed, gel buffer was 0.05 M sodium phosphate, pH 7.0. Samples were electrophoresed for 2.5 hours at 8 mA per gel and 25°. The mobility of the heavy chain in this run was 0.257 ± 0, corresponding to a molecular weight of 58,000. The light chain mobility was 0.778 ± 0.

agarose gels (12). These results are in general agreement with the values of 54,000 and 16,000 for the spinach beet carboxymethylated RuDP-case determined by chromatography on Sephadex G-200 in alkaline urea buffer (3).

In order to ascertain the quaternary organization of RuDP-case, it is necessary to determine the mass ratios of the two subunits in the intact enzyme. Heavy and light chains were prepared from aminoethylated RuDP-case on G-200 as previously described (1). The mass ratios of the two chains were calculated from ratios of total recovered A_{280}, Folin protein, biuret protein, and amino acid analysis as shown in Table 1. Because of the unusually high proportion of aromatic amino acids in the light chain, A_{280} values give a misleading high value

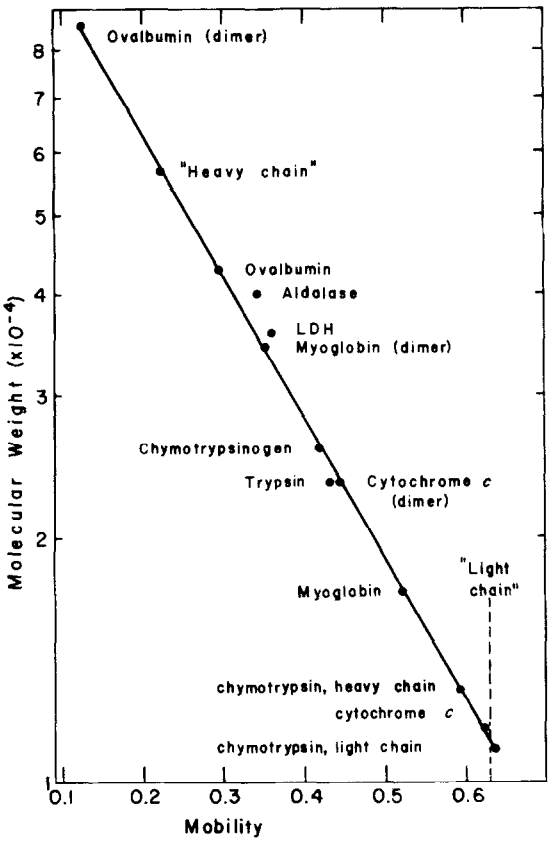

Fig. 3. Electrophoretic mobility of protein standards and native carboxylase. A 15% acrylamide-0.20% bis gel was employed. Other conditions as in fig. 2. The mobility of the light chain was 0.63 ± 0.016, corresponding to a molecular weight of 11, 300. The mobility of the heavy chain in 0.235.

for the percentage of light chain protein. The biuret, Folin and acyl amino acid weights data are the more reliable values. Using these values, it can be calculated that the native carboxylase consists of 7-8 heavy chains and 8-10 light chains (Table 1). Binding studies (6) at low ionic strength with ribulose diphosphate and 2-carboxyribitol diphosphate yield a value n = 8 sites, suggesting 8 catalytic subunits.

The data in this communication do not permit a choice to be made as to which subunit binds ribulose diphosphate. It is tempting to speculate that because of similarities in amino acid composition between the heavy chains of the

Table 1. Distribution of mass between heavy and light chains of aminoethylated RuDP-case resolved by SDS-gel filtration.

Method of analysis	Per cent of total protein		Mass in holo RuDP-case[a]		Number of subunits	
	H.C.[b]	L.C.[b]	H.C.[b]	L.C.[b]	H.C.[b]	L.C.[b]
A_{280}	64.5	35.5	359,300	197,700	6-7	15-17
	64.6[c]	35.4[c]	359,800	197,200	6-7	15-17
Biuret	75.8	24.2	422,200	134,800	7-8	10-12
Folin	77.9[c]	22.1[c]	433,900	123,100	7-8	10-11
Acyl Amino acid weight	85.0[c]	15.0[c]	473,400	83,600	8-9	6-7

[a] Molecular weight 557,000 (9).
[b] H.C. (heavy chain) corresponds to subunit 1, and L.C. (light chain) to subunit 2, previously described (1).
[c] Unpublished data (Rutner and Lane, 1967).

spinach (1), tobacco (2), spinach beet (3) and holoenzyme from bacteria (4), as well as the partial immunological cross reactivity of the plant enzymes (2, 13, 14), the large subunit is the one involved in catalysis. The light chain of the plant enzymes may not be essential for catalysis, but may be of significance in maintaining structural integrity or as binding site for an as yet unidentified allosteric small molecule effector.

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Addendum: Mole ratio of valine omitted from Table 1, ref. 1, is: native carboxylase, 1.60; subunit peak 1, 1.70; subunit peak 2, 1.17.

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