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Note

Rapid ion-exchange chromatography for preparative separations of proteins

II. Application to anaerobic purification of ribulose-1,5-bisphosphate carboxylase/oxygenase

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Recently we reported a rapid ion-exchange chromatographic method for the large-scale preparation of proteins[1]. The procedure gave a high flow-rate with maintained chromatographic resolution, by applying a gas pressure to a column packed with a fibrous DEAE-cellulose. It was thus possible to decrease the purification time by a factor of ten compared with that of conventional chromatography, which should be particularly advantageous during separation of unstable proteins. When nitrogen gas is used, proteins that are sensitive to oxidation can be chromatographed under anaerobic conditions.

The enzyme ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (EC 4.1.1.39) is the major soluble leaf protein in plants. It catalyses the primary step of carbon dioxide fixation in Calvin's reductive pentose phosphate cycle, as well as the initial reaction of the competing photorespiration[2]. Since problems with the oxidation of thiol groups of this enzyme leading to inactivation have been noted, the rapid ion-exchange chromatography method performed under non-oxidizing conditions should be very suitable in the preparation of the enzyme. In this paper we report a successful purification of RuBP carboxylase, where all the steps can be completed within one day.

EXPERIMENTAL

Chromatographic resins

Sephadex G-25, Sephacryl S-300 and DEAE-Sepharose CL 6-B were purchased from Pharmacia. DEAE-cellulose DE 23 was obtained from Whatman.

Enzyme purification

The enzyme was purified from spinach leaves, as described by Nilsson and Brändén[3], except that the final time-consuming chromatographic step on DEAE-Sepharose CL-6B was replaced by rapid ion-exchange chromatography on a DEAE-cellulose column (50 × 6 cm I.D., Whatman DE-23 for optimal flow-rate). The equipment is easily set up, as described previously[1]. The pressure in this case was

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achieved by connecting the chromatographic equipment to a nitrogen cylinder.

Before use, oxygen was removed from all solutions in the chromatography with nitrogen. The fractions containing RuBP carboxylase from the penultimate gel filtration step on Sephacryl S-300 in the procedure of Nilsson and Bränden[3] were pooled and applied to the DEAE-cellulose column. The sample was loaded onto the column under pressure at a flow-rate of 30 ml/min. The column was equilibrated with 50 m*M* Tris–HCl, (pH 7.7) containing 0.1 *M* sodium chloride and 2 m*M* dithiothreitol. The proteins were eluted at a flow-rate of 30 ml/min with a linear gradient formed by 0.1–0.6 *M* sodium chloride in the above buffer (2.5 l of each).

Activity measurements

The carboxylase assay was performed as described by Lorimer *et al.*[4], and the protein concentration was determined[5] from the absorption at 280 nm, using an $A^{1\%}_{280\ nm}$ of 16.4 cm^{-1}.

Isoelectric focusing

Electrofocusing was performed on a LKB 2117 Multiphor equipment with LKB ampholine (pH 3–10)[6].

RESULTS

After the initial preparation steps, the final purification of RuBP carboxylase was performed by rapid ion-exchange chromatography on a DEAE-cellulose DE 23 column. A chromatogram from a representative separation is shown in Fig. 1. The applied sample consisted of the pooled enzyme solution from the preceding gel filtration step corresponding to 1.0 g of protein. Fractions 48–80 were pooled and

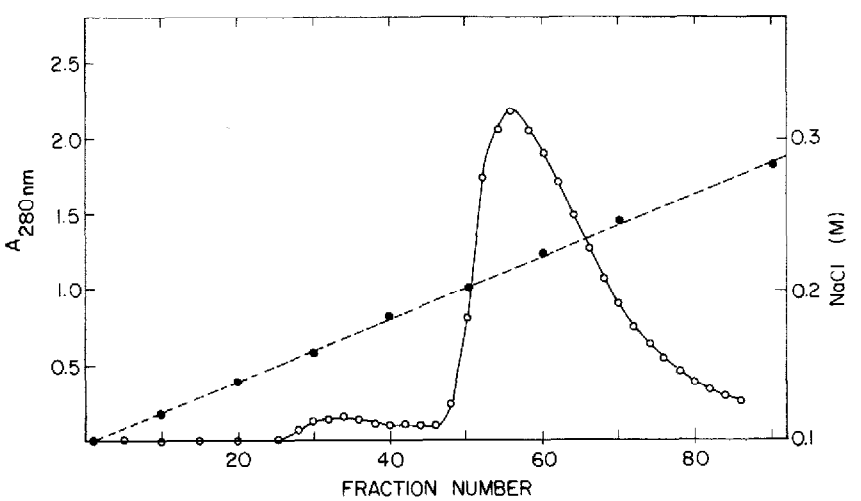

Fig. 1. The final chromatography in the purification of RuBP carboxylase on a DEAE-cellulose DE-23 column under nitrogen pressure (*ca.* 30 kPa). Gel bed dimensions, 50 × 6 cm I.D.; flow-rate, 30 ml/min; fraction volume, 34 ml; temperatures, 8–10°C. Open circles = $A_{280\ nm}$; filled circels = sodium chloride concentration.

contained 0.80 g of the enzyme, giving a yield of 80%. The total time for this chromatographic step was only 90 min. The resolution of the chromatogram is equivalent to that obtained by conventional chromatography on DEAE-Sepharose CL-6B as described by Nilsson and Brändén[3] run in the same column at one-tenth of the flow-rate used here. The specific enzyme activity was determined to 1.8 μmol CO_2/min mg at pH 8.3 and 25°C. This is equivalent to reported values of 1.5–2 μmol/min mg (ref. 3) in this assay. Analysis of the prepared enzyme by isoelectric focusing yielded only a single band with a p*I* value of 5.5.

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