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A SPECIFIC CONCANAVALIN A-MEDIATED BINDING OF BOVINE SERUM α-MANNOSIDASE TO CULTURED HUMAN SKIN FIBROBLASTS

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SUMMARY

A specific elevation of cell-associated α-mannosidase was observed in human skin fibroblasts cultured with concanavalin A for 12-72 hours. There was a latency of several hours before the increase of the enzyme activity occurred. When the cells were washed with α-methylmannoside, α-mannosidase activity was not increased. Other lysosomal enzymes including β-mannosidase showed a slight decrease in activity. It was concluded that the elevation of this enzyme activity was the result of a specific binding to the cell surface mediated by concanavalin A.

INTRODUCTION

Concanavalin A (Con A) has been used for the analysis of cell surface structure from morphological and biochemical aspects (1,2). This lectin is known to bind specifically to α-linked mannose residues which are unsubstituted at C-3, C-4 and C-6 in oligosaccharides of glycoproteins (3). It is mitogenic at low doses and cytotoxic at high doses. The precise mechanism of the effect on cell surface is obscure, although the changes of membrane microviscosity (4) and also of phospholipid metabolism (5,6) have been reported in some lectins. In the present study Con A was added to the culture medium to investigate the effects on biochemical functions of fibroblasts. A specific binding of α-mannosidase in the culture medium to Con A-treated cells was found.

MATERIALS AND METHODS

Skin fibroblasts were cultured in F-10 medium supplemented with 15% fetal calf serum and antibiotics. In some experiments serum-free medium was used for study. The control cells included normal and pathological strains of nonmetabolic or nonlysosomal metabolic diseases. Strains of I-cell disease and mannosidosis were also included in this study. Con A (Difco) was added to various concentrations and the fibroblasts were incubated in these culture madia for various periods up to 72 hours. Cells were

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harvested by scraping off directly or after washing with α-methylmannoside solution (50 mM), and washed three times with isotonic saline. The cell pellets suspended in water were homogenized by sonication. Lysosomal enzymes were assayed with synthetic substrates as described in previous reports (7,8). For most enzymes 4-methylumbelliferyl derivatives (Koch-Light) were used as substrates. Arylsulfatase A was assayed by the method of Baum et al (9) with p-nitrocatechol sulfate (Sigma) as substrate. β-Mannosidase was assayed with p-nitrophenyl β-mannopyranoside (Koch-Light) as substrate (5 mM) at pH 4.3. Protein was determined by the method of Lowry et al (10).

RESULTS

Table 1 shows a specific elevation of α-mannosidase after long-term incubation with Con A. Other enzymes including β-mannosidase showed a slight decrease in activity in sharp contrast to α-mannosidase. In this experiment the cells were not washed with α-methylmannoside. The elevation of α-mannosidase did not occur in the Con A-treated fibroblasts after washing with α-methylmannoside (Fig. 1). In all cell strains studied there was a

Table 1 Lysosomal enzymes in cultured skin fibroblasts with or without Con A

	without Con A (A)	with Con A (B)	B/A
α-mannosidase	26.0 ± 0.5	134 ± 16	5.63 ± 0.70
β-mannosidase	61.4 ± 10.0	49.4 ± 12.8	0.77 ± 0.07
α-galactosidase	41.0 ± 2.7	43.9 ± 4.5	1.06 ± 0.06
β-galactosidase	444 ± 49	281 ± 27	0.59 ± 0.05
α-glucosidase	106 ± 11	66.3 ± 6.4	0.64 ± 0.06
β-glucosidase	149 ± 8	89.6 ± 10.7	0.59 ± 0.04
α-fucosidase	59.0	45.9	0.77
β-glucuronidase	60.1 ± 6.3	45.8 ± 2.8	0.82 ± 0.06
β-N-acetylglucosaminidase	3050 ± 614	2411 ± 472	0.88 ± 0.05
arylsulfatase A	1337	1061	0.80

n=5 (n=2 for α-fucosidase and arylsulfatase A), mean ± S.E.M.
Enzyme activities were expressed as nmol/mg protein/hour.
Fibroblasts were cultured with or without Con A (50 μg/ml) for 72 hours.
Substrate concentrations and assay pH (50 mM citrate): α-mannosidase 1 mM, pH 4.0, α-galactosidase 2 mM, pH 4.0, β-galactosidase 1 mM, pH 4.5 (50 mM NaCl added), α-glucosidase 1 mM, pH 4.0, β-glucosidase 3 mM, pH 5.5 (0.6% sodium taurocholate added), α-fucosidase 1 mM, pH 4.5, β-glucuronidase 2 mM, pH 4.5, and β-N-acetylglucosaminidase 1 mM, pH 4.5

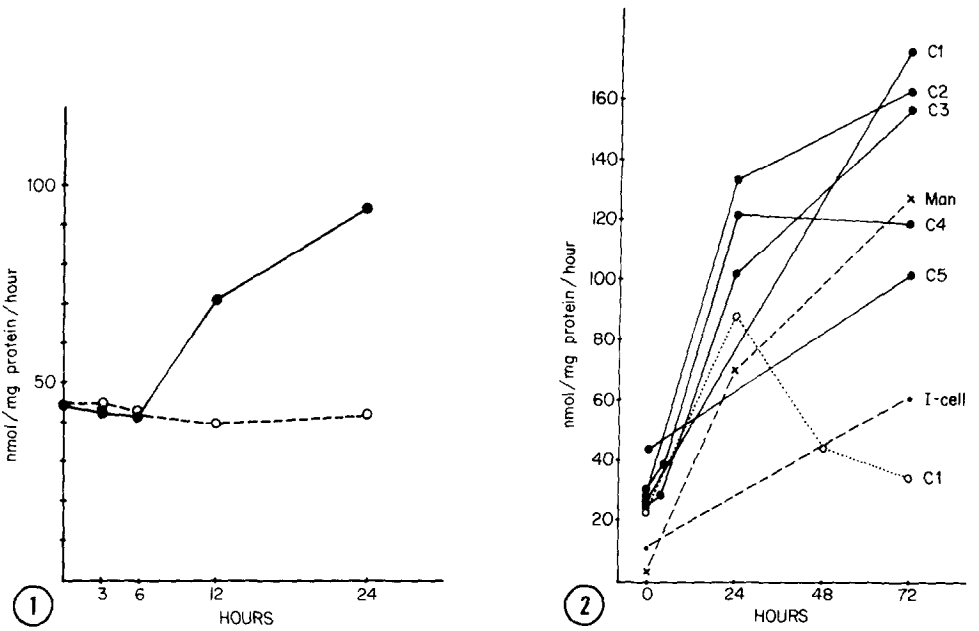

Fig. 1 Time course of α-mannosidase activity
The cells (adult control) were cultured with Con A and α-mannosidase activity was assayed. ●—● washed with isotonic saline, ○- - -○ washed with α-mehylmannoside (50 mM) and then with isotonic saline.

Fig. 2 α-Mannosidase activity after incubation with Con A (50 μg/ml)
●——● control cells. C1: heredodegenerative disease of central nervous system of unknown etiology, C2: glycogen storage disease type 3, C3: spinocerebellar degeneration, C4 and C5: normal adults. •- - -• I-cell fibroblasts (I-cell), x- - -x mannosidosis fibroblasts (Man). ○·····○ The cells (C1) were incubated with Con A for 24 hours, washed with α-methylmannoside and cultured for further 24 and 48 hours without Con A. The cells harvested at 24 hours were not washed with α-methylmannoside.

latency of 6-12 hours before the elevation of α-mannosidase activity (Fig. 2). In one strain the cells were washed with α-methylmannoside at 24 hours, and culture was further continued in Con A-free regular medium. α-Mannosidase activity decreased significantly for the following 24 and 48 hours, but was still higher than the initial activity without Con A. I-cell and mannosidosis fibroblasts also showed the same changes in α-mannosidase activity. The effective concentration for the elevation of α-mannosidase activity was 50 μg/ml. There was no change in the enzyme activity at lower concentrations. At higher concentrations Con A had a strong toxicity and the cells could not survive for a long period. When the cells were cultured in F-10 medium

without fetal calf serum, effect of Con A was not observed on the enzyme activity. This finding excluded the possibility of increased de novo synthesis of enzyme protein by the fibroblasts.

DISCUSSION

From the results described above it can be concluded that the effect of Con A is an increase in specific binding capacity of the cell surface to α-mannosidase in the medium, and not in uptake of enzyme proteins as the cell-associated additional activity was washed off by α-methylmannoside. α-Mannosidase-deficient cells (I-cell disease and mannosidosis) showed essentially the same response to Con A in this respect although the initial α-mannosidase activity was low.

Con A is known to be a tetravalent lectin at neutral pH (11), and may serve as a cross-linking agent between the cell surface and glycoprotein enzyme molecules in the culture medium. However, a specific binding of α-mannosidase with a latency of several hours can not be explained by this type of binding. Several lysosomal enzymes in fetal calf serum were adsorbed to Con A-Sepharose column and eluted with 50 mM α-methylmannoside solution in a preliminary experiment of this study. No selective adsorption of α-mannosidase was observed. At 37°C most lysosomal enzymes in culture medium were moderately inactivated after 24 hours, but N-acetyl-β-glucosaminidase was still as high as α-mannosidase which was least inactivated. At 4°C the cell-associated enzyme activities decreased except that α-mannosidase was slightly elevated. All enzyme activities were preserved at this temperature.

The selective binding of α-mannosidase may be due to a specific affinity to substrate analogue on the cell surface bound to Con A, or a modification of the cell surface induced by Con A. The latter hypothesis may be supported by the fact that the cells cultured with Con A for 24 hours and then washed with α-methylmannoside kept a reltively high α-mannosidase activity after 24-48 hours without Con A. Also the cells incubated with Con A in a serum-

free medium for 24 hours showed a significant increase in α-mannosidase activity within 6 hours after starting culture with fetal calf serum and Con A.

Although the biological significance of this phenomenon is not known, the interaction of α-mannosidase and cell surface may be analyzed by using Con A. Recent studies revealed that urinary lysosomal enzymes were less adsorbed to Con A-Sepharose 4B column in patients with mucolipidosis II and III, human neurometabolic diseases with multiple lysosomal enzyme deficiencies in cultured fibroblasts and an increase of several lysosomal enzyme activities in culture media (12). The aberrant interaction of these enzymes with Con A-Sepharose 4B may be the result of a defect of Golgi-associated α-D-mannosidase which participates in normal processing of lysosmal hydrolases (13). Further analysis of interactions between cell surface and enzyme molecules may be possible by using Con A-mediated binding of α-mannosidase in these diseases.

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