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ORIGINAL ARTICLE

Characterization of prolidase I and II purified from normal human erythrocytes: comparison with prolidase in erythrocytes from a patient with prolidase deficiency

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Abstract The effect of various sulfur-containing amino acids on the activities of prolidase isoenzymes I and II isolated from erythrocytes of healthy individuals, and erythrocyte lysates from a patient with prolidase deficiency was investigated. The activity of prolidase I against glycylproline was strongly enhanced by D-methionine. L-Methionine and D,L-methionine slightly enhanced the activity at low concentration, but *N*-acetyl-L-methionine had no effect. D-Ethionine, L-ethionine, and D,L-ethionine also enhanced the activity of prolidase I. D,L-Homocysteine enhanced the activity at low concentration, but inhibited the activity at 50 mM. The activity of prolidase II against methionylproline was enhanced by D-methionine, D,L-methionine, and L-methionine, but *N*-acetyl-L-methionine had no effect. D-Ethionine and D,L-ethionine strongly enhanced the activity of prolidase II compared with L-ethionine; D,L-homocysteine weakly enhanced the activity. D,L-Homocysteine-thiolactone inhibited the activities of prolidase I and II in a concentration-dependent manner. The effect of various sulfur-containing amino acids on prolidase activity against methionylproline in erythrocyte lysates from a patient with prolidase deficiency was almost the same as that on prolidase II. The kinetics of the activities of prolidase I, II, and patient prolidase were also studied. Their K_m values were changed by adding sulfur-containing amino acids, but V_{max} values were unchanged.

Keywords Sulfur-containing amino acids · Prolidase isoenzymes · Prolidase deficiency · Erythrocytes

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Introduction

Prolidase (peptidase D; EC 3.4.13.9) is a manganese-requiring homodimeric iminodipeptidase, cleaving only iminodipeptides with carboxy-terminal proline or hydroxyproline. Human prolidase, a polymorphic protein, is a genetic marker on chromosome 19 (Lewie and Harris 1969). Tanoue and co-workers reported that human prolidase is a homodimer with each subunit weighing 54.3 kDa. The prolidase gene is on the short arm of chromosome 19, which spans over 130 kb and consists of 15 exons (Endo et al. 1989). The lymphoblastoid cells taken from the patient used this experiment have abnormal mRNA with skipping of a 192-bp sequence corresponding to exon 14. Translation snf expression analyses using the mutant prolidase cDNA revealed that a mutant protein translated from the abnormal mRNA had an Mr of 49,000 and was enzymatically inactive (Tanoue et al. 1990a, b).

Prolidase deficiency is a rare autosomal recessive disease characterized by chronic ulcerative dermatitis, mental retardation, frequent infections, and massive urinary excretion of iminodipeptides (Powell et al. 1974; Der Kaloustian et al. 1982; Goodman et al. 1968). The disease

has been diagnosed by assay of prolidase activity in erythrocytes (Umemura 1978) and cultured skin fibroblasts (Arata et al. 1979; Buist et al. 1972), and confirmed to be due to hereditary prolidase deficiency (Jackson et al. 1975). It has been reported that the activity of the enzyme against glycylproline (Gly-Pro) was almost totally missing in patients with prolidase deficiency, whereas activity against other substrates remained close to normal (Butterworth and Priestman 1984; Priestman and Butterworth 1984).

Two forms of prolidase, Prolidase I, (PD I) and Prolidase II (PD II), have been isolated from normal human erythrocytes, leukocytes, and cultured skin fibroblasts, and the characteristics of these enzymes have been investigated (Kodama et al. 1989; Myara 1987). Purified PD I and PD II from normal human erythrocytes differed in molecular mass (PD I, 56 kDa, PD II, 95 kDa in SDS-PAGE), response to manganese, substrate specificity, and heat stability (Nakayama et al. 2003; Ohhashi et al. 1990). It has been reported that prolidase activity in erythrocytes from a normal human and a patient with prolidase deficiency was enhanced by pre-incubation with glycine, D-valine, D-leucine, and D-isoleucine, but L-branched amino acids and sulfur-containing amino acids, such as D,L-homocysteine strongly inhibited the prolidase activity (Wang et al. 2004; Uramatsu et al. 2007). However, the effect of various sulfur-containing amino acids on the activities of PD I and II isolated from erythrocytes of a normal human and a patient with prolidase deficiency has not been investigated in detail. In this work we studied the effect of various sulfur-containing amino acids on the activities of control, and patient, PD I and PD II against various iminodipeptides.

Materials and methods

Chemicals

Iminodipeptides including glycylproline (Gly-Pro), methionylproline (Met-Pro), serylproline (Ser-Pro), valylproline (Val-Pro), leucylproline (Leu-Pro), phenylalanylproline (Phe-Pro), and prolylproline (Pro-Pro) were purchased from Bachem (UK) (St Helens, Merseyside, UK). All other reagents were of analytical grade and obtained from Nacalai Tesque (Osaka, Japan) unless otherwise stated.

Blood samples from a normal control and from a patient with prolidase deficiency were collected into heparinized tubes. Erythrocyte lysates were prepared from the heparinized blood using a method described by Umemura (1978) with the minor changes. An aliquot of erythrocyte lysates from the patient with prolidase deficiency was used for enzyme activity assay.

Enzyme activity

Prolidase activity was assayed by using the method descried previously (Kodama et al. 1989) with minor changes. The reaction mixture, composed of 10 µl enzyme solution, 80 µl 50 mM Tris-HCl buffer (pH 7.8), 10 µl 1 mM $MnCl_2$, and the sulfur-containing amino acid, was pre-incubated at 37°C for 10 min, then incubated with 100 µl 10 mM substrate in 50 mM Tris-HCl buffer (pH 7.8) at 37°C for 30 min. The reaction was stopped by adding 200 µl 10% trichloroacetic acid. The mixture was centrifuged at 8,000 × *g* for 10 min, and the amount of proline liberated was determined by spectrophotometry using Chinard's method (Chinard 1952).

Kinetic studies of the activities of prolidase I and II from erythrocytes of a normal human and the patient with prolidase deficiency were performed by using the Lineweaver Burk equation (Lineweaver and Burk 1934). The concentration of protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Enzyme purification

The purification of PD I and PD II from erythrocytes was carried out by the method of Nakayama et al. (2003) with minor modifications. Erythrocytes from 50 ml normal human whole blood were washed three times with cold 0.9% NaCl followed by centrifugation at 1,000 × *g* for 10 min. The erythrocytes were hemolyzed by addition of three volumes of cold distilled water. The hemolysates were diluted with an equal volume of 50 mM Tris-HCl (pH 7.4) containing 10 mM mercaptoethanol (Buffer A) and were then centrifuged at 15,000 × *g* for 10 min to remove membranes. Diethylaminoethyl-cellulose, pre-equilibrated with Buffer A, was added to the hemolysate (200 g/l of hemolysate). After stirring for 3 h, the suspension was poured into a column (5 × 50 cm), and washed with Buffer A until the absorbance at 280 nm of the pass-through fraction decreased below 0.2. The enzymes were eluted by stepwise addition of Tris-HCl buffer (pH 7.4) from 150 to 250 mM. The enzyme-rich fractions were pooled and were subjected to ammonium sulfate fractionation (40–70%). The precipitates were dissolved in 10 mM Tris-HCl buffer (pH 7.4) containing 10 mM mercaptoethanol (Buffer B) and the solution was dialyzed against the buffer overnight to remove ammonium sulfate. The dialysates were applied to a Sephadex G-150 column (2 × 60 cm) equilibrated with Buffer A. The fractions containing PD I and PD II were collected and were brought to 70% saturation by adding ammonium sulfate; the precipitates were then dialyzed as described above. The dialysates were applied to a hydroxyapatite column (2 × 10 cm) pre-equilibrated with Buffer C,

a mixture of equal volumes of Buffer B and 2 mM Na-phosphate buffer (pH 7.4). The column was washed with Buffer C, and the enzymes were eluted with a high concentration phosphate buffer, a mixture of equal volumes of Buffer B and 100 mM Na-phosphate buffer (pH 7.4). The fractions containing PD I and PD II were pooled (each enzyme separately), concentrated by ultrafiltration, and used to study the effect of sulfur-containing amino acids on the activity of each enzyme.

Statistical analysis

The results are expressed as mean ± SD of six values. The effect on each condition was examined using one way analysis of variance. Individual difference between groups were evaluated using Dunnett's test, and those at $P < 0.05$ were considered significant.

Results and discussion

In our previous study, we found that the activity of PD I against Gly-Pro was significantly enhanced by glycine in the presence of $MnCl_2$, but not in the absence of $MnCl_2$. On the other hand, the activities of PD II and the patient's prolidase against Gly-Pro were very low, even in the presence of $MnCl_2$. The activities of both enzymes against Met-Pro were enhanced three fold by glycine in the presence of $MnCl_2$ (Nakayama et al. 2003). Recently, we have reported that the activity of prolidase I against Gly-Pro and Met-Pro was enhanced by the D-isomers of valine, leucine, and isoleucine, but the L-isomers of branched amino acids inhibited the activity of prolidase I. On the other hand, the activities of PD II and the patient's prolidase were enhanced by all the L- and D-branched amino acids (Liu et al. 2005). The activities of prolidase in erythrocyte lysates from a normal human and a patient with prolidase deficiency were also inhibited by the L-isoform of branched amino acids, for example L-methionine and some sulfur-containing amino acids, for example D,L-homocysteine.

In this study we examined the effect of various D- and L-isomers of sulfur-containing amino acids on the activities of PD I and PD II from the erythrocytes of a normal human and a patient with prolidase deficiency.

Figure 1a shows the effect of L-methionine, D-methionine, D,L-methionine and NAc-L-methionine on the activity of PD I against Gly-pro in the presence of 0.1 mM $MnCl_2$. The activity was significantly enhanced by D-methionine. L-Methionine, and D,L-methionine were also able to enhance the activity at low concentrations. The magnitude of their effect was: D-methionine > D,L-methionine > L-methionine. NAc-L-methionine had no effect. These results indicated that compounds containing free amino residue at the α-carbon position were essential for enhancement of prolidase activity, as reported in a previous paper (Liu et al. 2005). L-Ethionine, D-ethionine, and D,L-ethionine also enhanced prolidase activity. The magnitude of their effect was D-ethionine > D,L-ethionine > L-ethionine (Fig. 1b). D,L-Homocysteine enhanced at low concentrations, but inhibited the activity at 50 mM (Fig. 1c). On the other hand,

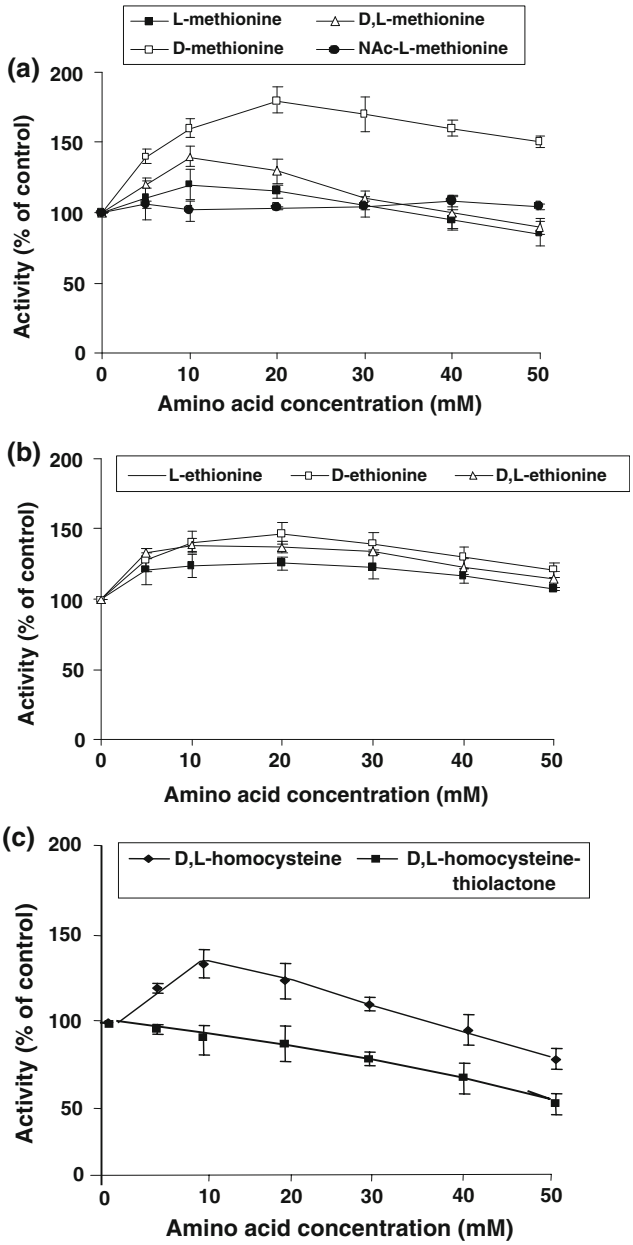

Fig. 1 Effect of various sulfur-containing amino acids on the activity of prolidase I from normal human erythrocytes against Gly-Pro in the presence of 1 mM $MnCl_2$. **a** L-methionine, D-methionine, D,L-methionine, and NAc-L-methionine; **b** L-ethionine, D-ethionine, and D,L-ethionine; **c** D,L-homocysteine, and D,L-homocysteine thiolactone. Each value is presented as the mean ± SD from six independent experiments

D,L-homocysteine thiolactone significantly inhibited in concentration-dependent manner.

The effect of L-methionine, D-methionine, and D,L-methionine on PD II against Met-Pro in the presence of 0.1 mM $MnCl_2$ is shown in Fig. 2a. L-Methionine, D-methionine, and D,L-methionine, but not NAc-L-methionine, enhanced the enzyme activity. D-Ethionine significantly enhanced the activity, while L-ethionine had less effect. D,L-Homocysteine also weakly increased the activity while D,L-homocysteine thiolactone had a concentration-dependent inhibitory effect.

L-Methionine, D-methionine, and D,L-methionine also resulted in positive modulation, but NAc-L-methionine was found to reduce enzyme activity (Fig. 3a). D-Ethionine, L-ethionine and D,L-ethionine increased this activity (Fig. 3b), and the enhancing effect of D-ethionine and D-methionine was more pronounced than that of the other isomer. D,L-Homocysteine significantly enhanced the activity (Fig. 3c), but D,L-homocysteine thiolactone inhibited the activity in concentration-dependent manner.

The effect of cystathionine on the activity of PD I, PD II and patient prolidase against Met-Pro is shown in Fig. 4. The activity of PD II from a normal human and from a patient with prolidase deficiency was enhanced more significantly than that of PD I. The activity of PD II and the patient's prolidase was not inhibited by 50 mM cystathionine. Although the relationship between the chemical structure and the effect on prolidase activity is unclear at present, it might be noteworthy that the substitutive residues linking to the sulfur position of homocysteine contribute to the effect on PD I, PD II, and patient prolidase.

Figure 5 shows the effects of 20 mM and 50 mM D,L-homocysteine on the activities of PD I, PD II, and the patient's prolidase against various iminodipeptides in the presence of 0.1 mM $MnCl_2$. The activities of PD II and the patient's prolidase against Gly-Pro were very low even in the presence of 0.1 mM $MnCl_2$ compared with other substrates, as reported in a previous paper (Nakayama et al. 2003). 20 mM D,L-homocysteine enhanced all prolidase activities compared with control (Fig. 5a–c). The activity of PD I against various iminodipeptides was inhibited by addition of 50 mM D,L-homocysteine compared with the activity of the control. On the other hand, the activities of PD II and the patient's prolidase against various iminodipeptides were inhibited by 50 mM D,L-homocysteine compared with the activity of 20 mM D,L-homocysteine, but the activities of th two prolidases against iminodipeptides tested were almost the same as that of the control.

The effects of 20 mM D-methionine and NAc-L-methionine on the activities of PD I, PD II, and the patient's prolidase against various iminodipeptides are shown in Fig. 6. All the activities of these three prolidases against the iminodipeptides examined were enhanced by 20 mM

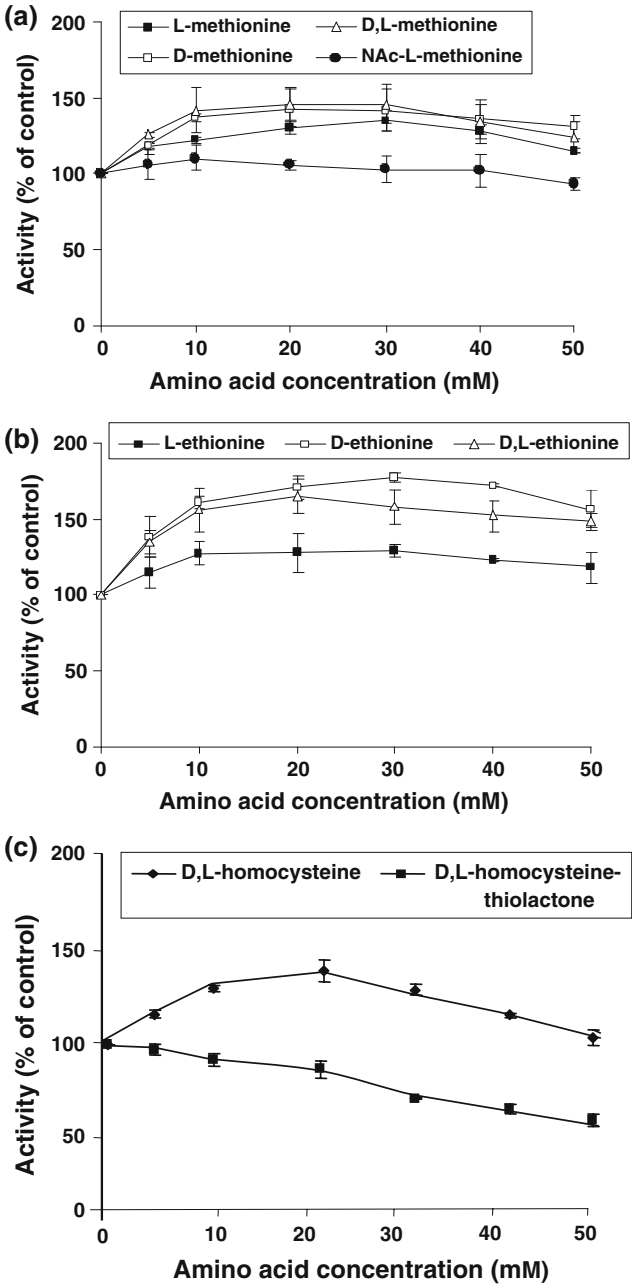

Fig. 2 Effect of various sulfur-containing amino acids on the activity of prolidase II from normal human erythrocytes against Met-Pro in the presence of 1 mM $MnCl_2$. **a** L-Methionine, D-methionine, D,L-methionine, and NAc-L-methionine; **b** L-Ethionine, D-ethionine, and D,L-ethionine; **c** D,L-Homocysteine and D,L-homocysteine thiolactone. Each value is presented as the mean ± SD from six independent experiments

D-methionine. On the other hand, NAc-L-methionine had no effect against any of the iminodipeptide tested.

The effects of 20 mM L-ethionine and 20 mM D-ethionine on PD I and II, and on the patient's prolidase against various iminodipeptides are shown in Fig. 7a–c. Both 20 mM L-ethionine and D-ethionine enhanced the activities of all these prolidases against the iminodipeptides tested.

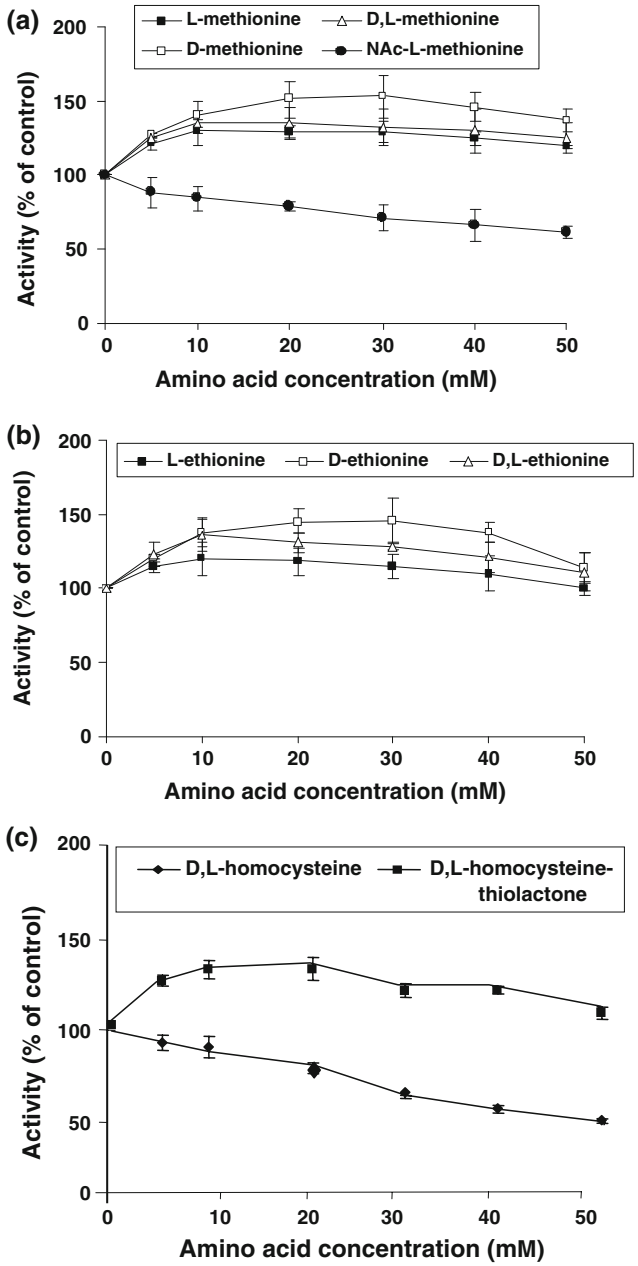

Fig. 3 Effect of various sulfur-containing amino acids on the activity of prolidase in erythrocytes from a patient with prolidase deficiency against Met-Pro in the presence of 1 mM $MnCl_2$. **a** L-Methionine, D-methionine, D,L-methionine, and NAc-L-methionine; **b** L-Ethionine, D-ethionine, and D,L-ethionine; **c** D,L-Homocysteine and D,L-homocysteine thiolactone. Each value is presented as the mean ± SD from six independent experiments

The enhancing effect of D-ethionine was more significant than that of L-ethionine. The effects of D- and L-ethionine on PD II and on the patient's prolidase against Gly-Pro were weak compared with those of PD I against other iminodipeptides.

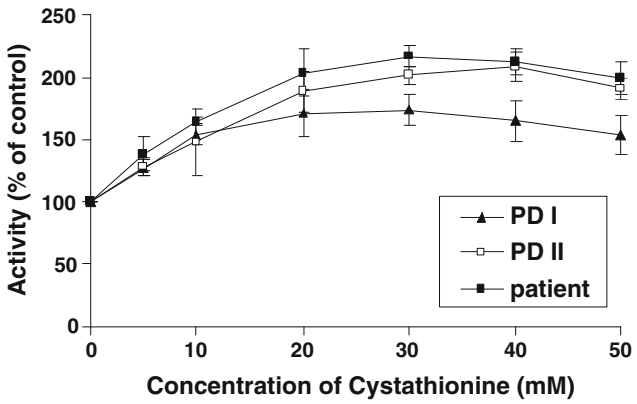

Fig. 4 Effect of L-cystathionine on the activity of prolidase I against Gly-Pro, and on the activity of prolidase II and prolidase in erythrocytes from a patient with prolidase deficiency against Met-Pro in the presence of 1 mM $MnCl_2$. Prolidase I and II were extracted from normal human erythrocytes. Each value represents the mean ± SD from six independent experiments

These results can be summarized as follows:

1 the activities of PD I, PD II, and the patient's prolidase against various iminodipeptides were prominently enhanced by the D-form, but not the L-form, of methionine and ethionine in the presence of 0.1 mM $MnCl_2$; and
2 the enzymatic properties of the patient's prolidase were similar to those of PD II.

In order to reveal the underlying mechanism of the effects that these amino acids and the stereoisomers exert on the prolidase activities, we studied the kinetics of these prolidases. As shown by the results listed in Table 1, K_m values of PD I for Gly-Pro and Met-Pro was reduced by adding 20 mM D-methionine, ethionine, and D,L-homocysteine, but the V_{max} value did not change, suggesting increased affinity of prolidase toward Gly-Pro and Met-Pro.

In contrast, 50 mM D,L-homocysteine did not affect the K_m value, but lowered the V_{max} value. These results were well consistent with the data shown in Fig. 5a. The kinetic values of PD II and the patient's prolidase were determined using Met-Pro as a substrate, because the activities of these prolidases against Gly-Pro were very low. K_m values of PD II and the patient's prolidase for Met-Pro were decreased by adding 20 mM methionine, ethionine, and D,L-homocysteine, but their V_{max} were not affected. K_m values of PD II and the patient's prolidase were increased by adding 50 mM D,L-homocysteine compared with adding 20 mM D,L-homocysteine, but the V_{max} values were not affected. This result are consistent with previous evidence from our studies (Kodama et al. 1989; Ohhashi et al. 1990; Liu et al. 2005).

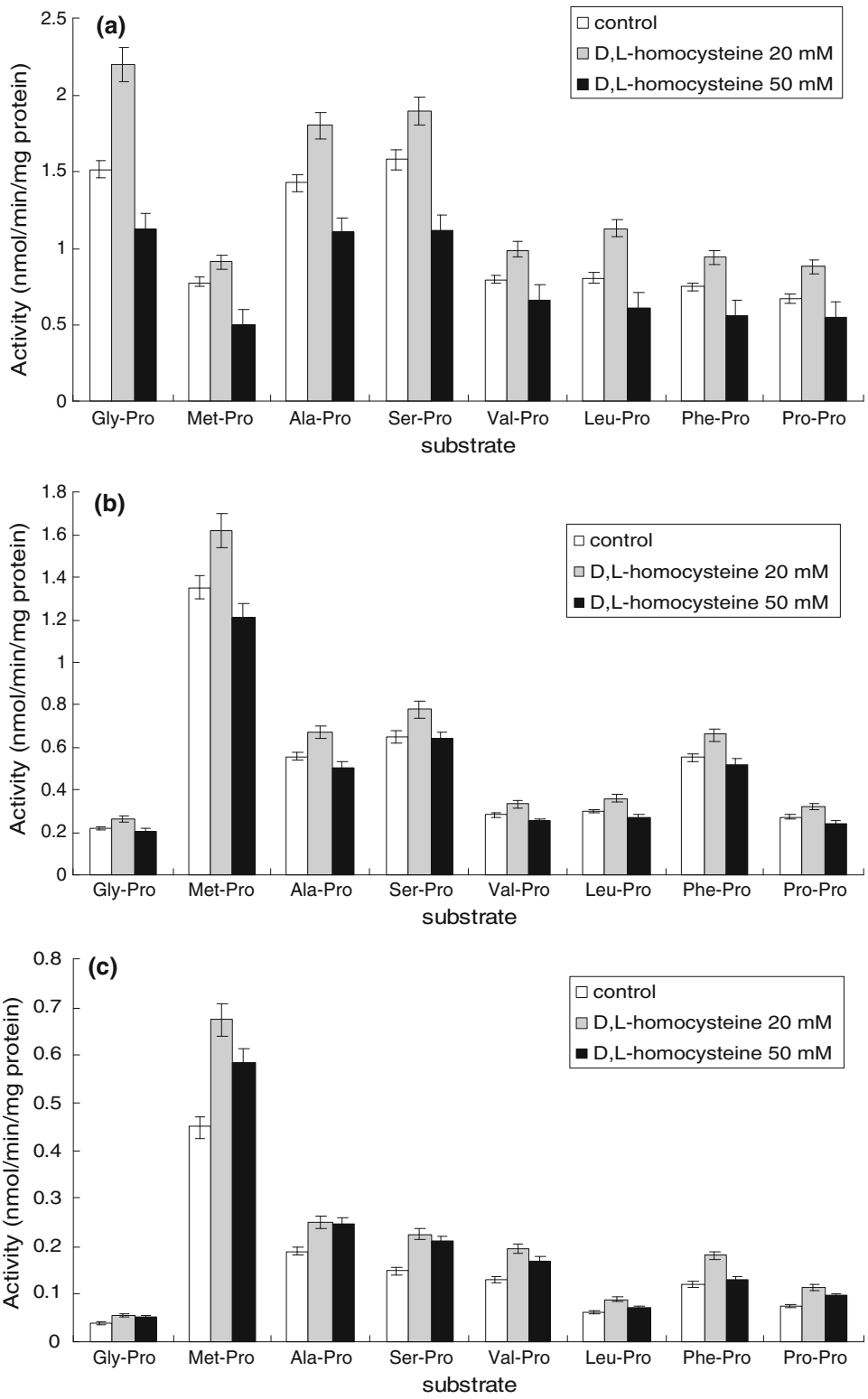

Fig. 5 Effect of D,L-homocysteine (10 mM or 50 mM) on the activity of prolidase I (**a**) and prolidase II (**b**) from normal human erythrocytes and on prolidase activity in erythrocyte lysates from a patient with prolidase deficiency (**c**) against various iminodipeptides (10 mM). Each value is presented as the mean ± SD from six independent experiments

One of the problems of prolidase deficiency is chronic recurrent leg ulcers that are recalcitrant to heal. Ulcers in prolidase deficiency have been attributed to the impaired recycling of proline (Pedersen et al. 1983). Arata et al. used 5% glycine 5% proline ointment to treat ulcers on the left leg, and the results were compared with those obtained using 5% proline ointment on the right leg. Three weeks later, there was an obvious difference with greater improvement observed with the 5% glycine and 5% proline ointment (Arata et al. 1986). Hechtman mentioned that

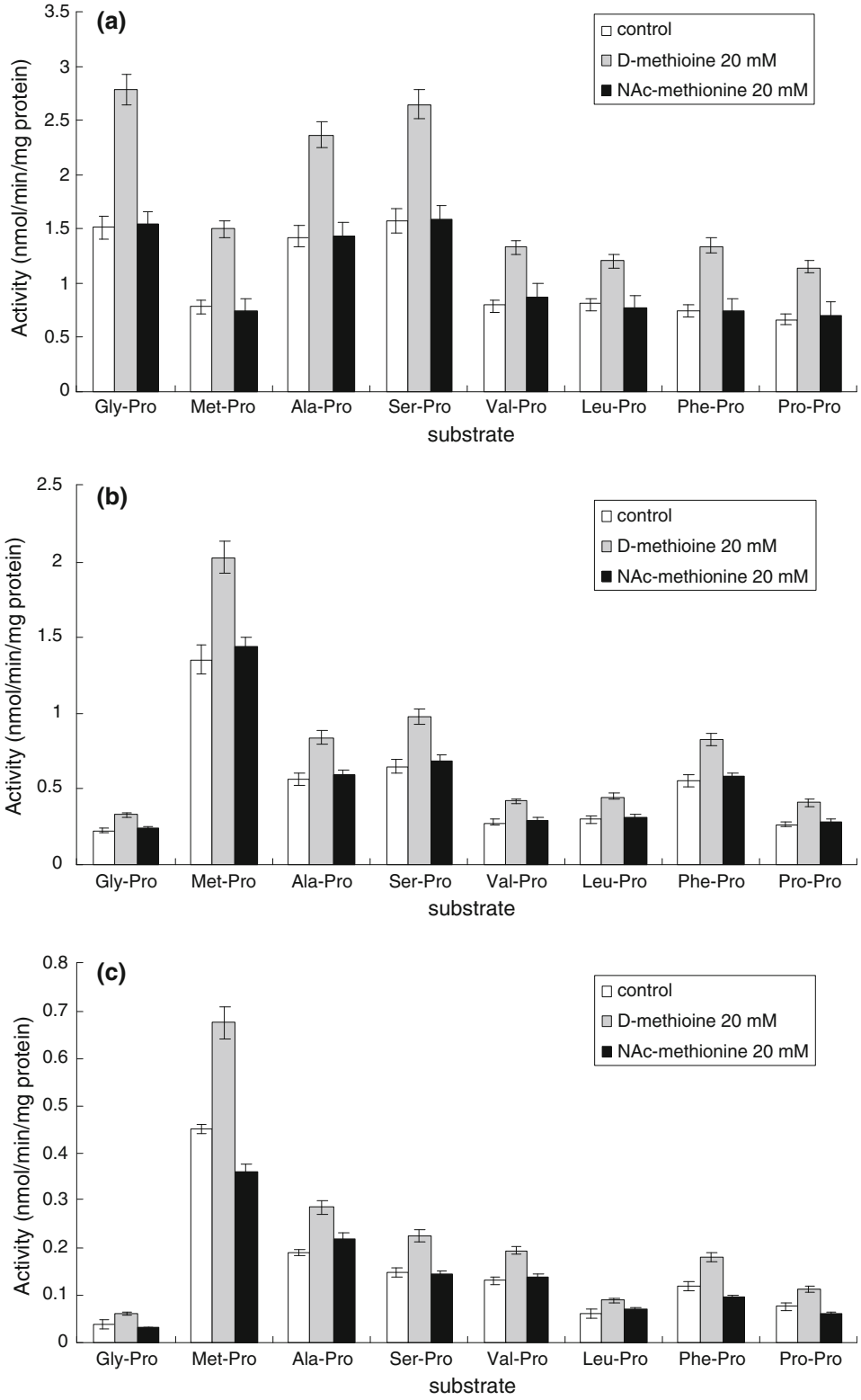

Fig. 6 Effect of D-methionine (20 mM) or NAc-L-methionine (20 mM) on the activity of prolidase I (**a**) and prolidase II (**b**) from normal human erythrocytes and on prolidase activity in erythrocyte lysates from a patient with prolidase deficiency (**c**) against various iminodipeptides (10 mM). Each value represents the mean ± SD from six independent experiments

donor erythrocytes activated with $MnCl_2$ before transfusion would play a significant role in the recovery of proline from dietary sources of iminodipeptides in a patient with prolidase deficiency (Hechtman et al. 1988). We reported that the sulfur-containing amino acids, L-methionine and D,L-homocysteine significantly inhibited the activities of prolidases in erythrocyte lysates from a normal human and the patient's prolidase with various substrates, but

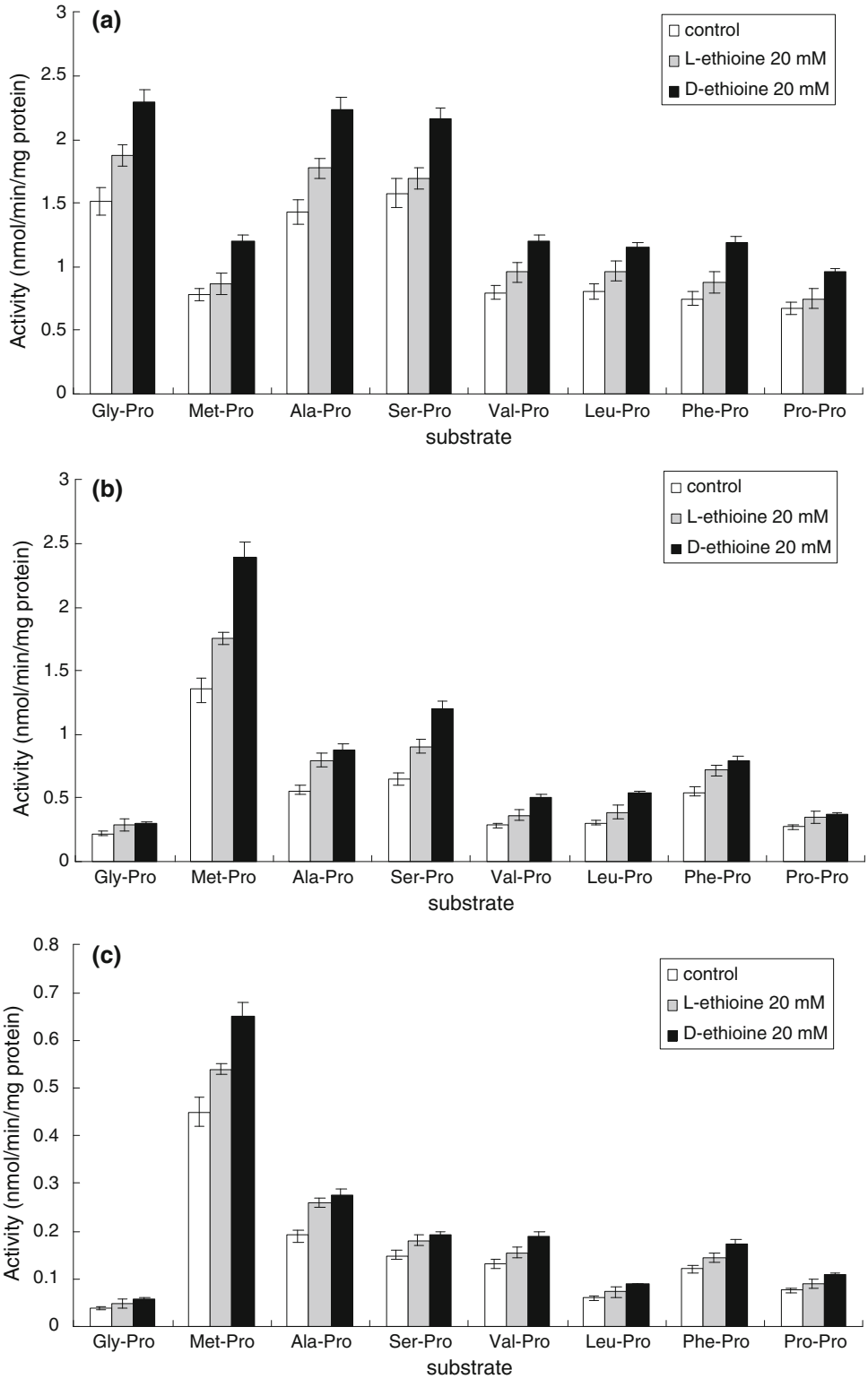

Fig. 7 Effect of L-ethionine (20 mM) or D-ethionine (20 mM) on the activity of prolidase I (**a**), prolidase II (**b**) from normal human erythrocytes, and prolidase activity in erythrocyte lysates from a patient with prolidase deficiency (**c**) against various iminodipeptides (10 mM). Each value is presented as the mean ± SD from six independent experiments

D-methionine enhanced the activities of the prolidases (Uramatsu et al. 2007). In the current study, the prolidase activity in erythrocyte lysates of a patient with prolidase deficiency against various iminodipeptides was significantly enhanced by adding D-methionine and D-ethionine. The detailed mechanism of this enhancing effect is currently uncertain and should be further investigated in that it may find important clinical applications.

Table 1 Effects of D,L-homocysteine, D-ethionine, and D-methionine on kinetic data for prolidase I, II, and prolidase in erythrocytes from a patient with prolidase deficiency, in the presence of 0.1 mM $MnCl_2$

	Prolidase I activity against Gly-Pro		Prolidase I activity against Met-Pro		Prolidase II activity against Met-Pro		Patient's prolidase activity against Met-Pro	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Control	2.99 ± 0.35	17.53 ± 1.67	10.21 ± 1.29	12.03 ± 2.56	8.38 ± 1.1	23.33 ± 4.67	9.32 ± 1.23	3.45 ± 1.56
D-Methionine 20 mM	1.64 ± 0.29	16.82 ± 2.53	5.21 ± 2.15	11.67 ± 1.98	5.04 ± 0.9	22.1 ± 2.53	5.63 ± 0.99	3.76 ± 0.78
D-Ethionine 20 mM	2.04 ± 0.31	18.47 ± 2.13	6.65 ± 1.76	13.57 ± 1.59	3.91 ± 1.9	24.5 ± 3.41	4.35 ± 1.03	3.27 ± 0.45
D,L-Homocysteine 20 mM	2.23 ± 0.49	16.96 ± 1.93	7.22 ± 1.42	12.53 ± 3.14	6.26 ± 2.9	22.84 ± 2.24	6.59 ± 1.31	3.02 ± 1.41
D,L-Homocysteine 50 mM	3.65 ± 1.08	12.16 ± 2.93	10.03 ± 1.95	7.67 ± 3.21	9.5 ± 2.43	21.01 ± 1.33	7.53 ± 1.98	3.52 ± 1.12

Assay conditions are described in the Materials and methods section. K_m and V_{max} values are shown in mM and nmol/min/mg protein, respectively. Each value represents the mean ± SD from three independent experiments

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References

Arata J, Umemura S, Yamamoto Y, Hagiyama M, Nohara N (1979) Prolidase deficiency; its dermatological manifestations and some additional biochemical studies. Arch Dermatol 115:62–67

Arata J, Hatakenaka K, Oono T (1986) Effect of topical application of glycine and proline on recalcitrant leg ulcers of prolidase deficiency. Arch Dermatol 122:626–627

Buist NRM, Strandholm JJ, Belinger JH, Kennaway NE (1972) Further studies on a patient with iminodipeptiduria: a probable case of prolidase deficiency. Metabolism 21:1113–1124

Butterworth J, Priestman D (1984) Substrate specificity of manganese activated prolidase in control and prolidase- deficient cultured skin fibroblasts. J Inherit Metab Dis 7:32–34

Chinard FP (1952) Photometric estimation of proline and ornithine. J Biol Chem 199:91–95

Der Kaloustian VM, Freij BJ, Kurban AK (1982) Prolidase deficiency: an inborn error of metabolism with major dermatological manifestations. Dermatologica 164:293–304

Endo F, Tanoue A, Nakai H, Hata A, Indo Y, Titani K (1989) Primary structure and gene localization of human prolidase. J Biol Chem 264:4476–4481

Goodman SI, Solomons CC, Muschenheim F, Mclntyre CA, Miles B, O'Brien D (1968) A syndrome resembling lathyrism associated with iminopeptiduria. Am J Med 45:152–159

Hechtman P, Richter A, Corman N, Leong YM (1988) In situ activation of human erythrocyte prolidase: potential for enzyme replacement therapy in prolidase deficiency. Pediatr Res 24:709–712

Jackson SH, Dennis AW, Greenberg M (1975) Iminodipeptiduria: a genetic defect in recycling collagen; a method for determining prolidase in erythrocytes. Can Med Assoc 113:759–763

Kodama H, Ohhashi T, Ohba C, Ohno T, Arata J, Kubonishi I, Miyoshi I (1989) Characteristics and partial purification of prolidase and prolinase from leukocytes of a normal human and a patient with prolidase deficiency. Clin Chim Acta 180:65–72

Lewie WHP, Harris H (1969) Peptidase D (prolidase) variants in man. Ann Hum Genet 32:317–322

Lineweaver H, Burk D (1934) The determination of enzyme dissociation constants. J Am Chem Soc 56:658–666

Liu G, Nakayama K, Sagara Y, Awata S, Yamashita K, Manabe M, Kodama H (2005) Characterization of prolidase activity in erythrocytes from a patient with prolidase deficiency: comparison with prolidase I and II purified from normal human erythrocytes. Clin Biochem 38:625–631

Lowry OH, Rosenhrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Myara I (1987) Effect of long preincubation on the two forms of human erythrocyte prolidase. Clin Chim Acta 170:236–270

Nakayama K, Awata S, Zhang J, Kaba H, Manabe M, Kodama H (2003) Characteristics of prolidase from the erythrocytes of normal humans and patients with prolidase deficiency and their mother. Clin Chem Lab Med 41:1323–1328

Ohhashi T, Ohno I, Arata J, Sugahara K, Kodama H (1990) Characteristics of prolidase I and II from the erythrocytes of a control, a patient with prolidase deficiency and her mother. Clin Chem Acta 187:1–10

Pedersen PS, Christensen E, Brandt NJ (1983) Prolidase deficiency. Acta Paediatr Scand 72:785–788

Powell GH, Rasco MA, Maniscalco RM (1974) A prolidase deficiency in man with iminopeptiduria. Metabolism 23:505–513

Priestman DA, Butterworth J (1984) Prolidase deficiency: characteristics of human skin fibroblast prolidase using colorimetric and fluorimetric assays. Clin Chim Acta 142:263–271

Tanoue A, Endo F, Akaboshi I, Oono T, Arata J, Matsuda I (1990a) Molecular defect in siblings with prolidase deficiency and absence or presence of clinical symptoms. J Clin Invest 87:1171–1176

Tanoue A, Endo F, Matsuda I (1990b) Structure organization of the gene for human prolidase (peptidase D) and demonstration of a partial gene deletion in a patient with prolidase deficiency. J Biol Chem 265:11306–11311

Umemura S (1978) Studies on a patient with iminopeptiduria II. Lack of prolidase in blood cells. Physiol Chem Phys 10:279–283

Uramatsu M, Liu G, Uramatsu S, Zhang M, Wang W, Nakayama K, Manabe M, Kodama H (2007) Different effects of sulfur-containing amino acids on prolidase and prolinase activity in normal and prolidase-deficient human erythrocytes. Clin Chim Acta 375:129–135

Wang W, Liu G, Yamashita K, Manabe M, Kodama H (2004) Characteristics of prolinase against various iminodipeptides in erythrocyte lysates from a normal human and a patient with prolidase deficiency. Clin Chem Lab Med 42:1102–1108