Carbohydrate Research, 86 (1980) 161–164
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Chromatography of phosphorylases on *N*-acylchitosan gels

TADASHI BABA, RYUJI YAMAGUCHI, YUJI ARAI, AND TATSURO ITOH
Institute of Applied Biochemistry, The University of Tsukuba, Ibaraki-ken 305 (Japan)
(Received February 4th, 1980; accepted for publication, March 5th, 1980)

Proteins and lipids have been isolated and purified on the basis of the interaction of their hydrophobic areas with hydrophobic groups coated on agarose[1–10]. *N*-Acyl- and *N*-arylidene-chitosan gels[11–15] have been used for immobilisation of an enzyme[16] and as media for gel chromatography[15].

We now report on the use of *N*-acylchitosan gels for hydrophobic-interaction chromatography and the purification of phosphorylase [(1→4)-α-D-glucan: orthophosphate D-glucosyltransferase, EC 2.4.1.1].

A series of *N*-acylchitosan gels having acyl groups $CO(CH_2)_nMe$ where n = 0–4 (chitosans-C_2/C_6) were prepared. Phosphorylase was subjected to chromatography on a column of each gel, and the results are shown in Fig. 1 and Table I. The phosphorylase was retarded by chitosan-C_2 and adsorbed on chitosans-C_3/C_6. The enzyme was eluted from chitosan-C_3 with M NaCl in a yield of over 82% (based on absorption at 280 nm). The adsorption of the enzyme increased as the length of the hydrocarbon chain in the acyl group increased, and the enzyme could not be eluted from chitosan-C_6 with 0.2M acetic acid, ethylene glycol (50%), Triton X-100

TABLE I

CHROMATOGRAPHY OF PHOSPHORYLASE *a* ON *N*-ACYLCHITOSAN GELS

Chitosan gel	*Applied enzyme (mg)*	*Unadsorbed enzyme (%)*	*Eluted enzyme*	
			M NaCl (%)	*0.2M AcOH (%)*
C_2	1.25	95[a]	5[a] (2)[b]	0[a]
C_3	1.04	0	82 (61)	17
C_4	1.16	0	40 (22)	60
C_5	1.35	0	0	6
C_6	1.25	0	0	0

[a]Based on absorption at 280 nm. [b]Based on activity measurement.

0008-6215/80/0000–0000/$ 02.25,

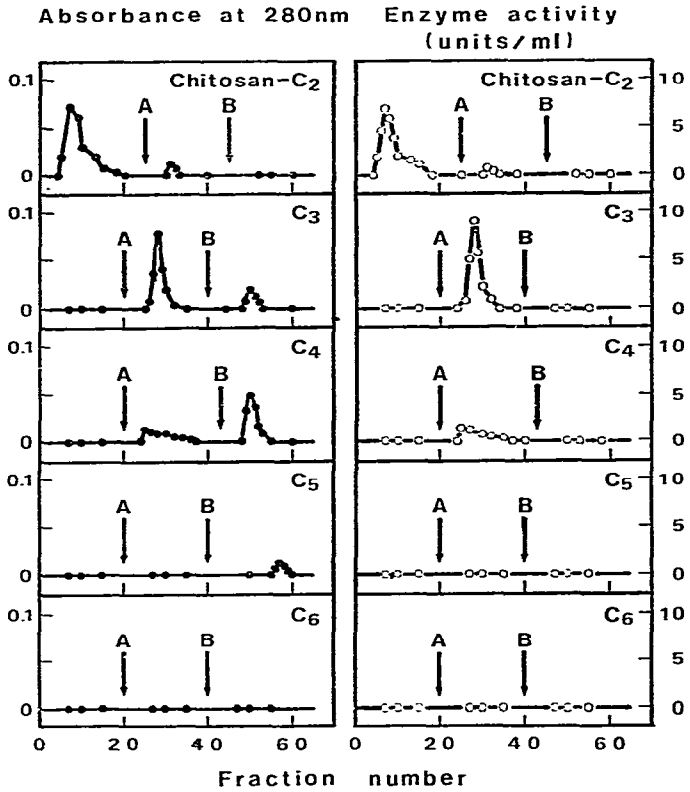

Fig. 1. Chromatography (1-ml fractions) of phosphorylase *a* on *N*-acylchitosan gels by elution with the equilibrating buffer (see Experimental) containing M NaCl (A), followed by 0.2M acetic acid (B) (see also Table I).

(5%), or 0.5M imidazole-HCl (pH 5.6). These data indicated that *N*-propionyl-chitosan gel (chitosan-C_3) was the most suitable for the purification of phosphorylase.

Shaltiel and co-workers[1] achieved a >95% yield of phosphorylase *b* and a 100-fold purification in one step from a rabbit-muscle extract, using butyl-Sepharose. In an attempt to purify rabbit-muscle phosphorylase *a* from a crude extract using chitosan-C_3, it was found that the enzyme could not be desorbed with NaCl and other eluents. However, a better result was obtained with maize phosphorylase, which could be purified from a crude extract by using chitosan-C_2 (Fig. 2), which gave a better result than chitosan-C_3. These results may be due to structural differences between rabbit-muscle phosphorylase *a* and maize phosphorylase. The latter phosphorylase (>60%, based on activity measurement) was eluted with a linear NaCl gradient up to 0.5M, and the specific activity was increased from 0.05 to 1.70 units/mg of protein in one step.

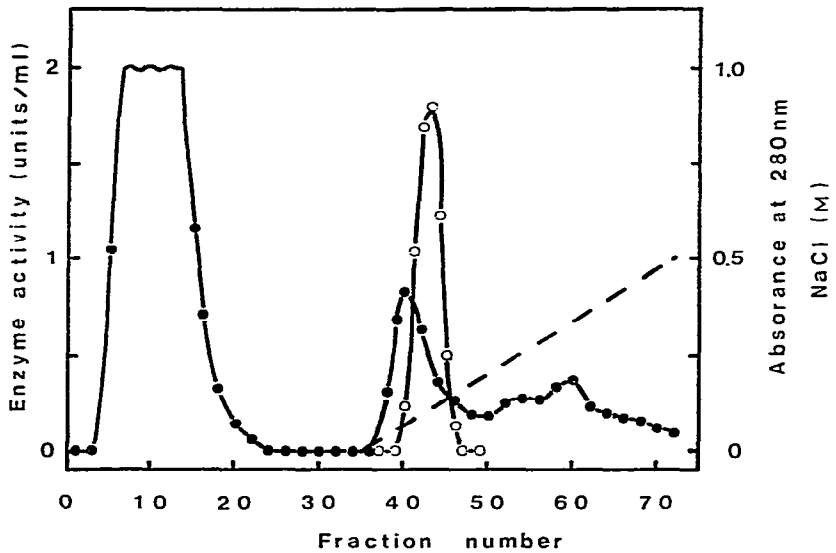

Fig. 2. Purification of maize phosphorylase on a column (1.6 × 10 cm) of chitosan-C_2 (see Experimental). The column was washed first with the equilibrating buffer, and the enzyme was then eluted with the same buffer containing a linear NaCl gradient up to 0.5M (–·–·–·–). The absorbance at 280 nm (—●—) of fractions (3.2 ml) was monitored. Maize-phosphorylase activity (—○—) was determined as described in the Experimental.

EXPERIMENTAL

Reagents. — Rabbit-muscle phosphorylase *a* (twice crystallised and lyophilised powder) and chitin from crab shells were purchased from Sigma.

Preparation of N-*acylchitosan gels.* — Solutions of chitosan[11] {1 g, $[\alpha]_D^{18}$ −15° (*c* 1, 10% acetic acid)} in 10% acetic acid (40 ml) was diluted with methanol (100 ml), and acetic, propionic, butyric, valeric, and hexanoic anhydrides (3 mol. per hexosaminyl residue) were severally added. Each mixture was stored at room temperature overnight, and the resulting gel was broken up into small pieces and homogenised, washed in succession with distilled water, 0.05M NaOH, distilled water, and 0.01M imidazole-HCl buffer (pH 6.8) containing 0.1mM dithiothreitol, and then packed into a column (1.2 × 4 cm).

Assay procedures. — A solution of phosphorylase *a* (~2 mg of protein/ml) was dialysed against 0.01M imidazole-HCl buffer (pH 6.8) containing 0.1mM dithiothreitol, and then applied to each column of *N*-acylchitosan gel that had been equilibrated with the above buffer at 0–4°. Unadsorbed enzyme was eluted with the above buffer and further elution was performed with the same buffer containing M NaCl, followed by 0.2M acetic acid. The absorbance of the eluate at 280 nm was monitored by an Isco U.V. monitor UA-5 (pathlength 5 mm). Phosphorylase *a* was assayed[17] in the absence of AMP.

Maize kernels (~10 g) were ground in a mortar with the above buffer (10 ml),

and then filtered through two layers of cheese cloth. The filtrate was centrifuged at 43,000*g* for 20 min, and then dialysed against the above buffer to give the crude extract of maize phosphorylase. All procedures were performed at 0–4°. The enzyme activity was assayed[18], and units of activity were expressed as μmol of inorganic phosphate released per min.

Proteins were determined as described by the method of Lowry *et al.*[19].

REFERENCES

1 Z. Er-el, Y. Zaidenzaig, and S. Shaltiel, *Biochem. Biophys. Res. Commun.*, 49 (1972) 383–390.
2 S. Shaltiel and Z. Er-el, *Proc. Natl. Acad. Sci. U.S.A.*, 70 (1973) 778–781.
3 Z. Er-el and S. Shaltiel, *FEBS Lett.*, 40 (1974) 142–145.
4 S. Shaltiel, *Methods Enzymol.*, 34 (1974) 126–140.
5 S. Grossman, M. Trop, S. Yaroni, and M. Wilchek, *Biochim. Biophys. Acta*, 289 (1972) 77–81.
6 D. G. Deutsch, D. J. Fogleman, and K. N. von Kaulla, *Biochem. Biophys. Res. Commun.*, 50 (1973) 758–764.
7 B. H. J. Hofstee, *Anal. Biochem.*, 52 (1973) 430–448.
8 H. Hokse, *Carbohydr. Res.*, 37 (1974) 390–392.
9 B. H. J. Hofstee, *Biochem. Biophys. Res. Commun.*, 50 (1973) 751–757.
10 Y. Imai and R. Sato, *J. Biochem. (Tokyo)*, 75 (1974) 689–697.
11 S. Hirano and R. Yamaguchi, *Biopolymers*, 15 (1976) 1685–1691.
12 S. Hirano, Y. Ohe, and H. Ono, *Carbohydr. Res.*, 71 (1976) 315–320.
13 S. Hirano, N. Matsuda, O. Miura, and H. Iwaki, *Carbohydr. Res.*, 71 (1979) 339–343.
14 S. Hirano, O. Miura, and R. Yamaguchi, *Agric. Biol. Chem.*, 41 (1977) 1755–1759.
15 S. Hirano, N. Matsuda, O. Miura, and T. Tanaka, *Carbohydr. Res.*, 71 (1979) 344–348.
16 R. Yamaguchi, Y. Arai, S. Hirano, and T. Ito, *Agric. Biol. Chem.*, 42 (1978) 1297–1299.
17 J. L. Hedrick and E. H. Fischer, *Biochemistry*, 4 (1965) 1337–1343.
18 E. Y. C. Lee and J. J. Braun, *Arch. Biochem. Biophys.*, 156 (1973) 276–286.
19 O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265–275.