Vol. 116, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 31, 1983 Pages 541-546

INTERACTION OF DICTYOSTELIUM DISCOIDEUM α-MANNOSIDASE WITH BEEF LIVER PHOSPHOMANNOSYL RECEPTOR[1]

Effect of Alkaline Phosphatase Treatment

Margarita H.Cladaras, Todd Graham[2], and Arnold Kaplan

Department of Microbiology, St. Louis University School of Medicine
St. Louis, Missouri 63104

Received September 13, 1983

Both intracellular and secreted α-mannosidase from D. discoideum bind quantitatively and specifically to immobilized phosphomannosyl receptor from beef liver. Almost all the intracellular enzyme molecules retain tight binding capacity subsequent to exhaustive alkaline phosphatase treatment. This supports the idea that uncovered phosphates are not essential for optimal recognition by the phosphomannosyl receptor. However, approximately 50% of the extracellular enzyme was converted to a weak binding form by the same treatment.

Mammalian acid hydrolases contain phosphorylated oligosaccharides (1,2,3) which are recognized by a membrane-bound receptor (4,5). The phosphorylated oligosaccharides contain an underlying high mannose backbone (1,2,6) and are thought to play a role in intracellular sorting (7,8). The phosphoryl groups are linked to 6-methoxy moieties of mannoses. Oligosaccharides which contain two phosphomonoesters (one of which is on the branch linked α-1,3 to the β-linked mannose) exhibit optimal binding to the receptor (9,10,11). However, because lysosomal enzymes contain numerous oligosaccharide chains each of which is subject to structural heterogeneity, it has been suggested that their tight binding to receptor may also be achieved by other oligosaccharides which bear two uncovered phosphates (11) or even by the combined interaction of several weakly binding phospho-oligosaccharides with partially or totally covered phosphates (12).

1 This research was supported in part by United States Public Health Service Research Grant #AM21424.
2 Todd Graham is an undergraduate studying for his B.S. in the Department of Biology, Maryville College, St. Louis, Missouri.

0006-291X/83 $1.50

Phosphomannosyl receptors will also interact with acid hydrolases from Dictyostelium discoideum and subsequently trigger their endocytosis by human fibroblasts (13). These enzymes have come into increasing use as reagents for purification of mammalian receptor and as analytical probes for the detection and study of the receptors in mammalian cells (10,11,12,14,15,16). A systematic study of the binding properties of D. discoideum α-mannosidase showed that the qualitative nature of its interaction with fibroblast receptor is identical to that of mammalian lysosomal enzymes (17). The findings of Freeze et al. indicate this is also true of slime mold β-glucuronidase (18).

Freeze et al. (19) found the phosphorylated oligosaccharides derived from D. discoideum acid hydrolases were almost entirely blocked by acid stable groups. We show here that the binding of intracellular α-mannosidase to beef liver receptor is unaffected by alkaline phosphatase treatment. This supports the idea that tight binding can be achieved even if all the involved phosphoryls are blocked. However, tight receptor binding to secreted α-mannosidase is partially destroyed by alkaline phosphatase treatment. Possible reasons for these differences are discussed.

MATERIALS AND METHODS

D. discoideum strain Ax-2 was grown in 12 liters of HL-5 (20) media until cells reached stationary phase. Cells (1.5 x 10^{11}) were separated from media and lysed in 5 mM phosphate, pH 6.5, with 1% Triton X-100. α-Mannosidase was purified from the cell lysate (intracellular enzyme) and from the growth media (secreted enzyme) as previously described (21). Both preparations were exhaustively dialyzed against 20 mM MES, pH 6.5, 0.1 M NaCl, and 0.01% sodium azide before alkaline phosphatase treatment. Beef liver phosphomannosyl receptor was purified from commercial acetone powders (Sigma Chemical Co., St. Louis, Missouri) by specific elution with Man-6-P from a column of immobilized phosphoglycoproteins, obtained from D. discoideum secretions (11), coupled to Affigel-10 as previously described (11). E. coli alkaline phosphatase (Type III-S) and other reagents were obtained from Sigma Chemical Co.

Alkaline phosphatase treatment and receptor binding studies were performed as follows: In each case, 100 units of α-mannosidase were incubated in 1 ml of 20 mM MES, pH 6.5, 0.1 M NaCl, 0.01% sodium azide (reaction buffer) and the indicated units of alkaline phosphatase, at 20°C for 24 hrs. (One unit of alkaline phosphatase is that amount of enzyme which will degrade 1 nM of 4-methylumbelliferyl-phosphate under the conditions described above.) Then 1-3 units of treated α-mannosidase were added to binding buffer (20 mM MES, pH 6.5, 1 mg/ml human serum albumin, 0.05% Triton X-100, 0.1 M NaCl, and 0.01% sodium azide) and applied at a

flow rate of 2 ml/hr to 0.4 ml of Affigel-10-phosphomannosyl receptor, pre-equilibrated in this buffer. The column was washed with 2 ml of binding buffer then with 3 ml of 5 mM Man-6-P in binding buffer. α-Mannosidase in each 0.25 ml fraction was assayed as previously described (21). Seventy to 100% of the applied units were recovered. Activity is reported as percent of units recovered per fraction.

RESULTS AND DISCUSSION

α-Mannosidase, purified from lysed D. discoideum cells, was bound quantitatively to a beef liver phosphomannosyl receptor column and was specifically eluted by 5 mM Man-6-P (Fig. 1A). This result is in agreement with our previous finding that nearly all molecules of this enzyme could be taken up by human fibroblasts (13). The interaction of the enzyme with receptor was essentially unaffected by different alkaline phosphatase treatments (Figs. 1B,C). (No more than 17% of tight binding capacity was lost). Since the chemical evidence (19) indicates that most of the phosphates on the enzyme exist as phosphodiesters, this resistance to

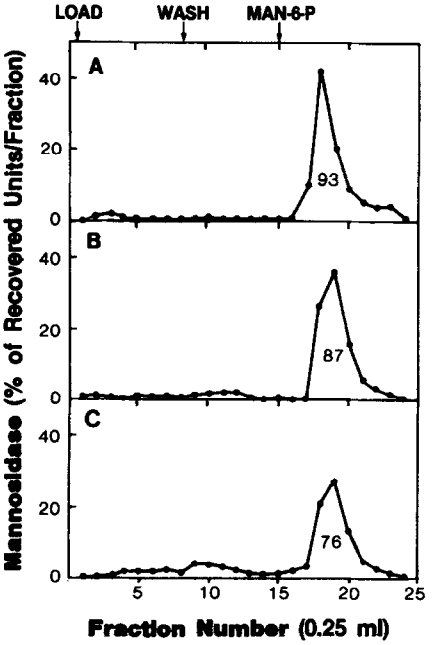

Figure 1. Effect of alkaline phosphatase on the interaction of intracellular α-mannosidase with phosphomannosyl-receptor. A) Incubated without added alkaline phosphatase. B) Incubated with 80 units of alkaline phosphatase. C) Incubated with 80 units of alkaline phosphatase during dialysis. Incubations were performed in test tubes (A and B) or in a dialysis bag which was stirred in 500 ml of reaction buffer (C). The number under the peak represents the percent of recovered units eluted by Man-6-P.

phosphatase treatment can most easily be explained by the idea that a phosphomonoester is not critical for recognition of enzyme by receptor. The view that the phosphates, while accessible to immobilized receptor, are unavailable to phosphatase, although formally possible seems unlikely. Most other trivial explanations tend to be obviated by the experiments with secreted enzyme presented below which, in addition to yielding further information, serve as a positive control for the results obtained with the intracellular enzyme.

α-Mannosidase, purified from the spent medium of stationary phase cells, was also tightly bound to phosphomannosyl receptor and could be eluted with 5 mM Man-6-P (Fig. 2A). Unlike intracellular enzyme, a major fraction of secreted enzyme (40-50%) was converted to weak binding forms by two different alkaline phosphatase treatments (Figs. 2B,C). It would appear that subsequent to phosphatase treatment the enzyme is heterogenous. Whether a particular molecule will bind tightly or weakly may depend on the

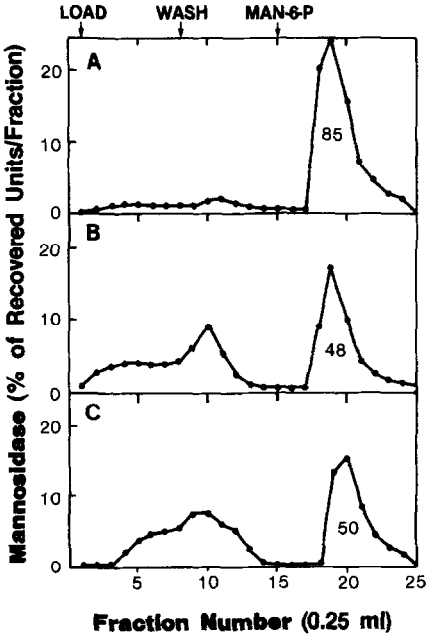

Figure 2. Effect of alkaline phosphatase treatment on the interaction of secreted α-mannosidase with phosphomannosyl receptor. A) Incubated without alkaline phosphatase. B) Incubated with 80 units of alkaline phosphatase. C) Incubated with 80 units of alkaline phosphatase during dialysis. See methods and Fig. 1 for details of experimental procedure.

number of appropriately phosphorylated oligosaccharides present or on the nature of the phosphate blocking groups.

It seems the extent of blockade of phosphoryls on intracellular α-mannosidase is greater than that on secreted enzyme. Freeze et al. (19)performed their chemical studies on enzymes secreted by starved cells. With regard to phosphate blockade, those enzymes behave more like intracellular α-mannosidase than that secreted during growth. This could be due to the presence of deblocking enzymes in spent growth media which catalyze a slow conversion of phosphodiesters to monoesters. The differences might also be generated during synthesis of α-mannosidase, if molecules with unblocked phosphates are preferentially secreted during growth. These alternatives are currently being evaluated.

The structure of the recognition marker on slime mold enzymes appears to be different from that on mammalian enzymes. Determination of its exact chemistry awaits analysis of structure binding studies. However, these fine points of chemical difference between mammalian and slime mold enzyme do not in anyway detract from the use of the latter as functional probes for the mammalian receptor.

REFERENCES

1. von Figura, K. and Klein, U., Eur. J. Biochem. 94, 347-354 (1979).
2. Distler, J., Hieber, V., Sahagian, G., Schmickel, R., and Jourdian, Proc. Natl. Acad. Sci., USA, 76, 4235-4239 (1979).
3. Natowicz, M.R., Chi, M.M.Y., Lowry, O.H., and Sly, W.S., Proc. Natl. Acad. Sci., USA, 76, 4322-4326 (1979).
4. Kaplan, A., Achord, D.T., and Sly, W.S., Proc. Natl. Acad. Sci., USA, 74, 2026-2030 (1977).
5. Sando, G.N. and Neufeld, E.F., Cell 12, 619-627 (1977).
6. Tabas, I. and Kornfeld, S., J. Biol. Chem. 255, 6633-6639 (1980).
7. Neufeld, E.F. and Ashwell, G., in The Biochemistry of Glycoproteins and Proteoglycans (Lennarz, W.J., ed) pp. 252-257, Plenum Press, New York (1980).
8. Sly, W.S. and Fischer, H.D., J. Cell. Biochem. 18, 67-85 (1982).
9. Creek, K.E. and Sly, W.S., J. Biol. Chem. 257, 9931-9937 (1982).
10. Fischer, H.D., Creek, K.E., and Sly, W.S., J. Biol. Chem. 257, 9938-9943 (1982).
11. Varki, A. and Kornfeld, S., J. Biol. Chem. 258, 2808-2818 (1983).
12. Talkad, V. and Sly, W.S., J. Biol. Chem. 258, 7345-7351 (1983).
13. Freeze, H., Miller, A.L., and Kaplan, A., J. Biol. Chem. 255, 11081-11084 (1980).
14. Goldberg, D.E. and Kornfeld, S., J. Biol. Chem. 258, 3159-3165 (1983).
15. Gabel, G.A., Goldberg, D.E., Kornfeld, S., Proc. Natl. Acad. Sci., USA, 80, 775-779 (1983).

16. Campbell, C.H., Fine, R.E., Squicciarini, J., and Rome, L.H., J. Biol. Chem. 258, 2628-2633 (1983).
17. Pannell, R., Wood, L., and Kaplan, A., Fed. Proc. 41, 519 (1982).
18. Freeze, H.H., Yeh, R.X., and Miller, A.L., J. Biol. Chem. 258, 8928-8933 (1983).
19. Freeze, H.H., Yeh, R.X., Miller, A.L., and Kornfeld, S., J. Biol. Chem. in press.
20. Watts, D.J. and Ashworth, J.M., Biochem. J. 119, 171-174 (1970).
21. Pannell, R., Wood, L., and Kaplan, A., J. Biol. Chem. 257, 9861-9865 (1982).