CHARACTERIZATION OF FIBROBLASTS-DERIVED PROLIDASE

-PRESENCE OF TWO FORMS OF PROLIDASE

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Prolidase deficiency is a rare metabolic disorder inherited as an autosomal recessive trait and is associated with characteristic manifestations such as recalcitrant leg ulcers of early onset and mental retardation. This report described the presence of two forms of prolidase in control cultured human skin fibroblasts and their alteration in prolidase deficient fibroblasts by high performance liquid chromatography.

MATERIALS AND METHODS

Fibroblasts derived from normal and control and two prolidase deficient patients(1) were cultured in a 150cm² flask, washed in a 0.9% NaCl solution, suspended in 0.05mol/l Tris-HCl buffer, sonicated for thirty seconds and centrifuged at 10,000Xg for ten minutes. The supernatant was subjected to an HPLC system(Pharmacia LKB,Ul trochrom GTi) equipped with an ion exchange column(TSK DEAE-5PW, 8x75mm, LKB) and eluted with a linear 0 to 250mmol/l NaCl gradient.

Prolidase was assayed as previously described(2).

RESULTS AND DISCUSSION

In control cultured skin fibroblasts, the activity of prolidase-I was eluted by about 90 mmol/l NaCl and the activity of prolidase-II was eluted by about 180 mmol/l NaCl.(Fig. 1). In cultured skin fibroblasts derived from prolidase deficient patients, the activity of prolidase-II was eluted by about 160 mmol/l NaCl. However, the activity of prolidase-I was not revealed against
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Met-Pro by a lower concentration of NaCl. (Data not shown). Recently two forms of prolidase were separated by ion exchange chromatography from most human tissues (3). And in prolidase deficient patients, the activity of prolidase-I was remarkably reduced in cultured skin fibroblasts (3). In this paper, we reported that two forms of prolidase derived from control cultured skin fibroblasts were separated by an HPLC system equipped with a TSK DEAE-5PW column and the activity of prolidase-I of the patients could not be determined. The results obtained in our patients were compatible with those in previous reports (3,4).

Fig. 1.

REFERENCES