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Different effects of sulfur amino acids on prolidase and prolinase activity in normal and prolidase-deficient human erythrocytes

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Abstract

Background: Prolidase and prolinase activity is known to be enhanced significantly in some diseases. Recently, the effect of amino acids on prolidase and prolinase activity in normal and prolidase-deficient human erythrocytes was investigated. It was reported that both enzymes were enhanced by glycine and alanine in the presence of $MnCl_2$.
Methods: Erythrocytes were isolated from heparinized blood from normal human and a patient with prolidase deficiency. Effects of various sulfur amino acids on prolidase and prolinase activities against iminodipeptides in the presence of 1 or 0.1 mmol/l $MnCl_2$ were investigated.
Results: Prolinase activity against prolylglycine in normal and prolidase-deficient erythrocyte lysates was inhibited by L-methionine, NAc-L-methionine and D,L-methionine in a concentration-dependent manner, but D-methionine enhanced the activity in low concentrations (0–20 mmol/l). D,L-Homocysteine inhibited the activity more strongly than other sulfur amino acids tested in a concentration-dependent manner. On the other hand, prolidase activity against glycylproline was enhanced by L-methionine, D-methionine, D,L-methionine, D,L-homocysteine thiolactone and D,L-ethionine. The rates of enhancement by these sulfur amino acids were in the following order: D,L-ethionine > D,L-methionine, D-methionine, D,L-homocysteine thiolactone > L-methionine (10 mmol/l).
Conclusion: The prolinase activity in normal and prolidase-deficient erythrocyte lysates was inhibited by L-methionine, D,L-ethionine and D,L-homocysteine. On the other hand, prolidase activity in their erythrocyte lysates was enhanced by D,L-ethionine, D-methionine and L-methionine. These results indicate the effects of these sulfur amino acids on prolidase and prolinase activities were different.
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Keywords: Prolidase; Prolinase; Erythrocytes; Sulfur amino acid; Prolidase deficiency

1. Introduction

Prolidase (EC 3.4.13.9) and prolinase (EC 3.4.13.8) are 2 enzymes that hydrolyze dipeptides containing proline or 4-hydroxyproline at the C- and N-terminal, respectively. Prolidase deficiency is a rare autosomal recessive disease characterized by chronic ulcerative dermatitis, mental retardation, frequent infections and massive urinary excretion of iminodipeptides [1–3]. It has been reported that the enzyme activity against glycylproline (gly-pro) was almost totally deficient in patients with prolidase deficiency, whereas the activity against other substrates was not as deficient [4,5]. Since then, 2 forms of prolidase were isolated from normal human erythrocytes and cultured skin fibroblasts using diethylaminoethyl (DEAE) cellulose column chromatography and the characteristics of these enzymes were investigated. These 2 forms differed in their responses to $MnCl_2$, substrate specificity and heat stability [6–9]. It has been reported that prolinase in skin fibroblasts and leukocytes from normal human has at least 2 forms when the enzyme is partial purified [9–11]. The prolidase and prolinase activities in erythrocytes or plasma from patients with several diseases

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were determined and found to be enhanced significantly in some diseases [12–14].

Recently, the effect of amino acids on prolidase and prolinase activities in erythrocytes from normal human and patients with prolidase deficiency was reported by Nakayama et al. [15–17]. However, the effect of various sulfur amino acids on prolidase and prolinase activities in erythrocytes has not yet been investigated.

In the present study, we described the effect of various sulfur amino acids on prolidase and prolinase activities in normal and prolidase-deficient human erythrocytes to investigate clinical use of sulfur amino acids for treatment of leg ulcers of patients with prolidase deficiency.

2. Materials and methods

2.1. Chemicals

Iminodipeptides, including glycylproline (gly-pro), methionylproline (met-pro), prolylglycine (pro-gly), prolylalanine (pro-ala), prolylmethionine (pro-met), prolylserine (pro-ser), prolylvaline (pro-val), prolylaspartic acid (pro-asp), prolylglutamic acid (pro-glu) and prolylphenylalanine (pro-phe) were from Backem (VK) Ltd. (St.Helens, Merseyside WA93A5, UK). All other reagents were of analytical grade and obtained from Nacalai Tesque Inc. (Osaka, Japan). Blood samples from 6 normal humans and a patient with prolidase deficiency were collected in heparinized tubes. The preparation of erythrocyte lysates from heparinized blood was carried out using a method based on that described by Umemura with only minor changes [4]. An aliquot of erythrocyte lysates was used as the enzyme solution.

2.2. Enzyme activity

Prolidase and prolinase activities were assayed using several iminodipeptides as substrate. The reaction mixture containing 10 μl of enzyme solution, 80 μl of 50 mmol/l Tris–HCl buffer (pH 7.8), 10 μl of 20 mmol/l or 2 mmol/l $MnCl_2$ and sulfur amino acid was preincubated at 37 °C for 10 min and then incubated with 100 μl of 10 mmol/l substrate in 50 mmol/l Tris–HCl buffer (pH 7.8) at 37 °C for 30 or 60 min. The reaction was stopped by the addition of 200 μl of 10% TCA. The mixture was centrifuged at 12,000 rpm for 5 min and the amount of proline liberated was determined by spectrophotometry using Chinard's method [18]. The kinetic examination of prolinase activity in erythrocyte lysates was determined by the Lineweaver–Burk equation [19]. The protein concentration was determined by the method of Lowry et al. [20] using bovine serum albumin as a standard.

3. Results

The effect of L-methionine, D-methionine, D,L-methionine and NAc-L-methionine on prolinase activity against pro-gly with 0.1 mmol/l $MnCl_2$ was investigated in erythrocyte lysates from both normal human and a patient with prolidase deficiency (Fig. 1A and B). L-Methionine and NAc-L-methionine strongly inhibited both prolinase activities in a concentration-dependent manner. However, the stereoisomer of L-methionine, D-methionine enhanced the activity at a low concentration.

The effect of L-, D-, D,L-methionine and NAc-methionine on prolidase activity against gly-pro in erythrocyte lysates from normal human was also studied (Fig. 1C). All these compounds

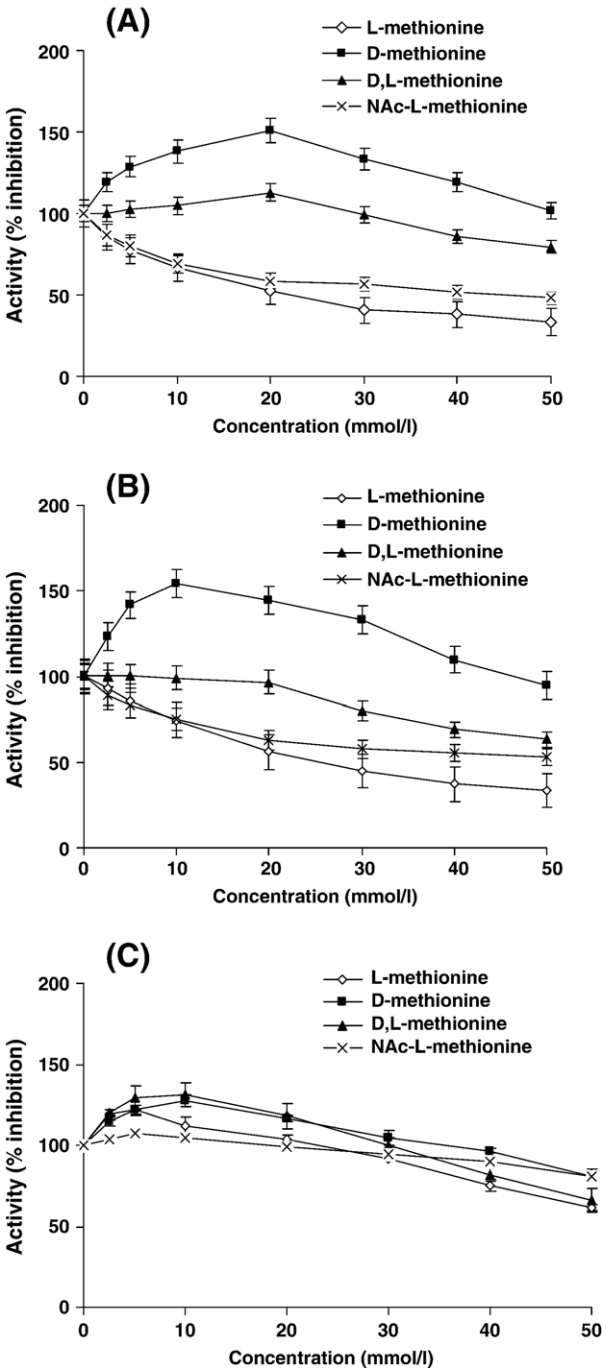

Fig. 1. Effect of D-, L-, D,L-methionine and NAc-L-methionine on prolinase activity against 5 mmol/l pro-gly in erythrocyte lysates from 6 normal humans (A), a patient with prolidase deficiency (B) and prolidase activity against 5 mmol/l gly-pro in erythrocyte lysates from normal human (C). Each value represented mean±S.D. from 6 independent experiments.

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