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Note

Purification of potato phosphorylase with hydrocarbon-coated Sepharose

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Recently, the interactions between the hydrophobic parts of protein molecules and hydrophobic groups chemically attached to a hydrophilic matrix such as Sepharose have been studied. Chromatographic separation of proteins, at least partly based on differences in the strength of these interactions, is possible[1–4].

Shaltiel *et al.*[1] purified phosphorylase b [(1→4)-α-D-glucan:orthophosphate D-glucosyltransferase, EC 2.4.1.1] from rabbit muscle by using columns of hydrocarbon-coated Sepharoses (Sepharose-C_n). Their results showed that lengthening of the hydrocarbon side-chain had a marked effect on the capacity of the derivative to bind phosphorylase, varying from zero binding with short chains to very strong binding with side chains containing six or more carbon atoms. The best results were obtained with Sepharose-C_4 (butyl-Sepharose).

We have purified potato phosphorylase by the above technique, although it must be pointed out that there are important structural differences between muscle phosphorylase b and the potato enzyme, as shown by Lee[5]. Potato phosphorylase does not contain serine phosphate, the molecular weight is 207,000 compared to 242,000 for the muscle enzyme, its activity is not influenced by 5′-AMP, and it contains fewer sulphydryl groups than the muscle phosphorylase.

The potato enzyme could be purified directly from the juice by using ethyl-, propyl-, and butyl-Sepharose. A typical elution pattern is shown in Fig. 1, and the full data are given in Table I. No Q-enzyme [(1→4)-α-D-glucan:(1→4)-α-D-glucan 6-D-glucosyltransferase (EC 2.4.1.18)] activity could be detected in the purified preparations. About 70% of the phosphorylase, based on activity measurements, could be eluted from the columns with a salt gradient. Compared to the muscle enzyme, which needs butyl-Sepharose, potato phosphorylase is bound sufficiently strongly to the ethyl-Sepharose. Adsorption to propyl- and butyl-Sepharose was stronger, but the elution patterns were essentially identical.

Shaltiel *et al.*[1] reported a 95% yield of muscle phosphorylase, using structure-deforming buffers containing imidazole. We have not been able to recover the remainder of the potato enzyme in this way. Hofstee[2] found that elution of proteins from columns of agarose-derivatives with salt solutions was only partly successful.

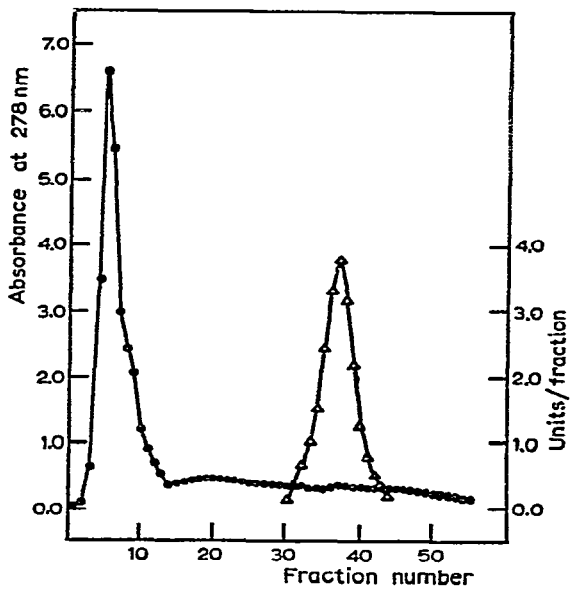

Fig. 1. Chromatographic purification (see Experimental) of phosphorylase from potato juice (10 ml) on propyl-Sepharose: fractions (6.5 ml); —●—, absorbance at 278 nm; —△—, enzymic activity.

TABLE I

CHROMATOGRAPHIC PURIFICATION OF PHOSPHORYLASE FROM POTATO JUICE WITH SEPHAROSE DERIVATIVES

Sepharose derivative	*Amount of juice (ml)*	*Total units of u.v.-absorbance at 278 nm*	*Units of activity*	*Yield of u.v.-absorbance (%)*	*Yield of activity (%)*	*Purification factor*
Ethylamine	5	110	7.0	4.9	72	15
Propylamine	5	105	11.0	3.2	70	22
Ethylamine	10	263	18.9	3.8	67	18
Propylamine	10	288	31.4	5.0	59	12
Butylamine	10	209	19.9	5.1	68	13

The presence of a high concentration of ethylene glycol in the salt solution greatly enhanced the yield. Elution with ethylene glycol in the absence of salts was inefficient. Hofstee concluded that the binding of the protein resulted from a combination of hydrophobic and electrostatic effects. Furthermore, the protein molecules did not all seem to be bound in the same way. These data can be explained simply by assuming different kinds of binding sites on the agarose derivatives; another explanation can be based on differences in protein structure.

When chromatography of purified potato phosphorylase on ethyl-Sepharose was repeated, the recovery of enzymic activity from a first chromatographic run was never more than ~70%, whereas the yield from the second run was 95% using only

a salt gradient. Thus, protein inhomogeneity may be an important factor in this type of enzyme purification.

EXPERIMENTAL

Derivatives of Sepharose-4B (Pharmacia) were prepared by the method of Cuatrecasas[6]. Phosphorylase activity was measured by the methods of Lee[5] and Whelan[7]. One unit of enzymic activity is defined as the amount of enzyme liberating 1 μmole of inorganic phosphate per minute at 35°. A Beckman DK-2 recording spectrophotometer was used for measurement of the u.v.-absorbance at 278 nm.

Potato juice was prepared with a Braun juice centrifuge, with addition of 1 g of sodium bisulphite per kg of potatoes ("Irene") to prevent blackening by phenol-oxidases[8]. The juice was dialysed against 10mM sodium citrate (pH 6.2) containing 0.1 g of sodium bisulphite per litre, and centrifuged for 30 min (4000 *g*) in a Martin Christ UJ III KS refrigerated centrifuge. Column chromatography was carried out at 4° in 10mM sodium citrate buffer (pH 6.2) containing 0.1 g of sodium bisulphite per litre at a flow rate of 20 ml/h. Potato juice (5 or 10 ml) was passed through the columns (1.2 × 17 cm), and elution was continued with 60 ml of buffer followed by a salt gradient composed of 175 ml of buffer and an equal volume of buffer containing 0.3M sodium chloride per litre.

The presence of Q-enzyme activity was determined as described by Gilbert *et al.*[9].

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