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Note

Isoelectric chromatography: a new approach in the use of ion exchangers for protein purification

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Polysaccharide ion exchangers have become widely used in the purification of proteins since the introduction of cellulose exchangers by Peterson and Sober in 1956[1]; their application has recently been reviewed[2].

The choice of the ion exchanger and of the eluent is determined by the pH stability range of the protein to be purified and by its isoelectric point (p*I*). It is generally suggested that ion-exchange chromatography should be performed under conditions such that the required protein can be absorbed on to the ion exchanger by choosing the initial working pH to be at least one unit below or above the p*I* of the protein when a cation or an anion exchanger, respectively, is employed[3]. Preliminary absorption tests are recommended when the p*I* of the protein is unknown[4].

Nowadays, however, this physico-chemical parameter can be determined easily and accurately by means of analytical isoelectric focusing techniques[5]. In this paper, we suggest a different strategy in the use of ion exchangers for protein purification. If the ion-exchange chromatography is performed consecutively on anion and cation exchangers at the isoelectric pH of the protein to be purified, the chromatographic process results in the absorption of the more acidic and the more basic mixture components, respectively, on the anion and on the cation exchangers. On the other hand, the required protein remains unabsorbed and, therefore, can be recovered in a short time and in a small volume. The method appears to be particularly suitable for a preliminary purification step but it is essential that the protein is soluble and stable at the isoelectric pH.

We used this procedure in the purification of two different cytoplasmatic aspartate aminotransferases and found it to be very satisfactory.

We believe that such a procedure can be of general interest for the purification of proteins and suggest the name "isoelectric" chromatography.

EXPERIMENTAL

Partially purified pig-heart and ox-heart cytoplasmatic aspartate aminotransferases were obtained after the heating and the ammonium sulphate steps from porcine and bovine heart muscle, respectively, following procedures described earlier[6,7] with some modifications as suggested by Christen[8]. After the ammonium sulphate

fractionation, the protein solutions (20 mg/ml) were dialyzed against the appropriate buffers and clarified by centrifugation. The buffers used were 20 m*M* sodium succinate (pH 6.3) for the bovine enzyme and 20 m*M* sodium acetate (pH 5.7) for the porcine enzyme. The ion-exchange resins, SP-Sephadex C-50 and QAE-Sephadex A-50 (Pharmacia, Uppsala, Sweden), were carefully equilibrated with the required buffers as reported by the manufacturer's instructions*.

Two columns (45 × 2.5 cm, SR 25/45, Pharmacia) were filled with the anion- and cation-exchange resins, respectively, and the outlet of the first column was connected with the inlet of the adaptor plunger of the second. The chromatographic process was started when the pH and the ionic strength of the eluate were identical with those of the eluting buffer. Volumes of 40–60 ml of the protein solution were applied on the top of the first column, which generally contained the anion exchanger. A flow-rate of 60 ml/h was used so that the chromatographic procedure lasted about 5 h. Each enzyme was eluted as a slightly tailed front peak in a volume of about 100–120 ml. The active fractions were collected and concentrated by ultrafiltration. Enzyme activities were measured as described by Karmen[9], and proteins were determined by the modified biuret procedure[10]. Gel isoelectric focusing was performed on an LKB 2117 Multiphor apparatus. (LKB, Bromma, Sweden).

RESULTS AND DISCUSSION

In addition to the general approach outlined in the introduction, there are several examples in the literature of the use of single ion exchangers at a pH at which the ampholyte to be purified remains unadsorbed[2]. However, to our knowledge, there are no examples of consecutive ion-exchange chromatography at the isoelectric pH of the ampholyte.

The effectiveness of isoelectric chromatography is related to the absorption of the more acidic and more basic components of a mixture, respectively, on the anion and cation exchangers, which should then work at the maximum actual capacity. Therefore, the exchangers should be completely ionized at the operating pH and the eluent should have the lowest ionic strength compatible with an adequate buffer capacity. Because of their very large pH range of ionization, SP- and QAE-Sephadex can be effectively employed for isoelectric chromatography from pH 3 to 10.

The usefulness of this approach has been demonstrated with the purification of two aspartate aminotransferases whose structural homologies are under investigation in our laboratory. As both of these enzymes are characterized by a pronounced microheterogeneity[11,12], we chose the pH of the eluents to be equal to the p*I* of the main and more active sub-forms[12,13].

Table I reports the results of this purification step for both the bovine and porcine enzymes. The specific activities were increased more than 4-fold in each instance, with a yield of about 85%. As mentioned under Experimental, the procedure took about 5 h. However, the elution peaks were tailed because the protein eluted with the solvent front, consisting of the main active sub-form, is accompanied by the more acidic and less active sub-forms, as shown by the isoelectric focusing pattern of several fractions tested on the tail.

* We had no difficulties in equilibrating the anionic exchangers with anionic buffers such as those mentioned above, provided that the resin had been thoroughly de-gassed in the presence of the acidic component of the buffers.

TABLE I

ISOELECTRIC CHROMATOGRAPHY ON BOVINE AND PORCINE CYTOPLASMATIC ASPARTATE AMINOTRANSFERASES

Enzyme	*Sample*	*Total protein (mg)*	*Total units*	*Specific activity (units/mg)*	*Yield (%)*
Bovine	Initial	1242.0	68,930	55.5	
	Eluate	228.0	58,125	255.0	84.3
Porcine	Initial	721.5	57,999	80.4	
	Eluate	164.2	48,197	293.5	83.1

The resolution power of isoelectric chromatography appears to be insufficient for the separation of proteins whose isoelectric points differ by 0.2–0.3 pH unit (the p*I* values of porcine enzyme sub-forms are 5.69, 5.53, 5.43 and 5.40)[12].

We have included this step routinely in our purification protocol and, although our experience so far is limited to these two proteins, we believe that such a procedure may be convenient as a preliminary and effective purification step for those proteins which are stable and soluble at their isoelectric points.

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