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Proteins of the kidney microvillar membrane

Enzymic and molecular properties of aminopeptidase W

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Aminopeptidase W is a newly discovered enzyme of the renal and intestinal brush borders, having been first isolated as a 130 kDa glycoprotein recognized by a monoclonal antibody [Gee & Kenny (1985) Biochem. J. **230**, 753–764]. It is particularly effective in the hydrolysis of dipeptides, Glu-Trp (K_m 0.57 mM; $k_{cat.}$ 6770 min^{-1}) being a favoured substrate. Dipeptides with tryptophan, phenylalanine or tyrosine in the P_1' position were rapidly hydrolysed, but the requirements in respect of the P_1 residue were not stringent. The activity of aminopeptidase W is markedly influenced by ionic conditions. The highest activity was observed in 100 mM-Tris/HCl, pH 8; phosphate ions were strongly inhibitory. Activity was also greatly affected by bivalent metal ions, and the magnitude and direction of the effects depended on the nature of the buffer anions and on pH. The most effective inhibitors were amastatin and bestatin. Some thiols also inhibited, but other chelating agents, EDTA and 1,10-phenanthroline, had no effect over the concentration range 1–10 mM. Other group-specific inhibitors, for cysteine, serine or aspartic peptidases, were also ineffective. Some molecular properties were studied. Deglycosylation by treatment with *N*-glycanase diminished the apparent subunit M_r from 130000 to 90000. The enzyme contained zinc, 1.2 atoms/subunit, and in spite of the atypical properties of this enzyme in respect of chelating agents, a zinc-catalysed mechanism is the most probable. Its roles in digestion and in renal function are not yet clear.

INTRODUCTION

The 130 kDa antigen in pig kidney microvilli recognized by monoclonal antibody GK5C1 (Gee & Kenny, 1985*a*) has been shown to be a new enzyme, named aminopeptidase W (Gee & Kenny, 1985*b*). Immunohistochemistry revealed it to be present in the brush borders of the renal proximal tubule and small intestine. An immunoradiometric assay confirmed these locations and indicated that the ileum had the highest activity. In both brush borders, aminopeptidase W is a significant component, amounting in kidney to 0.8% of the microvillar membrane protein. The structure and topology of aminopeptidase W purified by immunoaffinity chromatography have been investigated. It is a typical ectoenzyme, a globular protein, homodimeric in this case, anchored through a stalk of about 3 nm length to the membrane, and released by papain treatment. The purified protein hydrolysed Leu-Trp more rapidly than any of the other substrates then tested, including some extended peptides, and the mode of attack was established to be that of an aminopeptidase. Although aminopeptidase N (EC 3.4.11.2) hydrolysed some of the same peptides tested, the specificities of the two enzymes were strikingly different. In particular, aminopeptidase W preferred dipeptides, whereas the activity of aminopeptidase N was greater with extended peptides.

In the present paper, we report some further studies of this novel aminopeptidase, including its specificity for a wide range of dipeptides, some kinetic parameters, the effect of inhibitors and some molecular properties indicating that it is a glycoprotein containing zinc.

EXPERIMENTAL

Materials

Actinonin, pepstatin and phosphoramidon were obtained from the Peptide Institute, Osaka, Japan. Bestatin and amastatin were from Sigma Chemical Co. Dipeptides were from either Sigma Chemical Co. or Bachem, Bubendorf, Switzerland. Captopril was a gift from the Squibb Institute for Medical Research, Princeton, NJ, U.S.A. *N*-Glycanase (glycopeptide *N*-glycosidase, EC 3.2.2.18) was purchased from Genzyme Fine Chemicals, Haverhill, Suffolk, U.K.

Methods

Purification of aminopeptidase W. This was achieved by immunoaffinity chromatography, with a hypoosmotic elution buffer (2 mM-Tris/HCl buffer, pH 8) as described previously (Gee & Kenny, 1985*b*).

Enzymic assays. The assays with dipeptides as substrates were monitored by h.p.l.c. (Gee & Kenny, 1985*b*). The kinetic parameters were determined by Lineweaver–Burk plots using six points over the substrate concentration range 60 μM–0.5 mM, and were the means for two experiments. Velocity was assessed by the release of tryptophan in conditions where no more than 5% of the substrate was hydrolysed.

Deglycosylation of aminopeptidase W. Samples (2 μg) of the purified protein were incubated with *N*-glycanase at 37 °C for 18 h. The incubation mixture (vol. 30 μl)

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contained 0.25 M-Na_2HPO_4/NaH_2PO_4 buffer, pH 8.6, 12 mM-1,10-phenanthroline and 0.0028–0.28 unit of *N*-glucanase. Some samples of aminopeptidase W were heated to 100 °C with 0.5% SDS before treatment with *N*-glycanase, and in this case the incubation mixture also contained 1.25% (v/v) Triton X-100.

SDS/polyacrylamide-gel electrophoresis. This was performed by using the system of Laemmli (1970) with 7–17% polyacrylamide gradient. Gels were silver-stained as described by Dubray & Bezard (1982).

Determination of zinc. This was done in an atomic absorption spectrophotometer (Perkin–Elmer 303) essentially as described for another enzyme (Fulcher & Kenny, 1983). Milli-Q (Millipore) water was used for preparation of all solutions. Acid-washed glassware was employed. Dialysis tubing was boiled with 10 mM-EDTA and washed exhaustively with water before use. Blanks prepared from the dialysis medium were checked, but could not be distinguished from the Milli-Q water blanks.

Other methods. These were those previously described (Gee & Kenny, 1985*b*).

RESULTS

Hydrolysis of dipeptidase: comparison with aminopeptidase N

A series of dipeptide substrates are compared in Table 1. In the previous series of dipeptides (Gee & Kenny, 1985*b*), Leu-Trp was hydrolysed faster than any of the others, but in this more extensive survey one dipeptide, Glu-Trp, was found to be slightly superior. The identity of the P_1' residue among the more favoured dipeptides (those with rates > 20% compared with Glu-Trp) was tryptophan, phenylalanine or tyrosine. This point is developed in Table 2, where the data are presented in a different and more convenient form. For a given P_1 residue, the hydrolysis of the dipeptide diminishes in the order tryptophan, phenylalanine, tyrosine, leucine, glycine, where these are the P_1' residues. The requirements for the P_1 position are not stringent. Small residues such as glycine and alanine were acceptable, as were large side chains such as tryptophan, isoleucine and leucine. Polar residues of either charge (glutamic acid or arginine) were also accommodated. This tolerance contrasts with the somewhat stricter requirements for aminopeptidase N (Table 1).

The specific activities, determined at an arbitrary peptide concentration, may not necessarily reflect the true efficiency of hydrolysis. Five dipeptides were therefore studied in more detail and the kinetic constants determined (Table 3). The peptides are ranked in the order of their specificity constants ($k_{cat.}/K_m$). The two best substrates (Glu-Trp and Leu-Trp) in this comparison are those listed in Table 1, and indeed the ranking order of the five dipeptides is the same. The K_m values are in the range 0.5–2.0 mM. The K_m values for some dipeptides (Leu-Trp, Ala-Phe and Tyr-Gly) with aminopeptidase N were in a similar range (0.9–5.0 mM; S. L. Stephenson & A. J. Kenny, unpublished work).

Table 1. Hydrolysis of dipeptides by aminopeptidase W and aminopeptidase N

See the Experimental section for details. Substrates (0.5 mM) were incubated with purified enzymes for 20 min under conditions such that hydrolysis did not exceed 20% of the peptide, and hydrolysis was assessed by h.p.l.c. analysis (at A_{214}) of the amino acid produced. Abbreviation: N.D., not determined. Values as percentages of Glu-Trp hydrolysis are shown in parentheses.

	Specific activity (μmol/min per mg of protein)	
Peptide	Aminopeptidase W	Aminopeptidase N
Glu-Trp	22.2 (100)	0.28 (100)
Leu-Trp	19.8 (89)	1.66 (593)
Gly-Trp	13.4 (60)	0.28 (100)
Phe-Phe	13.1 (59)	N.D.
Asp-Phe	12.9 (54)	N.D.
Leu-Phe	11.8 (53)	1.82 (650)
Phe-Trp	11.1 (50)	N.D.
Ala-Trp	10.6 (48)	1.78 (636)
Ile-Trp	9.1 (41)	N.D.
Leu-Tyr	9.1 (41)	2.42 (864)
Arg-Trp	7.5 (34)	N.D.
Tyr-Phe	7.1 (32)	N.D.
Trp-Trp	5.8 (26)	0.20 (71)
Glu-Tyr	5.3 (24)	N.D.
Val-Trp	4.4 (20)	0.15 (54)
Phe-Tyr	4.0 (18)	N.D.
Tyr-Leu	4.0 (18)	0.78 (279)
Gly-Tyr	3.3 (15)	0.98 (350)
Ile-Phe	3.3 (15)	N.D.
Pro-Trp	3.1 (14)	0.05 (18)
Trp-Phe	3.0 (14)	N.D.
Ala-Phe	2.0 (9)	2.56 (914)
Phe-Leu	2.0 (9)	0.58 (207)
Ile-Tyr	1.8 (8)	N.D.
Ala-Tyr	1.6 (7)	N.D.
Val-Phe	1.3 (6)	N.D.
Phe-Gly	1.2 (5)	1.23 (439)
Trp-Tyr	1.2 (5)	N.D.
Trp-Ala	1.0 (4)	N.D.
Pro-Phe	1.0 (4)	0.03 (11)
Trp-Leu	0.4 (2)	0.52 (186)
Pro-Tyr	0.4 (2)	N.D.
Gly-D-Phe	0 (0)	0 (0)

Ionic effects on the hydrolysis of Leu-Trp

The pH optimum has been previously reported to be pH 8, in 0.1 M-Tris/HCl buffers (Gee & Kenny, 1985*b*). It became apparent that the activity was greatly influenced by the nature and concentration of the buffer ions used. The effects of four different buffer systems at pH 8 and pH 9 are shown in Table 4. Phosphate was strongly inhibitory at pH 8. At pH 9, Tris/HCl gave the highest activity compared with $NaHCO_3/NaOH$ and glycine/NaOH. The strong influence of the buffers did not, however, correlate with the ionic strengths of the buffers. The effect of concentration was also studied with respect to Tris at pH 8.0 and revealed a sharp optimum at 100 mM-Tris/HCl (Fig. 1).

Effects of metal ions and chelating agents

Among the cations tested (as chlorides at 1 mM in 100 mM-Tris/HCl buffer, pH 8), Ca^{2+}, Mg^{2+} and Mn^{2+}

Table 2. Rates of hydrolysis of dipeptides by aminopeptidase W

The substrate concentration was 0.5 mM and incubation time 20 min, as noted in the legend to Table 1. All rates are relative to the hydrolysis of Glu-Trp (= 100). The effect of the P_1' residue on the relative rates is shown in the bottom line of the Table, where the rates are compared with tryptophan.

		Relative rate of hydrolysis				
P_1 residue	P_1' residue ...	Trp	Phe	Tyr	Leu	Gly
Glu		100	–	24	–	–
Asp		–	54	–	–	–
Leu		89	53	41	–	–
Tyr		–	32	–	18	–
Gly		60	–	15	–	–
Phe		50	59	18	9	5
Ala		48	9	7	–	–
Ile		41	15	8	–	–
Arg		34	–	–	–	–
Trp		26	14	5	2	4
Val		20	6	–	–	–
Pro		14	4	2	–	–
Mean values for P_1' residues compared with Trp (±S.E.M.)			50±12	25±4.4	13	12

Table 3. Kinetic constants for some dipeptides with aminopeptidase W

Purified aminopeptidase W was incubated with dipeptides at 0.5 mM, 0.4 mM, 0.3 mM, 0.2 mM, 0.1 mM and 0.05 mM. The assay time was adjusted so that not more than 5% of the substrate was hydrolysed. Kinetic constants were obtained from plots of $1/v$ versus 1/[S]. Results are the means for two determinations. (In parentheses are $k_{cat.}/K_m$ values relative to that with Glu-Trp.)

Substrate	K_m (μM)	$V_{max.}$ (μmol/min per mg of protein)	$k_{cat.}$ (min^{-1})	$k_{cat.}/K_m$ ($min^{-1} \cdot \mu M^{-1}$)
Glu-Trp	568	52	6773	11.9 (100)
Leu-Trp	488	43	5557	11.4 (96)
Gly-Trp	1757	59	7590	4.3 (36)
Ala-Trp	625	19	2312	3.7 (31)
Pro-Trp	1700	13.7	1775	1.04 (9)

Table 4. Effects of buffers on hydrolysis of Leu-Trp by aminopeptidase W

Purified aminopeptidase W was diluted in 1 mM-Tris/HCl buffer, pH 8.0, and assayed in 100 mM buffers at either pH 8.0 or 9.0. Results are means for two experiments. The rates relative to those in Tris/HCl buffer, pH 8, are shown in parentheses.

Buffer	pH	Specific activity (μmol/min per mg of protein)
Tris/HCl	8.0	19.7 (100)
Na_2HPO_4/NaH_2PO_4	8.0	1.5 (8)
Tris/HCl	9.0	12.9 (65)
Glycine/NaOH	9.0	4.3 (22)
$NaHCO_3$/NaOH	9.0	6.6 (34)

ions had no effect, Ni^{2+} was slightly inhibitory (25%), and Zn^{2+}, Co^{2+} and Cu^{2+} were strongly inhibitory (65–90%). There was, however, a marked dependence on pH: Zn^{2+} and Co^{2+} showed weak inhibition at pH 6 and pH 10, and strong inhibition between these values with maximum effect at pH 8–9. The converse was observed with Cu^{2+}, which exhibited minimum inhibition at pH 9 and maximum at the extremes of pH (Fig. 2). However, these effects were also dependent on the nature of the buffer used (Table 5). In glycine/NaOH buffer, pH 8, these metal ions caused activation rather than inhibition, e.g. Co^{2+} activates strongly in glycine buffer, but inhibited in bicarbonate or Tris/HCl buffers. In phosphate buffer, pH 8, which itself was strongly inhibitory, the effects of Zn^{2+} and Co^{2+} were slight, although Cu^{2+} abolished the residual activity.

The inhibition by Zn^{2+} showed a strong dependence on pH in Mes/NaOH and Tris/HCl buffers (Fig. 3). IC_{50} values from this experiment were approx. 10 μM at pH 9,

50 μM at pH 8 and 1 mM at pH 7 (all in Tris/HCl), and no inhibition was observed at pH 6 in Mes/NaOH. The inhibition by Zn^{2+} was fully reversed by EDTA (Fig. 4), though full recovery required a 5–10-fold molar excess of the chelator.

The effects of EDTA and 1,10-phenanthroline on the untreated enzyme were also studied over the concentration range 1 μM–10 mM (preincubation in 0.1 M-Tris/HCl buffer, pH 8, for 1 h at 4 °C; the concentrations of the chelators in the assays were 90% of those in the preincubation. No inhibition was detected. Indeed, dialysis of the enzyme against 10 mM-EDTA for 3 days produced no loss of activity compared with a control dialysed against buffer.

Effects of inhibitors on the hydrolysis of Leu-Trp

Group-specific inhibitors for cysteine peptidases (iodoacetamide, iodoacetate, *p*-chloromercuribenzoate, *N*-ethylmaleimide), for serine enzymes (di-isopropyl phosphorofluoridate) and for aspartic peptidases (pepstatin) had no effect when preincubated with aminopeptidase W at various concentrations (Table 6). Phosphoramidon, a specific inhibitor of certain metalloendopeptidases (e.g. endopeptidase-24.11), also had no effect.

The only effective inhibitors (IC_{50} values 2 μM and 6 μM) were those known to inhibit many other aminopeptidases, amastatin and bestatin (Umezawa & Aoyagi, 1977; Aoyagi *et al.*, 1978; Rich *et al.*, 1984); actinonin, a more specific inhibitor for aminopeptidase N (Umezawa *et al.*, 1985), had no effect. Some inhibition was also observed with some thiol compounds, the dithiol, dithiothreitol, being more effective than 2-mercaptoethanol and captopril. The last-named is a potent inhibitor of peptidyl dipeptidase A (angiotensin-

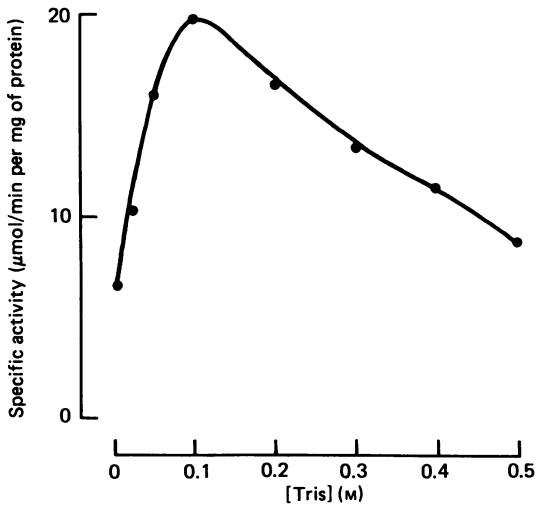

Fig. 1. Effect of Tris buffer concentration on the hydrolysis of Leu-Trp by aminopeptidase W

The pH was 8.0 and the substrate was 0.5 mM-Leu-Trp. Results are means for two experiments.

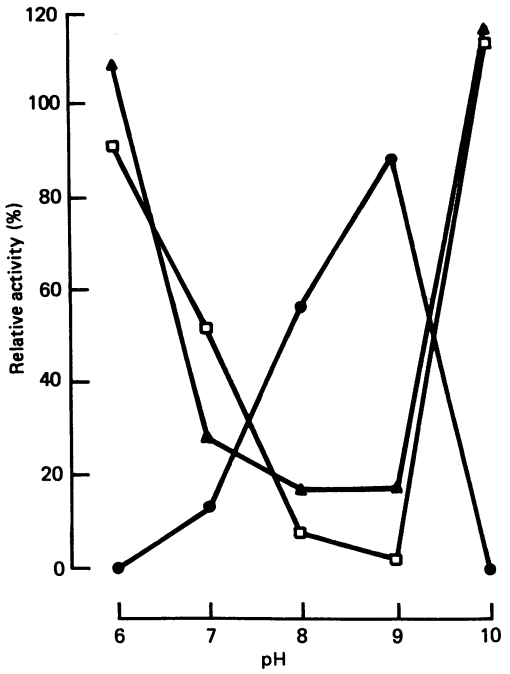

Fig. 2. pH-dependence of metal ion inhibition of aminopeptidase W

The metal ion salts (1 mM as chlorides) were preincubated with enzyme for 1 h at 4 °C before assay with 0.5 mM-Leu-Trp. Buffers used (0.1 M) in preincubation and assay were: pH 6, Mes/NaOH; pH 7, 8 and 9, Tris/HCl; pH 10, $NaHCO_3$/NaOH. □, Zn^{2+}; ●, Cu^{2+}; ▲, Co^{2+}. The activities are relative to those in the controls (at each pH value) without the addition of metal ion salts.

Table 5. Interactive effects of buffer and metal ions on the hydrolysis of Leu-Trp by aminopeptidase W

Chloride salts of metal ions (1 mM) were preincubated for 1 h at 4 °C with aminopeptidase W in 10 mM buffers as indicated and assayed in the same medium with 0.5 mM-Leu-Trp as substrate.

			Activity (μmol/min per mg of protein)			
Buffer	pH	Addition ...	None	Zn^{2+}	Cu^{2+}	Co^{2+}
Tris/HCl	8.0		20.3	1.1	9.8	4.3
Na_2HPO_4/NaH_2PO_4	8.0		1.5	1.4	0	1.4
Glycine/NaOH	8.0		4.2	10.9	4.8	22.0
Tris/HCl	9.0		13.2	0	9.0	2.3
$NaHCO_3$/NaOH	9.0		7.1	2.0	0	4.6

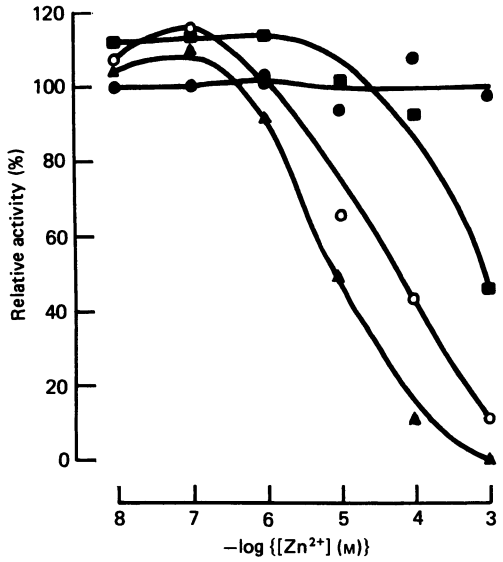

Fig. 3. pH- and concentration-dependence of Zn^{2+} inhibition of aminopeptidase W

Samples of the enzyme were preincubated with $ZnCl_2$ at various concentrations and at different pH values for 1 h at 4 °C before assay with 0.5 mM-Leu-Trp as substrate. Assay conditions were those used in preincubation. The preincubation buffers were as in Fig. 2 legend. ●, pH 6; ■, pH 7; ○, pH 8; ▲, pH 9.

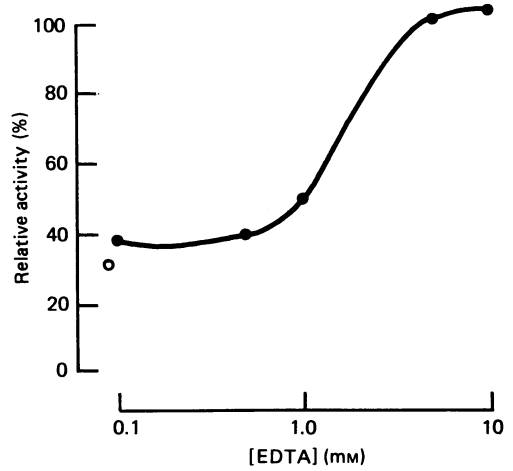

Fig. 4. Re-activation of aminopeptidase W by EDTA after inhibition by Zn^{2+}

A sample of aminopeptidase W was preincubated with 1 mM-$ZnCl_2$ for 1 h at 4 °C and then with various concentrations of EDTA for 1 h at 4 °C. The buffer in both steps was 0.1 M-Tris/HCl buffer, pH 8.0. ○, Activity before EDTA treatment. Activity was assayed with 0.5 mM-Leu-Trp as substrate.

converting enzyme, EC 3.4.15.1), but the high IC_{50} for aminopeptidase W (0.3 mM) should be contrasted with that for peptidyl dipeptidase A (3 nM) in hydrolysing hippuryl-His-Leu (Matsas *et al.*, 1984).

Table 6. Effects of inhibitors and potential inhibitors on aminopeptidase W

The enzyme was preincubated with the inhibitors for 1 h at 4 °C before assay with 0.5 mM-Leu-Trp as substrate in 0.1 M-Tris/HCl buffer, pH 8.0. Abbreviation: N.D., no inhibition observed.

Inhibitor	Concentration range tested (μM)	IC_{50}
EDTA	1–10000	N.D.
1,10-Phenanthroline	1–10000	N.D.
Iodoacetamide	1–1000	N.D.
Iodoacetate	1–1000	N.D.
p-Chloromercuribenzoate	1–1000	N.D.
N-Ethylmaleimide	1–1000	N.D.
Di-isopropyl phosphorofluoridate	1–100	N.D.
Phosphoramidon	1–10	N.D.
Pepstatin	1–1000	N.D.
Actinonin	0.1–100	N.D.
Amastatin	0.1–10	2 μM
Bestatin	0.1–1000	6 μM
Dithiothreitol	1–1000	0.1 mM
2-Mercaptoethanol	1–1000	0.6 mM
Captopril	1–1000	0.3 mM

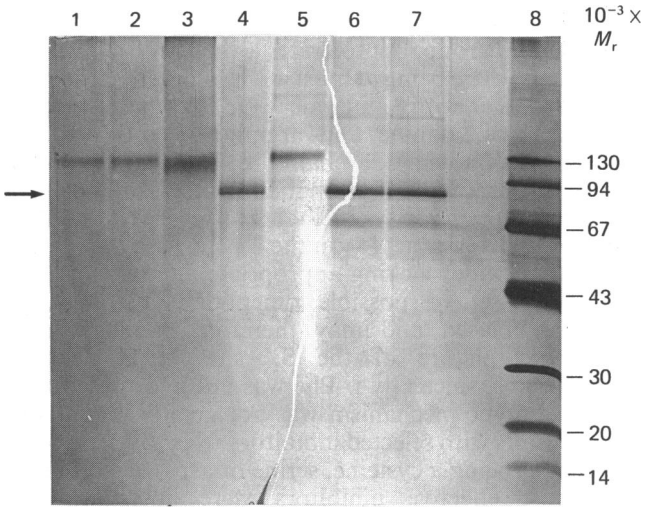

Fig. 5. SDS/polyacrylamide-gel electrophoresis of aminopeptidase W showing effect of *N*-glycanase treatment

The gel was stained for protein by the silver method (see the Experimental section for details). Tracks 1–4 contained samples of aminopeptidase (2 μg) pretreated with 0.5% SDS and then incubated with 0, 0.0028, 0.014 and 0.028 unit of *N*-glycanase respectively in the presence of 1.25% Triton (v/v) X-100. Tracks 5–7 contained native samples of aminopeptidase W (2 μg) incubated with 0, 0.14 and 0.21 unit of *N*-glycanase respectively. At the end of the incubation (18 h at 37 °C) all samples were subjected to SDS/polyacrylamide-gel electrophoresis. Track 8 contains M_r markers as indicated. Deglycosylation to a 90000-M_r polypeptide (arrow) was observed with 0.028 unit of *N*-glycanase (track 4, on the SDS-treated samples) and by 0.14 and 0.21 unit (tracks 6 and 7, on the untreated samples).

Deglycosylation of aminopeptidase W

The purified enzyme had been shown to stain positively with HIO_4/Schiff reagent (Gee & Kenny, 1985*b*), and further evidence for the carbohydrate content of aminopeptidase W was obtained by enzymic deglycosylation with *N*-glycanase. Fig. 5 shows an SDS/polyacrylamide-gel electrophoretogram. Treatment with *N*-glycanase diminished the apparent M_r from 130000 to 90000. Treatment of the aminopeptidase with 0.5% SDS before incubation with the glycanase gave a comparable result. Thus aminopeptidase W contains about 30% carbohydrate, removable by *N*-glycanase treatment.

Zinc content of aminopeptidase W

Analysis by atomic absorption spectrophotometry gave a value of 0.059% Zn (w/w of protein). A sample of crystalline insulin contained 0.36% Zn, in agreement with the expected value of 0.4% [Merck Index, 9th edn. (1976)]. From the apparent subunit M_r (from SDS/polyacrylamide-gel electrophoresis) of 130000, this sample of aminopeptidase W contains 1.2 atoms of Zn/subunit.

DISCUSSION

It is possible to visualize some aspects of the active site of aminopeptidase W. The site is probably not extensive, being best suited to accommodate dipeptides. It is, however, not a true dipeptidase, since extended peptides were hydrolysed, albeit more slowly than the original dipeptide (Gee & Kenny, 1985*b*). The S_1 site requires a free α-amino group, but tolerates a variety of different P_1 side chains, there being at most a 3-fold difference in rates of hydrolysis with glutamic acid, leucine, glycine, phenylalanine, alanine and arginine in this position. Bulky hydrophobic side chains in P_1' seem to favour hydrolysis, especially if aromatic (tryptophan > phenylalanine > tyrosine > > leucine). However, the need to monitor hydrolysis at A_{214} in the h.p.l.c. assay precluded testing dipeptides lacking appropriate amino acids, and hence our set of possible dipeptide substrates was inevitably biased and may therefore have given an incomplete picture of the S_1' site. It is certainly stereospecific, since Gly-D-Phe was not hydrolysed.

The catalytic mechanism was not greatly illuminated by the results with selected inhibitors. Negative responses excluded it being a cysteine, serine or aspartic peptidase. The most effective inhibitors were amastatin and bestatin, reagents that are effective inhibitors for many different aminopeptidases (Kenny *et al.*, 1987). The effect of thiols, especially dithiothreitol, is consistent with it being a metallopeptidase, as, too, are the inhibitory effects of phosphate buffers (compare, e.g., endopeptidase-24.11; Kerr & Kenny, 1974). Indeed, zinc analysis revealed the presence of 1 atom/subunit. But the complete resistance of the enzyme to EDTA and 1,10-phenanthroline is certainly not typical of a metallopeptidase. Nevertheless, a metal-catalysed mechanism, probably involving zinc, remains the most likely interpretation of our findings, especially if note is taken of the remarkable sensitivity of the enzyme to various metal ions. The highest activity of the enzyme was achieved by Co^{2+} ions in the presence of glycine/NaOH buffer. Most of the other peptidases in the kidney microvillar membrane are Zn-metalloenzymes, including endopeptidase-24.11, aminopeptidase N, carboxypeptidase P, peptidyl dipeptidase A and microsomal dipeptidase. Only one is known to contain Ca^{2+} (aminopeptidase A), and another (dipeptidyl peptidase IV) is a serine enzyme (for a review see Kenny *et al.*, 1987). Thus, with respect to its molecular properties (glycoprotein, zinc content, large subunits and dimeric structure) and its topology (a stalked ectoenzyme), aminopeptidase W is typical of this group of membrane peptidases.

The role of this aminopeptidase W among the battery of other exopeptidases in renal and intestinal brush borders has yet to be elucidated. It would appear to have advantages over aminopeptidase N in hydrolysing smaller fragments, especially dipeptides, generated in the course of intestinal digestion. Insofar as it has a predilection for dipeptides with a P_1' tryptophan residue, it might be important in scavenging peptides containing this essential amino acid. The same arguments may be applied to peptides containing phenylalanine, tyrosine and the branched chain amino acids, since some of these were also well hydrolysed by aminopeptidase W. It might have a similar role in the renal proximal tubule, though here the need, in relation to a much smaller load of filtered peptides, is less obvious.

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