Purification and characterization of the major aminopeptidase from human skeletal muscle

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The major aminopeptidase from human quadriceps muscle was purified (as judged by polyacrylamide-gel electrophoresis) by anion-exchange chromatography (two steps) and gel filtration (two steps). The enzyme showed maximum activity at pH 7.3, in the presence of 1 mM-2-mercaptoethanol and 0.5 mM- Ca^{2+} ions; activation of the enzyme occurred in the presence of several other bivalent cations. Inhibition of activity was obtained in the presence of metal-ion-chelating agents and inhibitors of aminopeptidases and thiol proteinases. The molecular weight of the enzyme was 102 000 (by gel filtration). The enzyme hydrolysed several amino acyl-7-amido-4-methylcoumarin derivatives; highest activity was obtained with alanyl-7-amido-4-methylcoumarin. The enzyme also degraded a series of dipeptides, alanine oligopeptides and some naturally occurring peptides. Of particular interest was the high activity of the enzyme towards the enkephalins.

The loss of muscle tissue associated with muscular dystrophy has been attributed to an increase in the activity of intramuscular proteolytic enzymes, resulting in an increased rate of protein degradation and an imbalance in muscle-cell protein turnover. The role of exopeptidases in protein degradation has received little attention; the object of the present study was to purify and characterize the aminopeptidases in human skeletal muscle, with a view to establishing the role of these enzymes in protein turnover. Although a number of these enzymes have been described in other tissues (McDonald & Schwabe, 1977), little is known about the muscle enzymes, particularly with respect to protein turnover. Most of the aminopeptidase activity (measured by the hydrolysis of aminoacyl-CONH-Mec derivatives) in human muscle can be accounted for by a single enzyme (D. Mantle, B. Lauffart & R. J. T. Pennington, unpublished work); the present paper describes the purification and characterization of this enzyme. A preliminary characterization of the aminopeptidases in rat (Parsons & Pennington, 1976; Parsons *et al.*, 1979) and human muscle (Bury & Pennington, 1973) has been carried out previously in this laboratory.

Abbreviations used: -CONH-Mec, -7-amido-4-methylcoumarin (NH_2 -Mec is the free base); RFI, relative fluorescence intensity; h.p.l.c., high-pressure liquid chromatography.

Experimental

Materials

All materials used were of analytical grade where available and were obtained as follows: DEAE-Sephadex A-50 and Sephacryl S-200 from Pharmacia; Ultrogel AcA 22 from LKB; Coomassie Brilliant Blue R from G. T. Gurr, London S.W.6, U.K.; NH_2 -Mec derivatives from Bachem, Bubendorf, Switzerland, and Cambridge Biochemicals, Cambridge, U.K.; soya-bean trypsin inhibitor from Merck; α_2 -macroglobulin from Boehringer; all other materials were obtained from Sigma and BDH.

Methods

Extraction and purification procedures. All experimental procedures were carried out at 6°C unless otherwise specified.

Initial extraction of enzyme. Human post-mortem quadriceps muscle was obtained within 12 h of death and stored in liquid N_2 . Approx. 6 g of muscle were minced, trimmed free from visible fat and connective tissue, and homogenized with an Ultra-Turrax homogenizer (two 30 s bursts at 15 000 rev./min, with cooling in ice). A 1:4 (tissue/buffer) homogenate (H) was prepared in the following extraction buffer: 50 mM-glycylglycine / 5 mM-EGTA / 1 mM-2-mercaptoethanol / 0.02% NaN_3 , pH 7.5 at 6°C; if necessary the pH of the homo-

genate was re-adjusted to 7.5 by dropwise addition of 0.1 M-NaOH. The homogenate was centrifuged at 17000g for 30 min, and the supernatant (S1) retained for enzyme purification (20 ml). The pellet was resuspended in extraction buffer containing 1 M-KCl, and rehomogenized as above (R).

Enzyme purification. Step 1: Anion-exchange chromatography of S1. Approx. 20 ml of S1 were applied to a column (30 cm $\times$ 1.5 cm) of DEAE-Sephadex A-50 ion-exchange resin equilibrated with extraction buffer containing 0.1 M-NaCl. The concentration of NaCl in S1 was adjusted to 0.1 M before loading on to the column. The column was washed with approx. 5 bed vol. of equilibration buffer, and then eluted with a linear salt gradient (0.1–0.5 M-NaCl, total volume 500 ml) in the same buffer at a flow rate of 10 ml/h; 3.6 ml fractions were collected. The NaCl concentration in the fractions was determined by Na⁺-ion flame photometry.

Step 2: Anion-exchange chromatography of the major enzyme peak from step 1. The fractions from step 1 comprising the major peak of aminopeptidase activity (122–132) were pooled (40 ml) and applied to a second ion-exchange column (30 cm $\times$ 1.5 cm) of DEAE-Sephadex A-50 equilibrated as in step 1. Elution conditions were as for step 1 (3.8 ml fractions collected), except that the NaCl concentration range covered by the gradient was from 0.2 to 0.3 M.

Step 3: Gel-filtration chromatography on Ultrogel AcA 22. The fractions comprising the peak of aminopeptidase activity in step 2 (87–97) were pooled (42 ml) and concentrated in an Amicon ultrafiltration cell with a 10000-mol.wt. exclusion membrane. The concentrated sample (4 ml) was applied to a column (88 cm $\times$ 1.5 cm) of Ultrogel AcA 22 equilibrated with 50 mM-Tris/acetate buffer, pH 7.5 at 6°C, containing 0.1 M-KCl, 1 mM-EDTA, 1 mM-2-mercaptoethanol and 0.02% NaN₃. The column was eluted at a flow rate of 10 ml/h; 3.8 ml fractions were collected. Fractions 37–42 (23 ml) were pooled.

Step 4: Gel-filtration chromatography on Sephacryl S-200. The enzyme peak from step 3 was concentrated as described above (2 ml) and applied to a column (88 cm $\times$ 1.5 cm) of Sephacryl S-200 equilibrated with 50 mM-Tris/acetate buffer, pH 7.5 at 6°C, containing 0.1 M-KCl, 1 mM-EDTA, 1 mM-2-mercaptoethanol and 0.02% NaN₃; the flow rate was 10 ml/h and 3 ml fractions were collected. Fractions 27–31 were pooled (15 ml).

Enzyme homogeneity: polyacrylamide-gel electrophoresis. Polyacrylamide-gel electrophoresis of the enzyme after the purification procedure was carried out at 6°C with 7.5% (w/v) polyacrylamide rod gels (10 cm long), with a continuous Tris/acetate buffer system (0.1 M, pH 7.5 at 6°C) containing 1 mM-2-mercaptoethanol. The gels were pre-electrophor-

osed at 8 mA/gel for 30 min; enzyme samples (approx. 5–10 μ g of protein per gel) were run at 8 mA/gel for approx. 2–3 h, with Bromophenol Blue as marker dye. The gels were fixed by immersion in 12.5% (w/v) trichloroacetic acid, followed by 15 min staining in Coomassie Brilliant Blue R stain (0.25%, w/v, in acetic acid/water/methanol, 1:5:5, by vol.). The gels were destained overnight in aq. 10% (v/v) acetic acid. Gels to be assayed for aminopeptidase activity were sliced immediately after the run (3 mm-thick slices), and the slices soaked for 24 h at 6°C in 50 mM-Tris/acetate buffer, pH 7.5, containing 1 mM-2-mercaptoethanol. Portions of this medium were then assayed for enzyme activity.

Assay of aminopeptidase activity. The enzyme (0.1 ml) was incubated for 1 h at 37°C in a medium containing 50 mM-Tris/acetate buffer, pH 7.5 at 37°C, 5 mM-CaCl₂, 1 mM-2-mercaptoethanol and 0.125 mM-Ala-CONH-Mec (total volume 0.3 ml); stock substrate solutions (2.5 mM) were prepared in dimethyl sulphoxide. The reaction was stopped by the addition of 0.6 ml of ethanol and the fluorescence of the liberated NH₂-Mec measured ($\lambda_{\text{excitation}}$ 380 nm, $\lambda_{\text{emission}}$ 440 nm) by reference to a fluorescence standard (RFI). Assay blanks were run in which the enzyme was added to the medium immediately before the addition of ethanol. Samples with a high enzyme activity (>15% substrate utilization) were assayed by using 0.01 ml of enzyme in a 5 min assay.

Protein. Protein was determined by the Coomassie Blue G binding method of Sedmak & Grossberg (1977), with bovine serum albumin as standard. An equal volume of 0.2 M-NaOH was added to homogenate samples before protein determination.

Effect of inhibitors on enzyme activity. Enzyme (0.09 ml) was preincubated for 2 min in ice with 0.01 ml of the appropriate concentration of inhibitor, followed by the addition of 0.2 ml of assay medium containing the same concentration of inhibitor. Controls were run in which the inhibitor was added at the end of the assay; where appropriate these incorporated the organic solvent used to prepare stock solutions of inhibitors.

Molecular-weight determination. The molecular weight of the purified aminopeptidase was estimated by gel filtration on Sephacryl S-200 (step 4 of the purification procedure). Myoglobin, pepsin, haemoglobin, creatine kinase, alcohol dehydrogenase and catalase were used as molecular-weight markers. The void volume of the column (V_0) was determined using Dextran Blue. Fraction volumes were determined by weight.

H.p.l.c. analysis of aminopeptidase activity towards peptide substrates. The activity of the purified major aminopeptidase on a series of alanine oligopeptides and naturally occurring peptides was

analysed by h.p.l.c. Enzyme (0.1 ml, 20 µg/ml) and the peptide (200 µg/ml) were incubated for 5 h at 37°C in a medium containing 50 mM-Tris/acetate buffer, pH 7.5 at 37°C, 5 mM-CaCl₂ and 1 mM-

2-mercaptoethanol (total volume 0.3 ml). The reaction was stopped by the addition of 0.3 ml of 2% (v/v) trifluoroacetic acid, the assay tubes centrifuged at 1000 g for 10 min, and the supernatants

Table 1. Purification of major aminopeptidase

Details of the fractionation procedure, determination of enzyme activity and protein concentration are given under 'Methods'.

Fraction	Total protein (mg)	Total activity (µmol/h)	Specific activity (µmol/h per mg)	Purification (fold)	Recovery of activity (%)
Homogenate	675	228	0.337	—	100
Soluble fraction S1	158	176	1.113	3.3	77
Major enzyme peak:					
Ion-exchange chromatography					
I	4.2	210	50.0	148	92
II	2.5	151	60.4	180	66
Gel filtration					
Ultrogel AcA 22	0.80	109	136.3	404	48
Sephacryl S-200	0.30	79	263.3	781	34

Fig. 1. Anion-exchange chromatography of muscle soluble fraction (S1)

Muscle soluble fraction (approx. 160 mg of protein) was applied to a column (30 cm x 1.5 cm) of DEAE-Sephadex A-50; fractions were eluted and assayed as described under 'Methods'. O, Activity (nmol/h per ml); ●, activity (nmol/h per ml)/100; —, A₂₈₀; ----, [NaCl].

retained for analysis. Assay blanks were run in which the enzyme was added immediately before precipitation with trifluoroacetic acid. The h.p.l.c. methods and equipment have been described in detail elsewhere (McDermott *et al.*, 1981). The chromatography was carried out on a μ -Bondapak C-18 column. Two linear gradients were employed: (i) 5–100% B (see below) over 10 min for the smaller peptides (<500 mol.wt.), and (ii) 3–70% B over 20 min for the larger peptides [in both cases A is 11 mM-trifluoroacetic acid; in gradient (i) B is 10% acetonitrile in 11 mM-trifluoroacetic acid and in gradient (ii) it is 70% acetonitrile in 11 mM-trifluoroacetic acid]. The flow rate throughout was 1 ml/min, and the column effluent was monitored at 206 nm. The degradation products were identified by comparison of their elution times with those of the appropriate standards.

Results and discussion

The results of a typical purification of the major aminopeptidase from human skeletal muscle are summarized in Table 1. The second (high-salt) extraction contained approx. 25% of the activity of

Fig. 2. Polyacrylamide-gel electrophoresis of purified enzyme

Approx. 10 μ g of purified enzyme was applied per gel; electrophoresis conditions, protein staining and assay of activity were as described under 'Methods'. The direction of migration is from cathode to anode. O, Activity (nmol/h per slice); the position of the stained band is shown.

the first extract, but was not purified. The percentage-recovery and specific-activity data based on values in the initial extracts are approximate, since inhibitors of the enzyme may be present in these fractions.

A preliminary survey of aminopeptidase activity in fraction S1 indicated that the highest activity was obtained at pH 7.5 with Ala-CONH-Mec as substrate in the presence of 5 mM- Ca^{2+} and 1 mM-2-mercaptoethanol; these conditions were therefore used in the assay of the fractions from the ion-exchange chromatography of fraction S1 (and for subsequent steps in the purification of the major aminopeptidase), the results of which are shown in Fig. 1. The preliminary assay (0.1 ml of fraction, 1 h incubation) showed the aminopeptidase activity to be fractionated into one major peak and several minor peaks. Modification of the assay (0.01 ml of fraction, 5 min incubation) showed that the major enzyme, which eluted from the gradient at a NaCl concentration of 0.27 M as a single peak of activity, accounted for more than 90% of the total aminopeptidase activity eluting from the column. The fractions comprising the major peak of activity were pooled for further purification. A single symmetrical peak of activity was obtained in each of the subsequent steps (2–4) of the purification procedure, i.e. ion-exchange chromatography, and gel-filtration chromatography on Ultrogel AcA 22 and Sephacryl S-200. The specific activity of the purified enzyme was 260 μ mol/h per mg of protein (Ala-CONH-Mec substrate), with a 780-fold purification and 34% recovery of activity. In one experiment, muscle obtained immediately after leg amputation was processed by the extraction and purification scheme described above. The elution profiles obtained for each of the purification steps were identical, within experimental error, with those obtained with post-mortem muscle.

Enzyme homogeneity was assessed by polyacrylamide-gel electrophoresis; a single band of protein which coincided with the enzyme activity was observed (Fig. 2).

Characterization of the purified enzyme

The assay method used throughout the studies described in the following sections was, unless otherwise specified, as outlined in the Experimental section; where appropriate, individual components in the assay were substituted (e.g. buffers, metal ions, thiol-group-containing reagents, substrates) in the medium, without preincubation with the enzyme.

(a) *Stability of the enzyme.* With increasing purification there was an increased dependence on the presence of a thiol-group-containing reagent to maintain enzyme activity. Maximum activity was obtained in the presence of 1 mM-2-mercaptoethanol; the routine incorporation of this reagent in

all buffers prevented the loss of activity of the purified enzyme when stored at 4°C for a period of several weeks. There was complete loss of activity after freezing at -70°C or freeze-drying.

Enzyme activity was measured over the temperature range 6–60°C by using the assay method described above, with a correction for the pH/temperature-dependence of the assay buffer. Maximum activity was obtained at 37°C; at temperatures greater than 50°C the enzyme was rapidly inactivated.

(b) *pH-dependence*. Enzyme activity was measured over the pH range 5–10 with the following buffer systems: pH 5–6, 50 mM-sodium acetate/acetic acid; pH 6–8.5, 50 mM-Tris/acetate; pH 8.5–10, 50 mM-glycine/NaOH. Highest activity was in the range pH 6.5–8.5, with maximum activity at pH 7.3.

(c) *Effect of ionic strength on activity*. The activity of the enzyme was unaffected by changes in ionic strength over the range 1–100 mM-NaCl; activity was decreased by approx. 50% in the presence of 1 M-NaCl.

(d) *Effect of metal ions*. Table 2 shows the effect of various bivalent cations (added as the chloride) on

enzyme activity over the concentration range 0.05–5 mM. Activation of the enzyme occurred in the presence of a number of metal ions; maximum activity was observed without preincubation of enzyme and metal ion. The highest levels of activity, over the whole of the concentration range studied were obtained in the presence of Ca²⁺ ions. The dependence of enzyme activity on Ca²⁺ ion concentration is shown in Table 2; maximum activity

Table 3. *Effect of potential inhibitors on enzyme activity*

Enzyme and inhibitor were preincubated at the concentration shown for 2 min at 0°C, followed by addition of assay medium incorporating the same concentration of inhibitor. The standard assay procedure was then used. Abbreviation used: Bu^t-Thr-Phe-Pro. t-butylthreonylphenylalanylproline.

Compound	Concentration	Activity remaining (%)
Leupeptin	2 µg/ml	85
	20 µg/ml	94
	100 µg/ml	85
Chymostatin	2 µg/ml	99
	20 µg/ml	99
	100 µg/ml	80
Antipain	1 µg/ml	91
	10 µg/ml	95
	50 µg/ml	86
Soya-bean trypsin inhibitor	0.2 mg/ml	91
	2 mg/ml	91
4-Chloromercuribenzoate	0.1 mM	75
	1 mM	43
	10 mM	5
4-Hydroxymercuriphenylsulphonate	0.01 mM	85
	0.1 mM	85
	1 mM	20
N-Ethylmaleimide	0.1 mM	60
	1 mM	17
	10 mM	6
Pepstatin	2 µg/ml	92
	20 µg/ml	111
	100 µg/ml	105
EDTA	0.5 mM	50
	5 mM	5
EGTA	0.5 mM	100
	5 mM	25
Puromycin	0.1 mM	10
	1 mM	2
	10 mM	0.5
Bestatin	0.1 µM	60
	1 µM	15
	10 µM	2
Bu ^t -Thr-Phe-Pro	0.01 mM	90
	0.1 mM	65
	1 mM	21
α ₂ -Macroglobulin	0.01 mg/ml	130
	0.1 mg/ml	120
	1 mg/ml	40
Phenylmethanesulphonyl fluoride	1 mM	103

Table 2. *Effect of (a) metal ions and (b) Ca²⁺ concentration on enzyme activity*

Activity was measured by using the standard assay method described under 'Methods', with the appropriate concentration of metal ion substituted in the assay medium.

Metal ion	Concn. (mM)	Activity (µmol/h per ml)		
		5	0.5	0.05
Ca ²⁺	...	4.24	5.30	2.38
Sr ²⁺		3.18	2.97	0.80
Ba ²⁺		1.33	0.90	0.27
Mn ²⁺		0.69	2.65	2.34
Mg ²⁺		1.59	1.69	0.37
Co ²⁺		0.10	0.80	2.32
Zn ²⁺		0.05	0.10	0.80
Cd ²⁺		0.05	0.05	0.16
Cu ²⁺		0.05	0.05	0.18
None		0.27		
EDTA		0.10		

(b)	
[Ca ²⁺] (mM)	Activity (µmol/h per ml)
0	0.15
0.025	1.00
0.050	2.27
0.100	3.18
0.250	4.61
0.500	5.30
1.000	5.30

was obtained at a concentration of approx. 0.5 mM. It is possible that the activity of the enzyme *in vivo* may be regulated by changes in intracellular metal-ion concentration.

(e) *Effect of potential inhibitors on activity.* Table 3 shows the effect of various potential inhibitors on enzyme activity. The activity was essentially unaltered in the presence of inhibitors of serine and carboxy proteinases; inhibition of activity occurred in the presence of inhibitors of thiol proteinases, aminopeptidases and metal-ion-chelating agents.

(f) *Estimation of molecular weight.* The molecular weight of the enzyme was estimated by gel-filtration chromatography on Sephacryl S-200 as described in the Experimental section; a value of $102\,000 \pm 10\,000$ (mean $\pm$ S.D., six determinations) was obtained.

(g) *Kinetic constants.* The K_m value for the reaction of the enzyme with Ala-CONH-Mec was estimated from a Lineweaver-Burk plot of $1/v$ against $1/[S]$ over the concentration range 10–125 μ M; a value of 70 μ M was obtained (V_{max} 260 μ mol/h per mg of protein). Marked substrate inhibition was found at concentrations greater than 125 μ M; the activity was unaffected by the presence of several L-amino acids, including alanine, over the concentration range 10 μ M–1 mM.

(h) *Enzyme specificity.* The activity of the enzyme on a series of L-amino acid CONH-Mec derivatives is shown in Table 4; assay conditions were as described in the Experimental section, with the appropriate substrate substituted in the assay

Table 5. Activity of enzyme on peptidyl-CONH-Mec derivatives

Activity was measured by using the standard assay procedure, with the appropriate peptidyl-CONH-Mec derivative substituted in the assay medium; activity is expressed relative to that on the Ala-CONH-Mec derivative. Abbreviations used: Bz, benzoyl; Cbz, benzyloxycarbonyl; Ac, acetyl; Suc, succinyl (3-carboxypropionyl).

Peptidyl-CONH-Mec derivative	Enzyme that hydrolyses substrate	Relative activity (%)
Alanine	Aminopeptidase	100
Gly-Arg	Dipeptidyl peptidase I	4.3
Lys-Ala	Dipeptidyl peptidase II	5.0
Arg-Arg	Dipeptidyl peptidase III	2.6
Gly-Pro	Dipeptidyl peptidase IV	9.2
Glutaryl-Phe	Chymotrypsin	0.7
Ala-Ala-Phe	Chymotrypsin	11.4
Glutaryl-Gly-Gly-Phe	Chymotrypsin	1.2
Bz-Arg	Trypsin	0.5
Cbz-Gly-Pro	Post-proline cleaving enzyme	1.1
Ac-Ala-Ala-Tyr	Elastase	0.2
Ac-Ala-Ala-Pro-Ala	Elastase	1.0
Suc-Ala-Phe-Lys	Plasmin	0.1
Cbz-Gly-Gly-Arg	Urokinase	0.8

Table 6. Activity of enzyme towards peptides

Activity was measured by using the standard assay procedure, modified for h.p.l.c. analysis as described under 'Methods'. Section (a) of the Table refers to complete peptide degradation, and section (b) to extent of initial peptide breakdown.

Peptide sequence	Degradation (% of total)
(a) Ala-Ala	100
Leu-Gly	100
Met-Ala	100
Ser-Tyr	60
Gly-Arg	35
Gly-Phe	30
Gly-Pro	0
Pro-Gly	0
Glu-Val	0
Ala-Ala-Ala	100
Ala-Ala-Ala-Ala	100
Ala-Ala-Ala-Ala-Ala	100
Ala-Ala-Ala-Ala-Ala-Ala	100
(b) Thyroliberin	0
Luliberin	0
Substance P	0
Proangiostensin	100
Angiotensin	100
Vasotocin	100
Oxytocin	100
Somatostatin-1–14-peptide	100
[Tyr ¹]somatostatin-1–14-peptide	100
[Leu ⁵]enkephalin*	100
[Met ⁵]enkephalin*	100

* Incubation for 2 min.

Table 4. Activity of enzyme on L-amino acid CONH-Mec derivatives

Activity was measured by using the standard assay procedure, with the appropriate aminoacyl-CONH-Mec derivative substituted in the assay medium; activity is expressed relative to that on the Ala-CONH-Mec derivative.

Aminoacyl-CONH-Mec derivative	Relative activity (%)
Alanine	100
Phenylalanine	51
Leucine	39
Methionine	27
Proline	26
Tyrosine	22
Lysine	15
Valine	11
Isoleucine	6
Arginine	5
γ -Glutamate	4
Serine	3
Glycine	3
α -Glutamate	1
Ornithine	1

medium. The highest activity was obtained with the Ala-CONH-Mec derivative, although the enzyme was clearly not specific for this derivative alone.

The low activity towards peptidyl-CONH-Mec substrates, which are normally used to measure enzymes other than aminopeptidases, is illustrated in Table 5. Although this enzyme is readily distinguishable from the enzymes listed in Table 5 under the stated assay conditions, it is possible that the aminopeptidase would interfere significantly in longer assays for several of these enzymes, for example, in histochemical estimations of enzyme activity, which may use incubation periods of several hours.

Table 6 shows the activity of the enzyme towards a series of peptide substrates. The results with the dipeptides indicate that the enzyme exhibits a degree of bond specificity, even with peptides of minimum complexity. The effect of the size of peptide on enzyme activity was examined by using a series of alanine-containing peptides. Each of these peptides was completely broken down to alanine under the standard assay conditions used (5h incubation); whether there is a limit to the size of peptide degraded has still to be established.

The activity of the enzyme on a series of natural peptides is also shown in Table 6; all were degraded under standard assay conditions, with the exception of Substance P, which was unaffected by the enzyme, and thyroliberin and luliberin which are blocked at the *N*-termini and immune to attack by aminopeptidases. Peaks that appeared during the initial degradation of these peptides have yet to be identified. Of particular interest was the susceptibility of [Leu]- and [Met]-enkephalin to degradation by the enzyme. Complete breakdown into amino acids occurred within 2–3 min when measured by the assay method described above; incorporation of puromycin (10 mM) or bestatin (10 μ M) in the assay prevented degradation of the enkephalins. Whether a

role will be found for the latter peptides in the functioning of muscle, or for this enzyme in the regulation of peptide hormones *in vivo*, remains to be seen.

There are a number of differences between the properties of this enzyme and similar enzymes characterized in human liver by Garner & Behal (1975) and rat skeletal muscle by Jacobs-Sturm *et al.* (1982). This may arise from species and/or tissue differences in the properties of these enzymes, or from the different experimental conditions employed in each case.

The present paper describes the purification and characterization of the major aminopeptidase from human skeletal muscle, as a first step in the investigation of the role of this enzyme in muscle protein turnover.

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