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Rubisco; easy Purification and Immunochemical Determination

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Abstract

Rubisco (Ribulose-1.5-bisphosphate carboxylase/oxygenase) from spinach was purified to homogeneity in one step by gel filtration. This enzyme is suitable for the generation of a specific antibody in rabbits. The enzyme concentration in spinach leaves amounted to 40 % of the total soluble protein. The specific antibody shows cross reaction with crude extracts from leaves of other higher plants. The enzymes subunits could be separated by denaturing preparative SDS gel electrophoresis.

Introduction

Rubisco (Ribulose-1.5-bisphosphate carboxylase/oxygenase) fixes globally about 10^{11} tons of carbon dioxide per year. The native enzyme consist of 8 large and 8 small subunits. In higher plants the large subunit is encoded by the chloroplast, whereas the small subunit is encoded by the nuclear genome. The small subunit is synthesised as a precursor protein in the cytoplasm and is transported into the chloroplast (Cornwell and Keegstra, !987). In red and brown algae both genes are encoded by the plastid DNA and are transcribed bicistronically (Bohnert and Jensen, 1988). The primary structure of the large subunit is conservative, whereas the amino acid composition of the small subunit varies in the plant kingdom. Thus, the large subunit has a molecular weight of about 55 kilo Dalton, whereas the small subunits amounts to 12 to 14 kilo Dalton in green plants (Parry et al., 1987).

Material and Methods

The activity of Rubisco was determined by incorporation of $H^{14}CO_3^-$ into acid stable products according to the method of Groß et al. (1993). Total soluble protein was measured by the method of Esen (1978). Partially purified Rubisco from spinach was purchased from Sigma and purified by gel chromatography with Sephacryl S400 (97·2.6 cm, Pharmacia) in 50 mM Tris-HCl, pH 8 and 50 mM NaCl at 4 °C. The flow rate was 24 ml · h^{-1} and fractions of 9.5 ml were collected. The recorder speed was 5 mm· h^{-1}· The gel was checked beforehand by chromatography of 50 mg cytochrome c. SDS-gel electrophoresis was carried out according to Laemmli (1970) using an acrylamid content of the separating gel of T = 12.5%. Specific antibodies against purified Rubisco were generated as described by Groß et al. (1993). Rubisco concentration was measured by positive ELISA according to the method of Catt and Millard (1988). The calibration curve for this assay was ranged from 0.01 to 1 µg · ml^{-1}.

Double radial immundiffusion was carried out as reported by Ouchterlony (1958). The subunits of rubisco were separated by preparative denaturing gel electrophoresis and eluted electrically from the gel (Groß, 1990).

Results

Partially purified powder of Rubisco from spinach can be bought from the chemical trade. It is available in large amounts as a lyophilised powder with stable activity. This enzyme can easily be purified about 7-fold by gel filtration (table 1). The exclusion limit of the column was clearly separated from the separation limit. Rubisco eluted from fraction 35 to 40 within the separation limit with the main protein peak (figure 1). The enzyme was more or less electrophoretically pure (figure 2) and was used for the production of a specific antibody in rabbits. The antibody produced a single band against leave extracts from spinach. It showed cross reactivity with leaves from other higher plants like peanut, carrot and the purified enzyme from peanut leaves (figure 3). No reactions occurred in diffusion tests against preimmuno serum (data not shown). Rubisco concentration from spinach leaves was determined by ELISA. It amounted to 400,4 ± 4,4 µg per mg total soluble protein (mean ± SD, n = 3).
Rubisco subunits were purified and separated from each other under preparative denaturing conditions. Figure 4 shows the results of this procedure. The two proteins were applied to different lanes of an analytical electrophoretic gel. The large subunit was applied to lane 2, and the small subunit to lane 3. The two parts of the native protein (lane 1) occurred in different lanes without any contamination of the other portion.

Table 1: Purification of Rubisco from spinach leaves by gel filtration on Sephacryl S400

	volume [ml]	protein [mg·ml^{-1}]	specific activity [nkat·mg^{-1}]	purification factor
Rubisco (Sigma)	1.3	75	0.76	1
Rubisco (purified)	57	0.82	5.63	7.4

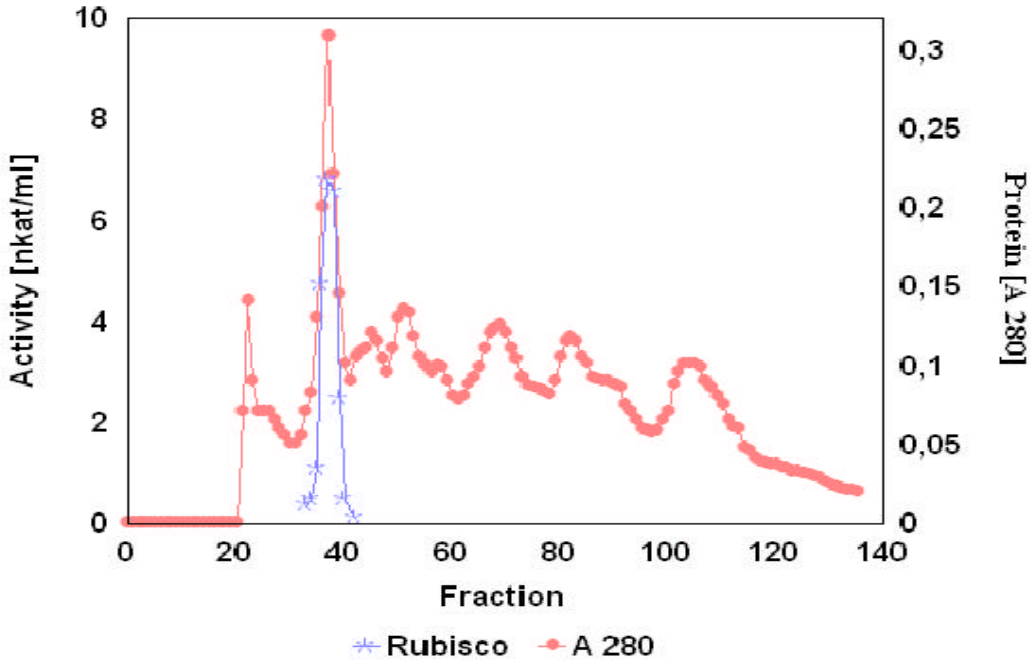

Figure 1: Elution profile of rubisco from spinach leaves on Sephacryl S400; A 280 = absorbance at 280 nm.

Figure 2: SDS-gel electrophoresis of purified rubisco from spinach (30 µg total soluble protein); LSU = large subunit, SSU = small subunit.

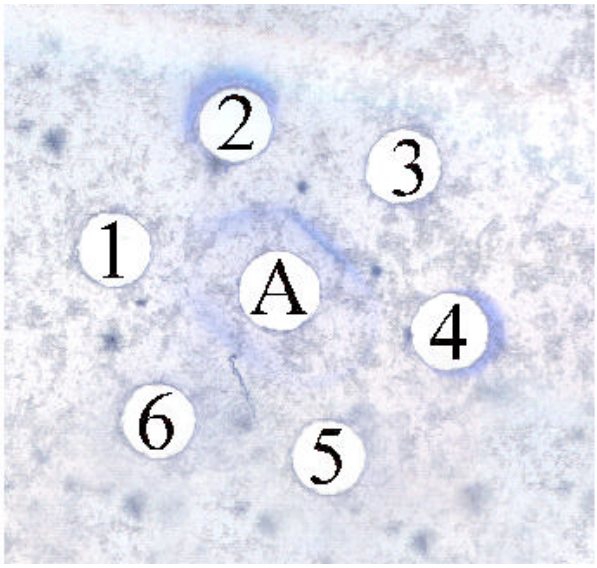

Figure 3: Double radial immunodiffusion: 1, H_2O; 2, crude extract from spinach leaves (4,48 mg ml $^{-1}$ total soluble protein); 3 and 6, crude extract from peanut leaves (6,41 mg ml $^{-1}$ total soluble protein); 4, purified rubisco from peanut leaves (0,7 mg ml $^{-1}$ total soluble protein); 5, crude extract from carrot leaves (3,51 mg ml $^{-1}$ total soluble protein); A, specific antibody against Rubisco from spinach.

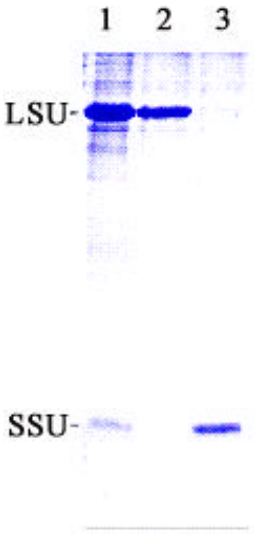

Figure 4: SDS gel electrophoretic pattern of partially purified rubisco from spinach (Sigma) and its separated subunits: 1, partially purified rubisco from spinach purchased from Sigma (30 µg total soluble protein); 2, large subunit (3 µg total soluble protein); 3, small subunit (10,5 µg total soluble protein); LSU = lage subunit, SSU = small subunit.

Discussion

Rubisco activity is regulated by pH and Mg^{2+} concentration. This means that a constant enzyme concentration can vary in ist activity. Furthermore, it has been reported in the literature that a sucrose supplement to the nutrition medium of photoautotrophic peanut cultures reduces rubisco activity without affecting its concentration (Groß et al., 1993). Thus, it is important to measure both enzyme activity and its concentration.
Rubisco from spinach was purified by an easy procedure. This preparation is suitable for the generation of a specific antibody in rabbits. A single precipitation line in an Ouchterlony immunodiffusion assay against crude extract from spinach leaves confirms the specificity of the antibody. Using this antibody, semi- or fully quantitative measurements of rubisco concentrations can be performed according to the method of Catt and Millard (1988). Furthermore, Rubisco obtained from other plant species can easily be purified by affinity chromatography using the immobilized antibody against spinach rubisco.
The two subunits of rubisco were separated and purified by preparative gel electrophoresis. This technique represents a means to investigate the interaction of the chloroplast and nuclear genome by concentration measurement of the enzyme subunits employing the specific antibody against the whole enzyme. Moreover, specific antibodies against the isolated subunits can be easily obtained by immunizing rabbits with the single proteins.

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