Genetic relationship between acylpeptide hydrolase and acylase, two hydrolytic enzymes with similar binding but different catalytic specificities

(acetylated peptide/carcinoma)

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Communicated by Alton Meister, December 19, 1990

ABSTRACT An 87% identity has been found between the reported cDNA sequence that encodes acylpeptide hydrolase (EC 3.4.19.1) [Mitta, M., Asada, K., Uchimura, Y., Kimizuka, F., Kato, I., Sakiyama, F. & Tsunasawa, S. (1989) *J. Biochem.* 106, 548–551] and a cDNA transcribed from a locus (*DNF15S2*) on the short arm of human chromosome 3, reported by Naylor *et al.* [Naylor, S. L., Marshall, A., Hensel, C., Martinez, P. F., Holley, B. & Sakaguchi, A. Y. (1989) *Genomics* 4, 355–361]; the *DNF15S2* locus suffers deletions in small cell lung carcinoma associated with a reduction or loss of acylase activity (EC 3.5.1.14). Acylpeptide hydrolase catalyzes the hydrolysis of the terminal acetylated amino acid preferentially from small acetylated peptides. The acetyl amino acid formed by acylpeptide hydrolase is further processed to acetate and a free amino acid by an acylase. The substrates for the acylpeptide hydrolase and the acylase behave in a reciprocal manner since acylpeptide hydrolase binds but does not process acetyl amino acids and the acylase binds acetylpeptides but does not hydrolyze them; however, the two enzymes share the same specificity for the acyl group. These findings indicate some common functional features in the protein structures of these two enzymes. Since the gene coding for acylpeptide hydrolase is within the same region of human chromosome 3 (3p21) that codes for the acylase and deletions at this locus are also associated with a decrease in acylase activity, there is a close genetic relationship between the two enzymes. There could also be a relationship between the expression of these two enzymes and acetylated peptide growth factors in some carcinomas.

The various types of exopeptidases that act on the free NH₂-terminal residues of polypeptides have been described in detail (1). The properties of a purified enzyme that cleaves an acetylated terminal amino acid from acetylated peptides (*N*-acylaminoacylpeptide hydrolase, EC 3.4.19.1, referred to here as acylpeptide hydrolase) have also been reported (2–12). For example, this enzyme catalyzes the hydrolysis of acetyltrialanine (Ac-Ala₃-OH) to acetylalanine (Ac-Ala-OH) and dialanine (Ala₂-OH). We reported that the rates of hydrolysis of different blocked peptide substrates varied considerably, depending on the nature of the first and second amino acids. Thus, there was a preference for Ac-Ala-, Ac-Met-, and Ac-Ser- at the blocked terminus (4). Comparison of this specificity with the sequences of about 100 known proteins acetylated at their NH₂-terminal residues indicated that most of them began with Ac-Ala-, Ac-Met-, or Ac-Ser- (13). Furthermore, in these blocked proteins there was a preponderance of charged amino acid residues at the second position. Hence, the characteristics of the terminal sequence of these blocked proteins appear to resemble the substrate

specificity of the acylpeptide hydrolase. The enzyme displays a broad spectrum with respect to the blocking group, since acetyl, chloroacetyl, formyl, and carbamoyl moieties are cleaved together with the first amino acid residue (2–5). In this communication we describe a systematic study of a family of blocked peptides and we present the *K_m* and *V_{max}* values for these blocked peptide substrates as a function of chain length and side-chain charge.

An enzyme (*N*-acyl-L-amino-acid amidohydrolase, EC 3.5.1.14, referred to here as acylase) that cleaves an acetyl-amino acid, a product of the acylpeptide hydrolase-catalyzed reaction, to acetate and the free amino acid does not cleave acetylated peptides, the substrates for the acylpeptide hydrolase (8, 14). Its broad range of specificity is the basis for a widely used procedure for the resolution of many racemic amino acids (15, 16). In the present study we compare some of the properties and substrate specificities of these two enzymes and we explore the possible genetic relationship between them.

MATERIALS AND METHODS

Source of Enzymes. The acylpeptide hydrolase was purified to apparent homogeneity from outdated human erythrocytes, as described previously (2–5). The final preparation was judged pure, since it showed only one band upon SDS/gel electrophoresis. The preparation was free of acylase, aminopeptidase, and carboxypeptidase (CPase)-like activity as indicated by prolonged incubation with the substrate Ac-Ala₃-OH (4); no products other than those formed by the acylpeptide hydrolase were detected. A partially purified acylase from pig kidney was purchased from Sigma. Further purification was achieved as described by Gade and Brown (14). The final preparation was about 95% pure as ascertained by SDS/gel electrophoresis. CPases A and Y were obtained from Boehringer Mannheim and from Calbiochem.

Assay for Acylpeptide Hydrolase. Ac-Ala₃-OH was usually employed as substrate for this enzyme. Incubations were performed in 100 mM [bis(2-hydroxyethyl)amino]tris-(hydroxymethyl)methane (Bis-Tris) buffer, pH 7.4, at 37°C as described previously (2–5). After an appropriate period, the amount of newly formed amino groups from the product Ala₂-OH was determined by the Fluram (fluorescamine) reagent. Amino acid analysis confirmed that the only ninhydrin-positive product in this reaction was Ala₂-OH.

Assay for Acylase. Ac-Met-OH or Ac-Ala-OH was incubated with the purified enzyme in 50 mM potassium phos-

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Abbreviations: Cm, carboxymethyl; CPase, carboxypeptidase; RP-HPLC, reverse-phase high-pressure liquid chromatography; Boc, butyloxycarbonyl; Fmoc, 9-fluorenylmethoxycarbonyl; Bis-Tris, [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane.

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phate, pH 7.5, containing 1 μ M ZnCl₂, as described by Gade and Brown (14). The rate of appearance of amino groups was determined by the Fluram reagent. The results with this assay correlated well with the appearance of free methionine or alanine determined by amino acid analysis.

Materials and Analytical Procedures. *N* $^{\alpha}$ -Fmoc-*N* $^{\epsilon}$ -Boc-Lys *p*-alkoxybenzyl alcohol resin and Ac-Ala₂-OMe were from Bachem (Fmoc, 9-fluorenylmethoxycarbonyl; Boc, *t*-butoxycarbonyl). Ac-Ala-OH, Ac-Ala₂₋₆-OH, Ac-Ala₃-OMe, Ala-Lys-HCl, and glyoxylic acid were from Sigma. Fluram was from Roche. Other reagents were trifluoroacetic acid (Sigma), trinitrobenzenesulfonic acid (Sigma), dicyclohexylcarbodiimide (Sigma), and piperidine (Fisher Scientific). High-performance liquid chromatography (HPLC) was carried out on a Beckman Altex system using an Ultrasphere-ODS C₁₈ column (5 μ m spheres; 0.46 $\times$ 25 cm, analytical; 1.0 $\times$ 25 cm, preparatory) and a gradient elution of increasing amounts of 1-propanol in aqueous 0.1% trifluoroacetic acid. Ac-Ala-Lys-OH, Ac-Ala-Lys(Cm)-OH, Ac-Ala-Lys(Ac)-OH, and Ac-Ala₄-Glu₃-Lys-OH each gave a single peak by HPLC and each had the correct amino acid analysis (Cm, carboxymethyl). Ac-Ala-Lys(Ac)-OH was also pure by elemental analysis, which was kindly performed by Robert Buzolich.

Protein samples for amino acid analysis were dialyzed extensively against water purified by reverse osmosis. The protein samples were heated in 6 M HCl at 110°C under reduced pressure for 20 or 70 hr prior to amino acid analysis. For analysis of cysteine as cysteic acid, the samples were treated with performic acid before acid hydrolysis. For analysis of tryptophan, the samples were hydrolyzed in methanesulfonic acid. Amino acid analysis was performed on an amino acid analyzer designed by Spackman *et al.* (17) or on a Beckman 6300 analyzer with System Gold enhancement. For determination of COOH-terminal amino acids, the protein samples were treated with CPase Y or CPase A for 2 or 20 hr at 25°C. The digests were then centrifuged in a Centricon device at 5000 $\times$ g; the filter was twice washed with water and again centrifuged. The combined filtrates were subjected to amino acid analysis. The appropriate control values with either CPase alone or protein alone were subtracted from the amino acid values found for the complete digest.

Synthesis of Ac-Ala-Lys-OH and Ac-Ala₄-Glu₃-Lys-OH. These peptides were prepared manually by Fmoc-solid phase strategy from Fmoc-Lys(Boc) *p*-alkoxybenzyl alcohol resin (0.67 mmol/g). Each synthetic cycle consisted of a 10 min of deprotection with 20% piperidine in dimethylformamide, washing with dimethylformamide, and coupling with Fmoc-Glu(OBu^t) or Ala pentafluoro ester (2 eq) [in the case of final coupling, symmetrical anhydride of Ac-Ala-OH (2 eq)] in dimethylformamide for 1 hr (Obu^t, *t*-butoxy). Completion of coupling was confirmed by a ninhydrin test; the final wash was with dimethylformamide. The protected peptide resins were treated with 90% trifluoroacetic acid for 3 hr. After filtration and evaporation of the solvent, the crude peptide was washed with diethyl ether and purified by reverse-phase (RP)-HPLC. Amino acid analysis for Ac-Ala₄-Glu₃-Lys-OH: Ala 4.00, Glu 2.98, Lys 0.80.

Synthesis of *N* $^{\alpha}$ -Acetylalanyl-*N* $^{\epsilon}$ -carboxymethyllysine. Ac-Ala-Lys(Cm)-OH was prepared by reaction of Ac-Ala-Lys-OH, sodium glyoxylate, and recrystallized sodium cyanoborohydride. The reagents were incubated in a molar ratio of 1:3:10 in 50 mM Hepes, pH 8, at 50°C. The amount of di-carboxymethyl derivative was minimized by employing a low ratio of sodium glyoxylate to Ac-Ala-Lys. The percentage of free amino groups during the reaction was monitored by Fluram assay. After 21 hr the samples were acidified with HCl and dried. A pure product, which was Fluram-negative, was obtained by RP-HPLC. Amino acid

analysis after hydrolysis with 6 M HCl at 110°C for 24 hr gave a Ala-to-Lys(Cm) ratio of 1.00:0.95.

Synthesis of *N* $^{\alpha}$ -Acetylalanyl-*N* $^{\epsilon}$ -acetyllysine. Ala-Lys-HCl (200 μ mol) in 1 ml of glacial acetic acid was acetylated with Ac₂O added dropwise over a period of 1 hr. After addition of water the sample was dried and the crude material was purified by preparative RP-HPLC. Amino acid analysis of the main peak gave a Ala-to-Lys ratio of 1:0.99; the yield was 40 μ mol (20%).

***K*_m and *V*_{max} Determination.** For kinetic studies, the reaction was initiated by addition of the appropriate amount of enzyme to different concentrations of substrates in 0.2 M Bistris, pH 7.4, at 37°C. The rate of hydrolysis was monitored by measuring the formation of amino groups with Fluram as a function of time; an appropriate aliquot of the reaction was diluted in 0.5 M sodium borate, pH 8.9, and 0.5 ml of 0.02% Fluram in acetone was added with vigorous stirring on a Vortex mixer. The fluorescence was measured on a Hitachi/Perkin-Elmer fluorescence spectrophotometer MPF-2A (excitation wavelength 390 nm, emission wavelength 480 nm). The absorbance values were converted to nmol by using standard calibration curves obtained with the individual amino acids or peptides that were expected to be released from each peptide substrate.

RESULTS AND DISCUSSION

Effect of Chain Length and Charge on Catalytic Efficiency of the Acylpeptide Hydrolase. The study of a series of related peptides of increasing length and different charge as substrates for the acylpeptide hydrolase is shown in Table 1. Ac-Ala-OH, the substrate for the acylase (see below), is not hydrolyzed even with prolonged incubation and large amounts of the enzyme. The efficiency of each peptide substrate is expressed in terms of *K*_m and *V*_{max} values. The diminution in catalytic efficiency (*V*_{max}/*K*_m) by the presence of any charge on the side chain of the second residue is clearly demonstrated by comparison of peptide substrate 2 with peptides 5 or 6. Thus, either a positively or a negatively charged side chain has a major influence on both *K*_m and *V*_{max} such that the overall catalytic efficiency is reduced about 10-fold. The presence or absence of an α -carboxyl group on peptides 2–4 does not reduce the catalytic efficiency very greatly. A systematic study of the effects of peptide length on the catalytic efficiency shows a large decrease between Ac-Ala₃-OH (substrate 7) and Ac-Ala₄-OH (substrate 8). There is little further change in catalytic efficiency up to Ac-Ala₆-OH (substrate 10). The peptide substrate 11 is also hydrolyzed but at a further reduced rate and affinity. The efficiency of cleavage of this octapeptide is about the same as that of the shorter peptide substrate 6. The presence of internal negatively charged side chains, as in peptides 6 and 11, results in a lowered but limiting degree of catalytic

Table 1. Efficiency of cleavage of acetylated alanine peptides by acylpeptide hydrolase

Substrate	<i>V</i> _{max} , mM·min ⁻¹	<i>V</i> _{max} / <i>K</i> _m , min ⁻¹
1. Ac-Ala-OH	0	0
2. Ac-Ala ₂ -OH	2.00	8.33
3. Ac-Ala-Lys(Ac)-OH	0.85	4.25
4. Ac-Ala-Ala-OMe	2.44	2.60
5. Ac-Ala-Lys-OH	0.29	0.37
6. Ac-Ala-Lys(Cm)-OH	0.16	0.15
7. Ac-Ala ₃ -OH	2.90	7.63
8. Ac-Ala ₄ -OH	1.10	1.17
9. Ac-Ala ₅ -OH	1.33	1.40
10. Ac-Ala ₆ -OH	0.84	0.99
11. Ac-Ala ₄ -Glu ₃ -Lys-OH	0.40	0.10

efficiency. Thus, this acylpeptide hydrolase might prove useful for sequencing peptides with blocked terminal residues. Indeed, we demonstrated that even peptides that are hydrolyzed at relatively inefficient rates could be cleaved in amounts adequate for sequencing when enough enzyme was added or when the incubation time was extended (3).

Relationship of the Acylpeptide Hydrolase with Erythrocyte "Butyryl Esterase." As described previously (2–5), the acylpeptide hydrolase also possesses esterase activity towards *p*-nitrophenyl ester. An enzyme referred to as "butyryl esterase" has been purified to homogeneity from human erythrocytes; α -naphthyl butyrate is the substrate usually used for assay of this enzyme (18–20). This enzyme is used for various types of genetic analyses. A comparison of the properties of this enzyme (molecular weight, number of subunits, and amino acid composition) with the corresponding values for the acylpeptide hydrolase indicates that these properties are practically identical. Furthermore, we have found that α -naphthyl butyrate is hydrolyzed very rapidly by the purified acylpeptide hydrolase with a $V_{\max}/K_m$ of about 6 min^{-1} . This value indicates a high efficiency of binding and cleavage of α -naphthyl butyrate by the acylpeptide hydrolase when compared with the best acetylated peptides in Table 1. Thus it appears that the two enzymes are identical.

Comparison of the Properties of the Acylpeptide Hydrolase and the Acylase. Purified acylpeptide hydrolase does not cleave acetylated amino acids, such as Ac-Met-OH or Ac-Ala-OH (Table 1). Nevertheless, acetylated amino acids bind tightly to the acylpeptide hydrolase and act as competitive inhibitors with acetylated peptide substrates (2–5, 8, 14). Acylpeptide hydrolase is a tetramer composed of subunits having a molecular weight of 75,000 each (732 amino acids per subunit). The amino acid sequences of the enzymes from porcine liver (9) and from rat liver (11) have been reported from the sequence of its cDNA and partial protein sequence information.

The acylases from bovine liver (8, 14), from pig kidney (21), and from bacteria (22) have been purified and partially characterized. This enzyme does not catalyze the hydrolysis of acetylated peptides (8, 14); however, we find that acetylated peptides bind to the acylase and act as competitive inhibitors with substrate acetyl amino acids. The subunit molecular weight of the acylase from both sources is in the range of 43,000–45,000 and the enzyme is made up of two such subunits. A partial NH_2 -terminal sequence has been reported for the bacterial enzyme (22), but there is no sequence information for the porcine enzyme.

We determined the number of amino acids per subunit of purified porcine acylase. The average of our four determinations and reported values (21) is that each subunit contains about 377 ± 10 residues, a value in full agreement with its molecular weight determined by independent means (8, 14, 21). Attempts to determine the NH_2 -terminal sequence of the protein by Edman degradation indicated that the NH_2 terminus was likely blocked. Analysis of the COOH terminus of porcine acylase by five separate digestions with two types of CPases gave the results shown in Table 2. Digestion with CPase Y for 2 hr and 20 hr in duplicate reproducibly released five amino acids in good yield; these were glycine, valine, leucine, phenylalanine, and lysine. No other amino acids were consistently released in the quadruplicate analyses with CPase Y except for a small amount of methionine and threonine. Digestion with CPase A released three amino acids—leucine, phenylalanine, and valine.

Genetic Relationship Between the Two Enzymes. Naylor *et al.* (23) reported a 3.3-kilobase (kb) cDNA sequence mapped to the *DNF15S2* locus on the short arm of human chromosome 3 (Fig. 1, top line). Deletions of this locus are found in various types of carcinomas, including small cell lung carcinoma and renal cell carcinoma (23–28). There is a good

Table 2. COOH-terminal analysis of porcine acylase by digestion with carboxypeptidases

Amino acid	Amount released, nmol/nmol of subunit	
	By CPase Y*	By CPase A
Gly	0.97, 0.74	0
Val	0.55, 0.37	0.18
Leu	0.49, 0.56	0.40
Phe	0.43, 0.52	0.31
Lys	0.32, 0.20	0

*Digestions with CPase Y were performed in duplicate for 2 and 20 hr, each with separate enzyme preparations from Calbiochem and Boehringer Mannheim; the values shown are the maximum amounts of amino acid released by treatment with the two different preparations. No other amino acids were consistently released by the CPase Y digestion except for threonine and small amounts of methionine ($<0.2 \text{ nmol/nmol}$ of subunit). Blank values for the acylase alone or carboxypeptidase alone were subtracted.

correlation between the presence of this locus and the expression of an acylase in small cell lung carcinoma (23, 28). However, the open reading frame for the acylase within this 3.3-kb cDNA sequence was not elucidated.

We find that there is a very strong degree of similarity between the *DNF15S2* coding sequence (Fig. 1, top line) and the acylpeptide hydrolase cDNA from pig (9) (Fig. 1, middle line), and rat (11), with at least 87% identity over a length of about 2.4 kb measured by employing the GENALIGN program (29). R. Erlandsson of the Karolinska Institute and colleagues (personal communication) have made a similar correlation and have suggested possible consequences of deletions at this site in renal cell carcinoma. Naylor *et al.* (23) and Miller *et al.* (28) found that deletions of this coding sequence were associated with the partial or total loss of acylase activity. Furthermore, Miller *et al.* (34) have mapped the gene coding for acylase to 3p21 of human chromosome 3, the same region that codes for acylpeptide hydrolase. This observation together with our results (2–5) as well as those of Gade and Brown (8, 14), which indicate that substrates for the acylase bind to but are not hydrolyzed by the acylpeptide hydrolase and substrates for the acylpeptide hydrolase act as competitive inhibitors with the acylase, suggest that there may be some relationship of the protein sequences of the two enzymes; however, conclusive evidence is still needed. Tentative location of the acylase as a segment of the acylpeptide hydrolase is supported by several observations:

(i) The five amino acids released from porcine acylase by CPase Y are residues 434–438 of the acylpeptide hydrolase protein sequence from porcine liver, Lys-Val-Gly-Phe-Leu (Fig. 1, lower line). The digestion with CPase A places the Leu released by CPase at position 438 rather than at position 433.

(ii) Since the total number of amino acids in porcine acylase is 377 ± 10 , the NH_2 -terminal Met of the acylase is placed at position 58 of the acylpeptide hydrolase for a total of 381 amino acids. Actually, Met-58 is encoded by the first start codon of the *DNF15S2* cDNA (23); a translational start at this site conforms to Kozak's rules (30).

(iii) The NH_2 terminus of porcine acylase is blocked. However, a thermostable bacterial acylase, which catalyzes the same reaction as porcine acylase, has a free NH_2 -terminal Met and begins with the sequence Met-Xaa-Asn-Xaa₂-Ile-Xaa-Arg-Leu-Val (22). From Met-58 of acylpeptide hydrolase, the corresponding sequence is Met-Xaa-Asn-Ile-Xaa₃-Arg-Xaa₂-Leu-Val. This homology over the first 12 amino acids is consistent with the assignment of the NH_2 terminus of porcine acylase as residue 58 of the acylpeptide hydrolase.

(iv) Proteolytic digestion of porcine acylase was employed to generate internal peptides that could be sequenced. A major peptide indicated the tetrapeptide sequence -Arg-Ser-

1 CGGT CAGGTGCTGCTGAGCGAGCCGAGGAGCGCGGCTCTGTATCGGGGCTTAGC GCCAGCCCGCGTGA GCCGCTGCTGGGCCGgA GTCAACACGAGTACGGcGGcCaaTACCGGAC
-9 agagcaaacatggagCGG CAGGTGCTGCTGAGCGAGCCGAGGAGCGCGGCTGTATCGGGGCTTAGC GCCAGCCCGCGTGA GCCGCTGCTGGGCCGgA GTCAACACGAGTACGGgGGTcGcTACCGGAC
1 M E R Q V L L S E P E E A A A L Y R G L S R Q P A L S A A C L G P E V T T Q Y G G R Y R T

126 nGTGCACACTGAGTGGACCCAGA GGACCTGGAAACGATGGAGAACATcGATTTcTnCGCCaAaTACCTGGTGTTCATGAcGGGACTCAGTGGTGTTCGAGGACCTGAGGACCAAGTGTGGAGACCGGGGG AaCTGCT
135 tGTGCACACTGAGTGGACCCAGA GGACCTGGAAACGATGGAGAACATcGATTTcTnCGCCaAaTACCTGGTGTTCATGAcGGGACTCAGTGGTGTTCGAGGACCTGAGGACCAAGTGTGGAGACCGGGGGgAgCTGCT
46 V H T E W T Q R D L E R M E N I R F C R Q Y L V F H D G D S V V F A G P A G N S V E T R G E L L

268 GAGCAGAGAGTCTCTTTCAGGCACCATGAAGGcTGTGCTGCGCAAGGCTGGAGGACCGaCcTcGgGAAGAGAGCAGTTCcTGGAGTCTGGGAGAAGACCGGAAGCTCAAGAGCTTCAACCTGTGACGCTGGAGAAACA
279 GAGCAGAGAGTCTCTTTCAGGCACCATGAAGGcTGTGCTGCGCAAGGCTGGAGGACCGGCaCcTcGgGAAGAGAGCAGTTCcTGGAGTCTGGGAGAAGACCGGAAGCTCAAGAGCTTCAACCTGTGACGCTGGAGAAACA
94 S R E S P S G T M K A V L R K A G G T G T A E E K Q F L E V W E K N R K L K S F N L S A L E K H

412 TGGGCTcGTTTATGAGGATGACTGCTTTGGCTGCTGTCTGCTGCACTCGGAGACACACTTgTtTgTATGTCGACAGAGAAGCGCCCAAGGcGAGTcTTCCTTTACAGCAAAAGCCTGGAGCTCAGTgCAGCGATGA
423 TGGGCTcGTTTATGAGGAcGACTGCTTTGGCTGCTGTCTGCTGCTGCACTCGGAGACACACTTtTtTATGTCGACAGAGAAGCGCCCAAGGCTGAGTcTTCCTTTACAGCAAAAGCCTGGAGCTCAGTgCAGCGATGA
142 G P V Y E D D C F G C L S W S H S E T H L L Y V A E K K R P K A E S F F Q T K A L D V T G S D D

556 TGAGATAGCCAGG ctGAAGaagCAGACCAAGCCATcAAGGGGATCAGTTTgTGTtTATGAAGA ctGGGAG AAACATGGTTTCCAAAAGcAtcCCTGT nTCTGCTGCTGGATgTcGAGAGtGGaAACATCTCTGTG
567 TGAGATgGCCAGGacaaagAG cCAGACCAAGCCATcAAGGGGATCAGTTTtTGTtTATGAAGAtgGGGAGAAACATGGTTTCCAAAAGtActCCTGTgTCTGCTGCTGGATgTcGAGAGtGGaAACATCTCTGTG
190 E M A R T K K P D Q A I K G D Q F L F Y E D W G E N M V S K S T P V L C V L D I E S G N I S V

694 CTTGAGGGGCTcCCTGAGAAcTGTGTCCCTGGACAGGCATTtTGGGCCCTGGAGATgCtGGTGTGGTGTtTGTGGGCTGGTGGCATGAGCC TTCGGTcTGGG ATcCGCTTtTgCcACCAATCGcAGGTGACCCCTGTATcTAc
709 CTTGAGGGGCTcCCTGAGAAcTGTGTCCCTGGACAGGCATTtTGGGCCCTGGAGAAcCaGGTGTGGTGTtTGTGGGCTGGTGGCATGAGCCcTTCGGcTGGGcAtTcCGCTTtTgCcACCAATCGcAGGTGACCCCTGTATcTAc
237 L E G V P E S V S P G Q A F W A P G D T G V V F V G W W H E P F R L G I R F C T N R R S A L Y Y

836 GTGACCTCAcTcGGGGGAAGTGTGAGCTCTCTcTcGATGAcTcTcTGGCTGTCTcTcTCTCCCGGCTgAGcCCAGAcCAATGTGCAcTcTcTACCTGCAcTcCCTCTCTGAcTCCCACTCAcCAaTGAcGCCAGcTGTGC
853 GTAGACCTCAcTcGGGGGAAGTGTGAGCTCTCTcTcGATGAcTcTcTGGCTGTCTcTcTCTCCCGGCTgAGcCCAGAcCAATGTGCAcTcTcTACCTGCAcTcCCTCTCTGAcTCCCACTCAcCAaTGAcGCCAGcTGTGC
285 V D L T G G K C E L L S D E S V A V T S P R L S P D Q C R I V Y L R F P S L V P H Q Q C G Q L C

980 CTGTATGACTGGTATACCAaGGTtACCTCAgTGGTGGTgATgTGTGCTCGGACGTGGGAGAGaACTTCTTGGGATCTACTGACGCTTCTGCTTcTGGGATGTGGTGTGAGTGTGAGTGGTcTTTGACTGcTg
997 CTGTATGACTGGTATACCAaGGTtACCTCAgTGGTGGTgATgTGTGCTCGGACGTGGGAGAGaACTTCTTGGGATCTACTGACGCTTCTGCTcTGGGATGTGGTGTGAGTGTGAGTGGTcTTTGACTGcTg
333 L Y D W Y T R V T S V V V D I V P R Q L G E D F S G I Y C S L L P L G C W S A D S Q R V V F D S

1124 gCTCAGCGCAcGCGGAGGACCTGTTTGTGTGGACCCCAaTGGGCAcTGTGACCTCccTcAcAGCTGGAAGGCTCAGGTGGgAGCTGGAAGTGTCTACAATGACCAAGGACCTATGGTGGcAcAGTtTCCCAcCCAGc
1141 cCTCAGCGCAcGCGGAGGACCTGTTTGTGTGGACCCCAaTGGGCAcTGTGACCTCccTcAcAGCTGGAAGGCTCAGGTGGgAGCTGGAAGTGTCTACAATGACCAAGGACCTATGGTGGcAcAGTtTCCCAcCCAGc
381 P Q R S R Q D L F A V D T Q M G S V T S L T A G G S G G S W K L L T I D R D L M V V Q F S T P S

1268 ctaCCTCCAAcCCTGAAAGTGGGTTCCTGCTTcTcGcAGGGAAGGAGCAGTcCaGtTtTgTGGT gtCCTGAGGAGGCGGAGCCCAcTTCcGACATCcaTGGGcATCCTGGGgTgCTACAGCCACCCCAcAGCAAGAGaAT
1285 gTACCcCCAAcCCTGAAAGTGGGTTCCTGCTTcTcGcAGGGAAGGAGCAGGcGgGTAaTcTGGGtTatcCCTGAGGAGGCGGAGCCCTTTCCTGACATCctTGGaGATCCTGGGgTgCTACAGCCACCCCAcAGCAAGAGcAT
429 V P P S L K V G F L P P A G K E Q A V S W V S L E E A E P F P D I S W S I R V L Q P P P Q Q E H

1411 GTGCACTATGCTGGCCTGACTTTGAAGCAATCTcTgTgCAGCTcGcAGcCcTCCAGATcAAGACCAaGTGCCATGCTGGTGTGATGCCCCACGGGGG CCCATTTCATCTTTGTGACTGCTGGATGTGTTCACGCCATG
1429 GTGCACTATGCTGGCCTGACTTTGAAGCAATCTcTcTcAGCCcCaGCAcTcTCCAGAGaAGACCAaGTGCCATGCTGGTGTGATGCCCCACGGGGGacCCATTTCATCTTTGTGACTGCTGGATGTGTTCACGCCATG
477 V Q Y A G L D F E A I L L Q P S N S P E K T Q V P M V V M P H G G P H S S F V T A W M L F P A M

1553 CTTTGCAAGATGGGcTTTGcGtTACTACTAGTGAACATCTGCTGGCTCCACgGGCTTTGGCCAGGACAGCATCTcTcTcTCCcAGGCAATGTGGGCCACAGGATGTGAAGGATGTCCAGTTTGTGAGTgGAaCAGGTGTCCAG
1573 CTTTGCAAGATGGGcTTTGcGtTACTACTAGTGAACATCTGCTGGCTCCACcGGCTTTGGCCaAGACAGCATCTcTcTcTCCcTGGCAATGTGGGCCACAGGATGTGAAGGAcGTCCAGTTTGTGAGTgGAaCAGGTGTCCAG
525 L C K M G F A V L L V N Y R G S T G F G Q D S I L S L P G N V G H Q D V K D V Q F A V E Q V L Q

1697 GAGGAACACTTTGATGCAaGCCaTGTGcncCTTATGGGTGGTTCOCATGGTGGCTTcTaTcTCTGOCATcTGATTTGGTCAAGTcCCAGAGACCTACAGGcGCTGCTcTg cGAACCC GTGATCA CaaTGCTcCATGtTG
1717 GAGGAACACTTTGATGCAaGCCcTGTGcncCTTATGGGTGGTTCOCATGGTGGCTTcTcTcTCTGOCATcTGATTTGGTCAAGTcCCAGAGACCTACAGTcGCTGCTGtTgagGAACCCcGTGATCAaCaTGCTcCATGtTG
573 E E H F D A G R V A L M G G S H G G F L S C H L I G Q Y P E T Y S A C V V R N P V I N I A S M M

1835 GGCTCCACTGACATCC TGACTGGTGGcTGGTGGAGGCTGGCTTC CtcCAGCAGcGACTGCTGCCAGCTCAGcGTGTGGGCTGaGATGCTGGACAAcTcGcCCATCAGaTA CtcCCTCAGGTGAAGAcCCACTGTTA
1861 GGCTCCACTGACATCCcTGACTGGTGGcTGGTGGAGGCTGGCTTC CtcCAGCAGcGACTGCTGCCAGCTCAGTGTGTGGGCTGcGATGCTGGACAAcTcCOCATCAAGTgTgCccCTCAGGTGAAGAcCCACTGTTA
621 G S T D I P D W C M V E A G F S Y S S D C L P D L S V W A A M L D K S P I K Y A P Q V K T P L L

1974 CTGATGcTgGG CAGGAGGAC GcGcGTGTGCC TC AGCAGG CAT GAGTAT acGtcCTCAAG aCCGGAATGTGC tGTcCGGCTCTGCTcTATCCCAAAAGCACCCAcGCATcTATCAGAGTGGAGGTGGA
2005 CTGATGcTgAGcCAGGAGGACagG GTGTGCCcTcTCAAGCAGGcGATgGAGTATcaccGTtCTCAAGgCCGGAATGTGCcagTcCGGCTCTGCTcTATCCCAAAAGCACCCAcGCATcTATCAGAGTGGAGGTGGAac
669 L M L G Q E D R R V P F K G M Y E A G Y R L K A R N V P V R L L L Y P K S L A C E V E V E S

2101 GcCAGCTTCATGAATGCTGTCTGCTGcTAcGCACACACTTGGGAGCTGAAGCCCTGcCAtTCTGcATGAGCTGatcagcctGTGCCaACACTTcGCTcTTGAGGAG CtcAacGGTCTGGCAG ggGCAgGAGcCTTcT
2148 aGACAGCTTCATGAATGCTGTCTGCTGcTAcGCACACACTTGGGAGCTGAAGCCCTcTcTGTCTGCTcTGAGCTG gTGGCCcCACTTgGCTaTTGgaGAGTcCcCa tGGTCTGGCAGagcTGAAGAcGCTTcTcT
717 D S F M N A V L W L C T H L G S

2241 GGGCTCTGGACTCCAcGGATGcGTG GcAGAGGAATGTGGGCTaTgTAgTcATAATAAATAGGACAgcagcAgctggtcAAtgActgcgtgA.....
2284 GGGCTCTGGACTCCAcGGATGcGTG GcAGAGGAATGTGGGCTgTATcTcATAATAAATAGGACAcagaaAaaaaaaAaaAaaaaaaA

FIG. 1. Comparison of *DNF15S2* transcript of chromosome 3 with acylpeptide hydrolase cDNA. In each set of three, the *DNF15S2* transcript (top line) is from ref. 23. The cDNA (middle line) and protein sequence (bottom line) of porcine kidney acylpeptide hydrolase are from ref. 9. Lowercase letters indicate nucleotides that differ in the two sequences.

Ala-Leu, corresponding to amino acids 279–282, and a minor peptide had a sequence -His-Gly-Pro-Val-, corresponding to amino acids 141–144 of the acylpeptide hydrolase.
(v) The calculated amino acid composition of segment 58–438 of porcine acylpeptide hydrolase (9) is in agreement with our amino acid analysis of porcine acylase and that from another laboratory (21) for the following amino acids (number

per subunit): Thr₁₇, Pro₁₉, Met₇, Leu₃₂, Tyr₉, Phe₂₀, and Arg₂₂. The values for the other amino acids differ between the two acylases and also for the 58–438 segment of the acylpeptide hydrolase.
The complete acylpeptide hydrolase cDNA reported by Mitta *et al.* (9) strongly resembles the first part of *DNF15S2* cDNA (23), although there are some minor deletions (Fig. 1).

Since deletions at this locus that codes for the acylpeptide hydrolase within this region also lead to a reduction or loss in acylase activity (28), there may be some homology that could be explained by a gene duplication event or by alternative splicing of a common primary transcript for the acylpeptide hydrolase and the acylase; proteolytic processing of a protein translation product is also a possibility. Such examples have been described for proteins and peptides (31–33) but not for two cognate enzymes of different specificity.

A question of particular interest is whether the acylpeptide hydrolase is actually expressed by the *DNF15S2* locus and may be absent in some cases of renal cell carcinoma (24) and in small cell lung carcinoma (23). The absence of either the acylpeptide hydrolase or the acylase could lead to accumulation of an acetylated peptide growth factor with a possible role in cell transformation. Thus, as shown in Table 1, the acylpeptide hydrolase is catalytically most efficient with blocked peptides. Indeed, biologically active blocked peptides are cleaved by this enzyme (3). Studies on the biological functions of the acylpeptide hydrolase and the acylase in normal and carcinoma cell lines and further genetic comparisons are in order. This system may be an example of a gene that is required for supplying an enzyme that controls a factor for cell growth (35).

We are grateful to Adelaide Acquaviva for her skillful help with the manuscript. We are also indebted to Sheenah Mische and Joseph Fernandez of the Rockefeller University Protein Sequencing/Biopolymer Facility and Rachael Kolb and Thomas O'Brien of the Rockefeller University Computing Services for their assistance. We thank Dr. F. Sakiyama of Osaka University for discussions. This work was supported in part by National Institutes of Health Grant HL-18819.

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