Prolinase and non-specific dipeptidase of human kidney

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Human kidney prolinase, assayed with Pro-Ala, and non-specific dipeptidase, assayed with Gly-Leu, were purified by using DEAE-cellulose, gel-filtration, metal-ion-chelate, hydrophobic and adsorption chromatography and chromatofocusing. Both enzymes gave single peaks of activity that were congruent and the ratio of their activities was constant throughout the purification. Gel filtration indicated an M_r of 100000 and chromatofocusing a pI of 5.4. Ni^{2+} -chelate chromatography demonstrated the presence of exposed histidine residues on the enzyme and was an effective separative procedure. Polyacrylamide-gel electrophoresis of the final preparation showed the two enzyme activities to be coincident. Both enzyme activities decayed at the same rate at 53 °C and were inhibited to the same extent by *p*-hydroxymercuribenzoate. Of six non-specific dipeptidase substrates tested Gly-Leu gave the highest activity, and of six prolinase substrates Pro-Leu had the highest activity. Gly-Leu was hydrolysed at double the rate of Pro-Leu. Pro-Ala was a competitive inhibitor of activity towards Gly-Leu, and Gly-Leu was a competitive inhibitor of activity towards Pro-Ala. Mixed-substrate studies strongly suggested that Gly-Leu and Pro-Ala were hydrolysed at a common active site. The data are consistent with prolinase and non-specific dipeptidase activity in human kidney being due to a single enzyme.

INTRODUCTION

Prolinase (EC 3.4.13.8) has been purified from a number of animal tissues (Sarid *et al.*, 1962; Mayer & Nordwig, 1973; Akrawi & Bailey, 1976; Reith & Neidle, 1979), and in all cases the final preparations had activity against non-specific-dipeptidase (EC 3.4.13.11) substrates, which was attributed to a contaminating enzyme. However, it has been proposed that the two activities may be due to a single enzyme (Hayman & Patterson, 1971). As kidney has been widely used in studies on dipeptidases from animals (Sarid *et al.*, 1962; Mayer & Nordwig, 1973; Akrawi & Bailey, 1976) and had the highest prolinase activity of the human organs tested (Butterworth & Priestman, 1982), prolinase was purified from human kidney. Increasing lability of dipeptidases with purification appears to preclude purification to homogeneity (Hayman & Patterson, 1971; Mayer & Nordwig, 1973). To ascertain whether human kidney prolinase and non-specific dipeptidase were the same enzyme, we studied properties of the enzyme, including substrate kinetics (Dixon & Webb, 1964, pp. 84–87), after purification by the use of a wide range of separative techniques.

MATERIALS AND METHODS

Chemicals

DEAE-cellulose was obtained from Whatman, Ultrogel AcA-44 was from LKB, butyl-agarose from Miles Laboratories, spheroidal hydroxyapatite was from BDH Chemicals, 4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside and 4-methylumbelliferyl α -D-mannopyranoside were from Koch-Light Laboratories, and all other chemicals were from Sigma Chemical Co.

Enzyme assays

Prolinase was assayed in 25 mM-barbital/HCl buffer, pH 9.2, containing 12.5 μ M-MnCl₂ and 10 mM-prolyl-L-

alanine (Pro-Ala), prolyl-L-glycine (Pro-Gly), prolyl-L-leucine (Pro-Leu), prolyl-L-phenylalanine (Pro-Phe), prolyl-L-valine (Pro-Val) or hydroxyprolylglycine (Hyp-Gly), and the liberated amino acid was determined fluorimetrically by using *o*-phthalaldehyde (Butterworth & Priestman, 1982). Non-specific dipeptidase was assayed in 25 mM-barbital/HCl buffer, pH 8.0, containing 10 mM-glycyl-L-leucine (Gly-Leu), L-alanyl-L-leucine (Ala-Leu), L-valyl-L-leucine (Val-Leu), L-seryl-L-leucine (Ser-Leu), L-phenylalanylglycine (Phe-Gly) or L-leucylglycylglycine (Leu-Gly-Gly), and the liberated L-leucine or L-phenylalanine was determined spectrophotometrically by the L-amino acid oxidase/peroxidase-coupled oxidation of *o*-dianisidine (Butterworth & Priestman, 1982). Prolidase (EC 3.4.13.9) was assayed against glycyl-L-proline (Gly-Pro) by determination of liberated proline and against L-phenylalanyl-L-proline (Phe-Pro) by determination of liberated L-phenylalanine (Priestman & Butterworth, 1984). Proline iminopeptidase (EC 3.4.11.5) activity was determined by determination of liberated proline from prolylglycylglycine (Pro-Gly-Gly) (Butterworth & Priestman, 1984).

Column fractions were diluted appropriately before enzyme assay with bovine serum albumin (0.25 mg/ml) in 10 mM-barbital/HCl buffer, pH 7.4, containing 50 mM-NaCl. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. One unit of enzyme activity was defined as the formation of 1 μ mol of product/min at 37 °C.

Extract preparation

Human kidney was obtained at autopsy 24 h after death and stored at –70 °C. A 20% (w/v) extract was prepared by homogenizing in 10 mM-barbital/HCl buffer, pH 7.4, containing 50 mM-NaCl (buffer A). The homogenate was sonicated at 8–12 μ m (MSE mark II ultrasonic disintegrator) and centrifuged at 100000 *g* for 60 min.

Chromatographic procedures

All procedures were carried out at 4 °C unless otherwise stated.

DEAE-cellulose. Kidney extract (50 ml) was applied to a 3.5 cm × 45 cm DEAE-cellulose column equilibrated with buffer A. After a washing with 200 ml of buffer A, the column was eluted with 500 ml of a 50–300 mM-NaCl linear gradient, and 10 ml fractions were collected.

Gel filtration. The DEAE-cellulose peak (95 ml) was concentrated 10-fold by using a PM10 ultrafiltration membrane system (Diaflo), applied to a 2.5 cm × 75 cm Ultrogel AcA-44 column, eluted with buffer A at 20 ml/h and 10 ml fractions were collected.

Metal-ion-chelate chromatography. Iminodiacetic acid was coupled to epoxy-activated Sepharose 6B (Porath *et al.*, 1975) and converted into a metal-ion-chelate gel by the passage of 5 ml of 50 mM-NiCl₂ or 50 mM-MnCl₂ in water through 2.5 ml of gel in a 1 cm × 3.5 cm column. The Ni²⁺-chelate gel was equilibrated with buffer A, and 40 ml of Ultrogel prolinase peak was applied to the column. After washing with 20 ml of 10 mM-barbital/HCl buffer, pH 7.4, containing 10 mM-NaCl (buffer B), the column was eluted with 60 ml of a 0–100 mM-imidazole linear gradient (in buffer B), and 2 ml fractions were collected.

Hydrophobic chromatography. The prolinase peak (16 ml) from the metal-ion-chelate chromatography was applied to a 1.4 cm × 25 cm butyl-agarose column equilibrated with buffer B. The column was washed with 75 ml of buffer B, eluted with 100 ml of a 10–300 mM-NaCl linear gradient, and 4 ml fractions were collected.

Hydroxyapatite chromatography. The prolinase peak (30 ml) from the hydrophobic chromatography was applied to a 1.3 cm × 6.5 cm hydroxyapatite column equilibrated with buffer A. The column was washed with 50 ml of buffer A, eluted with 100 ml of a 0–150 mM-sodium phosphate linear gradient in buffer A, and 5 ml fractions were collected.

Chromatofocusing. The prolinase peak (15 ml) from the hydroxyapatite chromatography was concentrated 10-fold by ultrafiltration, diluted (1+1) with 25 mM-imidazole/HCl buffer, pH 7.4, and applied to a 1.3 cm × 5 cm PBE 94 column equilibrated with this buffer. Elution was carried out with 60 ml of Polybuffer 74 titrated with HCl to pH 4.0, and 2 ml fractions were collected.

M_r determination

A 1% (w/v) kidney extract (10 ml) in buffer A was applied to a 2.5 cm × 75 cm Ultrogel AcA-44 column, eluted with buffer A at 20 ml/h, and 10 ml fractions were collected. The elution volumes of prolinase, *N*-acetyl- β -D-glucosaminidase (Butterworth *et al.*, 1976) and α -D-mannosidase (Butterworth, 1980) were determined. A mixture (10 ml) containing 1 ml of the enzyme peak from hydrophobic chromatography and five protein standards was applied to the same column, and 3 ml fractions were collected. The elution volumes of the protein standards were determined after detection at 280 nm.

Polyacrylamide-gel electrophoresis

Discontinuous electrophoresis (4.5% stacking, 7.5% separating gel, 0.75 mm thick) was performed with a Bio-Rad Protean slab electrophoresis cell (Davis, 1964). Concentrated samples (10 μ l) of the original kidney extract and the hydroxyapatite peak were applied and electrophoresed at 14 mA for 4.5 h. The gel lanes were cut into 0.25 cm strips and bisected, one half for prolinase assay and the other half for non-specific-dipeptidase assay. For prolinase each gel strip was put into 100 μ l of 50 mM-barbital/HCl buffer, pH 9.2, containing 0.025 mM-MnCl₂, incubated for 60 min after the addition of 100 μ l of 10 mM-Pro-Phe, and liberated phenylalanine was determined (Butterworth & Priestman, 1982). For non-specific dipeptidase each gel strip was put into 100 μ l of 50 mM-barbital/HCl buffer, pH 8.0, incubated for 60 min after the addition of 100 μ l of 25 mM-Gly-Leu, and liberated leucine was determined (Butterworth & Priestman, 1982). Protein bands were detected by using the silver staining method of Merril & Goldman (1984).

Enzyme characteristics

Studies were performed on the dipeptidase peak eluted after hydrophobic chromatography and after chromatofocusing following equilibration with buffer A by ultrafiltration.

Heat-lability. Enzyme samples were incubated at 53 °C for up to 30 min before the assay of prolinase (Pro-Ala) and non-specific dipeptidase (Gly-Leu).

p-Hydroxymercuribenzoate treatment. Enzyme samples were treated with 1–50 μ M-*p*-hydroxymercuribenzoate for 5 min at 37 °C before the assay of prolinase (Pro-Ala) and non-specific dipeptidase (Gly-Leu).

Substrate specificity. The enzyme preparation after chromatofocusing was tested against a number of potential substrates (see above under 'Enzyme assays').

Substrate kinetics. The type of inhibition and *K_i* values were determined (Dixon & Webb, 1964, pp. 327–329) by studying the hydrolysis of Pro-Ala (2 or 5 mM) at pH 9.2 in the presence of Gly-Leu (0.5–10 and 1–25 mM respectively) and of Gly-Leu (0.5 or 2 mM) at pH 8.0 in the presence of Pro-Ala (0.1–2 and 1–10 mM respectively). As a test of the activities being due to a single enzyme (Dixon & Webb, 1964, pp. 84–87), Pro-Ala (5 mM) hydrolysis at pH 9.2 with or without Gly-Leu (5 mM) and Gly-Leu (5 mM) hydrolysis at pH 9.2 with or without Pro-Ala (5 mM) were monitored, and the *K_i* value for Pro-Ala at pH 9.2 was determined.

RESULTS

The purification of prolinase and non-specific dipeptidase is summarized in Table 1. To assay the two enzyme activities in the original kidney extract, it was necessary to dilute to a low protein concentration (< 0.01 mg/ml), at which the enzyme was unstable. Addition of bovine serum albumin (0.25 mg/ml) stabilized the enzyme to dilution. During the purification procedure the protein concentration of the active fractions up to and including the hydroxyapatite column chromatography was sufficient to maintain stability, but addition of bovine serum

Table 1. Purification of human kidney prolinase and non-specific dipeptidase

For experimental details see the text.

	Protein (mg)	Prolinase			Non-specific dipeptidase		
		Activity (units)	Specific activity (units/ml)	Yield (%)	Activity (units)	Specific activity (units/ml)	Yield (%)
Crude extract	948	631	0.657	100	4888	5.16	100
100 000 g supernatant	354	573	1.62	91	4444	12.6	91
DEAE-cellulose fraction	56.2	319	5.68	50	2333	41.5	48
Ultrogel AcA-44 fraction	15.6	283	18.1	45	2151	138	44
Ni ²⁺ -chelated-Sepharose fraction	3.7	148	40.0	23	1194	323	24
Butyl-agarose fraction	1.27	127	100	20	1050	827	21
Hydroxyapatite fraction	0.27	87	319	14	700	2564	14
Chromatofocusing fraction	—	17	—	2.6	116	—	2.4

albumin was required for dilution of the column fractions before assay. A preparation retained after hydrophobic chromatography was stable for 9 months at 4 °C in the presence of bovine serum albumin. Co-elution of both of the enzyme activities as a single peak was observed for all the separative procedures, either performed singly with a crude kidney extract or during the sequential purification. The ratio of the two activities was similar after each of the separative procedures.

During the sequential purification procedure the two enzyme activities were co-eluted with the highest activity at 110 mM-NaCl for the DEAE-cellulose column, 30 mM-imidazole for the Ni²⁺-chelate column, 100 mM-NaCl for the butyl-agarose column and 30 mM-phosphate for the hydroxyapatite column. A Mn²⁺-chelate gel did not retain either enzyme activity. The relative elution volumes (V_e/V_0) of *N*-acetyl- β -D-glucosaminidase and α -D-mannosidase lay on the gel-filtration M_r calibration curve and give M_r values of 110 000 and 180 000 respectively. The position of the enzyme, as detected by its prolinase and non-specific dipeptidase activity, indicated an M_r of 100 000 (Fig. 1). Elution from the chromatofocusing column indicated a pI of 5.3 for the enzyme. Silver staining of the polyacrylamide gel after electrophoresis of the pooled enzyme activity from the hydroxyapatite column revealed the presence of two major and three minor protein bands (Fig. 2a). Band I (Fig. 2a) was the marker Bromophenol Blue and band II was a staining artifact. The gel showed a coincident band of prolinase and non-specific dipeptidase activity (Fig. 2b). A crude kidney extract gave the same position for the two enzyme activities.

As recovery of enzyme activity was poor from the chromatofocusing column and involved exposure to acidic pH, properties of the enzyme were studied after the hydrophobic-chromatography as well as the chromatofocusing procedures. However, the results were the same for both enzyme preparations. Heat treatment of the enzyme at 53 °C resulted in a similar rate of loss of activity against Gly-Leu and Pro-Ala, with a 50% loss of activity after about 12 min (Fig. 3). The thiol-blocking reagent *p*-hydroxymercuribenzoate was markedly inhibitory of enzyme activity against both substrates with 50% inhibition at about 14 μ M (Fig. 4).

The activity of the final enzyme preparation against a range of potential substrates (10 mM) is given in Table 2.

Fig. 1. Calibration of Ultrogel column for M_r determination

The relationship between the relative elution volumes (V_e , elution volume; V_0 , void volume) and the logarithms of the M_r values of certain proteins is shown for Ultrogel AcA-44. For experimental details see the Materials and methods section. Key: (1) α -D-mannosidase; (2) alcohol dehydrogenase; (3) *N*-acetyl- β -D-glucosaminidase; (4) haemoglobin; (5) ovalbumin; (6) chymotrypsinogen A; (7) cytochrome c; (8) prolinase and non-specific dipeptidase.

Of the non-specific dipeptidase substrates the highest activity was against Gly-Leu; Phe-Gly was not hydrolysed. Three of the prolinase substrates (Pro-Leu, Pro-Phe and Pro-Val) gave activities that were higher than the activity towards Pro-Ala, the substrate used to monitor the enzyme purification. The enzyme had no activity towards Pro-Gly-Gly, the substrate for proline iminopeptidase, and towards Gly-Pro and Phe-Pro, substrates for prolidase. Analysis of substrate interactions by Dixon (1953) plots showed that Gly-Leu was a competitive inhibitor of enzyme activity towards Pro-Ala with a K_i of 1.1 mM at pH 9.2 (Fig. 5), and Pro-Ala was a competitive inhibitor of enzyme activity towards Gly-Leu with a K_i of 0.22 mM at pH 8.0 (Fig. 6) and 2.2 mM at pH

Fig. 2. Polyacrylamide-gel electrophoresis of human kidney dipeptidase

The gel was prepared, run and stained or the enzyme activities were determined for the hydroxyapatite peak as described in the Materials and methods section. (a) Silver-staining for protein in the separating gel (10 cm). O, Origin; +, anode end of gel. Band I was the tracking dye Bromophenol Blue and Band II was a staining artifact. (b) Dipeptidase relative activity in gel slices (0.25 cm) against Gly-Leu and Pro-Phe.

Fig. 3. Heat treatment of human kidney dipeptidase

The enzyme peak after chromatofocusing was equilibrated with 10 mM-barbital/HCl buffer, pH 7.4, containing 50 mM-NaCl, heated at 53 °C for up to 30 min and remaining prolinase (●) and non-specific dipeptidase (○) activities were determined as described in the Materials and methods section.

Fig. 4. Inhibition of human kidney dipeptidase by *p*-hydroxymercuribenzoate

The enzyme peak after chromatofocusing was equilibrated with 10 mM-barbital/HCl buffer, pH 7.4, containing 50 mM-NaCl, treated with 1–50 μM-*p*-hydroxymercuribenzoate and remaining prolinase (●) and non-specific dipeptidase (○) were determined as described in the Materials and methods section.

Table 2. Hydrolysis of dipeptides and tripeptides by the purified human kidney enzyme

The activity of the enzyme preparation after chromatofocusing was determined against potential substrates (10 mM) as described in the Materials and methods section.

Substrate	Enzyme activity (units/ml)	Relative activity (%)
Gly-Leu	3.23	100
Ser-Leu	1.29	40
Val-Leu	0.96	30
Ala-Leu	0.89	28
Phe-Ala	0.32	10
Phe-Gly	0	0
Leu-Gly-Gly	0.12	4
Pro-Leu	1.55	48
Pro-Phe	1.42	44
Pro-Val	1.30	40
Pro-Ala	0.43	13
Pro-Gly	0.29	9
Hyp-Gly	0.12	4
Pro-Gly-Gly	0	0
Gly-Pro	0	0
Phe-Pro	0	0

9.2. The K_m for the enzyme against Pro-Ala was 0.47 mM at pH 9.2, and the K_m against Gly-Leu was 0.79 mM at pH 8.0 and pH 9.2. The expected hydrolysis of equimolar concentrations of two substrates by (a) one or (b) two enzymes in an extract can be calculated by using experimentally determined values of V_{max} , K_m and K_i for

Fig. 5. Determination of K_i for Gly-Leu inhibition of prolinase activity against Pro-Ala

The hydrolysis of (a) 2 mM- and (b) 5 mM-Pro-Ala in the presence of a series of concentrations of Gly-Leu was determined. The K_i was determined by the method of Dixon (1953).

Fig. 6. Determination of K_i for Pro-Ala inhibition of non-specific dipeptidase activity against Gly-Leu

The hydrolysis of (a) 0.5 mM- and (b) 2 mM-Gly-Leu in the presence of a series of concentrations of Pro-Ala was determined. The K_i was determined by the method of Dixon (1953).

the two substrates (Dixon & Webb, 1964, pp. 84–87). Whether the activity against both substrates is due to one or two enzymes can be assessed by comparison of the two calculated rates of hydrolysis with the observed rate. The present data gave a calculated total activity of 8.9

units/ml for one enzyme and 16.6 units/ml for two enzymes working on equimolar concentrations (5 mM) of the two substrates. As the observed total activity was 9.2 units/ml, it seems highly probable that the preparation contained a single enzyme hydrolysing both substrates.

DISCUSSION

Non-specific dipeptidases purified from pig and guinea-pig intestinal mucosa (Noren *et al.*, 1973; Piggott & Fottrell, 1975) and mouse ascites tumour (Hayman & Patterson, 1971) hydrolysed prolinase substrates. Conversely, prolinases purified from pig kidney (Sarid *et al.*, 1962; Mayer & Nordwig, 1973), bovine kidney (Akrawi & Bailey, 1976) and mouse brain (Reith & Neidle, 1979) hydrolysed non-specific dipeptidase substrates. Also, the major peak of human skin-fibroblast prolinase coincided with a peak of non-specific dipeptidase on DEAE-cellulose chromatography (Butterworth & Priestman, 1984). Generally, the two activities had been considered to be due to at least two enzymes, and whether there is an enzyme capable of hydrolysing both prolinase and non-specific-dipeptidase substrates is undecided, particularly for human tissues.

Prolinase and non-specific dipeptidase from human kidney were soluble, in agreement with results for human skin fibroblasts (Butterworth & Priestman, 1982) and pig kidney (Mayer & Nordwig, 1973). The elution position of the human kidney enzyme on DEAE-cellulose chromatography corresponded to that of the major peak of human skin-fibroblast prolinase (Butterworth & Priestman, 1982, 1984). The accuracy of the M_r calibration curve was demonstrated by obtaining the expected M_r values for α -D-mannosidase and *N*-acetyl- β -D-glucosaminidase (Marinkovic & Marinkovic, 1976a, b). The determined M_r value of 100000 for the human kidney dipeptidase was similar to the value reported for bovine kidney prolinase (Akrawi & Bailey, 1976), but was lower than the value of 300000 estimated for pig kidney prolinase (Mayer & Nordwig, 1973).

Metal-ion-chelate chromatography separates proteins on the basis of their affinity for heavy-metal ions (Porath *et al.*, 1975; Porath & Olin, 1983). Histidine and cysteine form complexes with Zn^{2+} , Co^{2+} , Hg^{2+} and Ni^{2+} , and proteins containing these amino acids on their surface can potentially bind to metal-ion-chelated Sepharose (Porath *et al.*, 1975). Although Mn^{2+} activates prolinase (Butterworth & Priestman, 1982) and a number of other enzymes (Dixon & Webb, 1964, pp. 672–785), the weak capacity of gel-immobilized Mn^{2+} for protein binding (Porath *et al.*, 1975) could explain why the Mn^{2+} -chelate gel did not adsorb prolinase. Affinity elution of human kidney dipeptidase from a Ni^{2+} -chelate gel by imidazole strongly suggests the presence of histidine residues on the surface of the enzyme (Porath & Olin, 1983). The results obtained with human kidney dipeptidase demonstrate the usefulness of the inclusion of a metal-ion-chelate-chromatography step in enzyme purification.

The binding of human skin-fibroblast prolinase to butyl-agarose (Butterworth & Priestman, 1982) and of pig intestinal non-specific dipeptidase to hydroxyapatite (Noren *et al.*, 1973) was the same as that of the human kidney enzyme. Although bovine (Akrawi & Bailey, 1976) and pig (Mayer & Nordwig, 1973) kidney prolinase gave a pI of 4.3 on isoelectric focusing, the pI value of 5.3 for

the human kidney preparation was within the range 5.05–5.3 obtained by chromatofocusing of preparations of human skin fibroblasts, liver and kidney (Butterworth & Priestman, 1984). The marked loss of human kidney enzyme activity on chromatofocusing was probably due to exposure to acidic pH, which is known to inactivate the enzyme (Butterworth & Priestman, 1982). The presence of multiple bands of activity, probably due to separation into subunits and re-aggregation, on polyacrylamide-gel electrophoresis of purified pig kidney prolinase (Mayer & Nordwig, 1973) was not a feature of the human kidney enzyme.

The inhibition of human kidney dipeptidase activity by *p*-hydroxymercuribenzoate indicates the presence of a thiol group at the active site, as previously suggested for monkey intestinal (Das & Radhakrishnan, 1972) and pig kidney enzyme (Sarid *et al.*, 1962). Although Gly-Leu was the best of the substrates tested for human kidney dipeptidase, the hydrolysis of non-specific-dipeptidase substrates varies widely, depending on conditions of assay and source of enzyme (Hayman & Patterson, 1971; Mayer & Nordwig, 1973; Noren *et al.*, 1973). The ability of the human kidney enzyme to hydrolyse prolinase substrates was similar to that of human skin-fibroblast prolinase (Butterworth & Priestman, 1982). In contrast with the human enzyme, pig kidney (Mayer & Nordwig, 1973) and guinea-pig intestinal-mucosal (Piggott & Fottrell, 1975) prolinase had very little activity towards Pro-Leu and Pro-Phe. The failure of the human kidney enzyme to hydrolyse Pro-Gly-Gly, the substrate for proline iminopeptidase and Gly-Pro and Phe-Pro, substrates for prolidase, agrees with the previous findings for pig and bovine kidney prolinase (Sarid *et al.*, 1962; Mayer & Nordwig, 1973; Akrawi & Bailey, 1976).

Throughout the purification procedures the human kidney prolinase and non-specific dipeptidase activities could not be separated. The enzyme peaks were congruent and the ratio of their activities was constant after each of the separative procedures. In addition, the responses of the two activities to *p*-hydroxymercuribenzoate and to heat treatment were the same. These data, together with the competitive inhibition of prolinase and non-specific dipeptidase activity by Gly-Leu and Pro-Ala respectively and the analysis of substrate interaction (Dixon & Webb, 1964, pp. 84–87), provide firm evidence

for the existence of an enzyme hydrolysing both prolinase and non-specific dipeptidase substrates in human kidney.

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