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Purification and Characterization of an Aminopeptidase from *Lactococcus lactis* subsp. *cremoris* AM2

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An aminopeptidase was purified from cell extracts of *Lactococcus lactis* subsp. *cremoris* AM2 by ion-exchange chromatography. After electrophoresis of the purified enzyme in the presence or absence of sodium dodecyl sulfate, one protein band was detected. The enzyme was a 300-kilodalton hexamer composed of identical subunits not linked by disulfide bridges. Activity was optimal at 40°C and pH 7 and was inhibited by classical thiol group inhibitors. The aminopeptidase hydrolyzed naphthylamide-substituted amino acids, as well as dipeptides and tripeptides. Longer protein chains such as the B chain of insulin were hydrolyzed, but at a much slower rate. The Michaelis constant (K_m) and the maximal rate of hydrolysis (V_{max}) were, respectively, 4.5 mM and 3,600 pkat/mg for the substrate L-histidyl-β-naphthylamide. Amino acid analysis showed that the enzyme contained low levels of hydrophobic residues. The partial N-terminal sequence of the first 19 residues of the mature enzyme was determined. Polyclonal antibodies were obtained from the purified enzyme, and after immunoblotting, there was no cross-reaction between these antibodies and other proteins in the crude extract.

Lactococcus lactis subsp. *cremoris* is a common constituent of mesophilic lactic starters used in the cheese industry. Aside from its property of acidification, this species is characterized by being very fastidious for amino nitrogen. It is incapable of synthesizing most of the amino acids it requires and must thus find them in its growth medium. Milk, however, is relatively poor in free amino acids and small peptides that can be used directly by the cells. To attain optimal growth, therefore, lactic acid bacteria must hydrolyze milk proteins (19, 23). This is done via a complex proteolytic system which is only now beginning to be more thoroughly understood. A proteinase located in the cell wall is the first enzyme active in the degradation of caseins, releasing peptides which can be used as substrates for cell wall, membrane-bound, or cytoplasmic peptidases (see reference 24 for a review).

We (1) have previously shown that *L. lactis* subsp. *cremoris* AM2 has high peptidase activity toward the substrate L-histidyl-L-phenylalanine-β-naphthylamide (His-Phe-β-NA). This activity was used to specifically observe the growth of *L. lactis* subsp. *cremoris* AM2 cocultivated with *Leuconostoc lactis* CNRZ 1091, which lacks aminopeptidase activity against this substrate (1). The present work reports the purification and characterization of this enzyme and indicates that it differs from other aminopeptidases described in lactic acid bacteria (3, 4, 8).

MATERIALS AND METHODS

Bacterial strain and culture conditions. *L. lactis* subsp. *cremoris* AM2, also called CNRZ 380, was obtained from the culture collection of the Centre de Recherches de Jouy-en-Josas, France, and was stored at −18°C in litmus milk. Cultures of 5 liters were grown in a medium containing lactose at 40 g/liter, tryptone (Difco Laboratories, Detroit, Mich.) at 20 g/liter, and yeast extract at 7 g/liter. Growth was at 30°C, pH was controlled at 6.5 by the addition of 10 N sodium hydroxide, and mechanical agitation was at 100 rpm. Growth was observed spectrophotometrically in a culture sample diluted 1/10 in 0.2% EDTA (pH 12), as well as by determining the consumption of 10 N NaOH. At the end of exponential growth, the cells were centrifuged at 6,000 × *g* for 10 min and washed three times in 0.05 M β-glycerophosphate buffer (pH 8.0).

Determining peptidase activity. The activity of whole cells was maximal with the substrate His-Phe-β-NA (Bachem, Bubendorf, Switzerland) (1), and protein extracts obtained during purification were routinely tested with this substrate. Other β-NA derivatives were also used, including His-Pro-β-NA, Gly-Pro-β-NA, Leu-β-NA, Ala-β-NA, Lys-β-NA, Pro-β-NA, Phe-β-NA, His-β-NA, and Glu-β-NA (Bachem). Enzyme activity was measured by assaying the quantity of naphthylamine produced from the substrates, using the method of Goldbarg and Rutenburg (9), adapted by Boquien et al. (1). The unit of activity used was the katal, the quantity of enzyme releasing 1 mol of naphthylamine per second.

The activity of the purified extract was also tested against several dipeptides and tripeptides: Leu-Tyr, Met-Pro, Met-Ala, Leu-Gly, Leu-Leu, Leu-Leu-Leu, Ala-Leu-Gly, Leu-Gly-Gly, carboxybenzoyl (CBZ)-Gly-Tyr (Sigma Chemical Co., St. Louis, Mo.), CBZ-Phe-Phe (Cyclo Chemicals, Los Angeles, Calif.), and His-Phe, Phe-Gly-Gly, Met-Leu-Gly (Interchim, Montluçon, France). Enzyme extracts were incubated at 37°C and pH 7 in the presence of 0.4 mM substrate. A volume of ninhydrin solution (20 g of ninhydrin, 750 ml of methylcellosolve [ethylene glycol monomethyl ether], 250 ml of 4 M acetate buffer [pH 5.51], 7 ml of titanium chloride) equal to the reaction volume was then

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added. After the solution was heated at 100°C for 10 min and 3 volumes of 50% ethanol were added, the absorbance of the solution was determined at 440 nm for proline and at 570 nm for the other amino acids.

Preparation of subcellular fractions. Spheroplasts were obtained after suspending washed cells (30 g [wet weight]) in 250 ml of 0.03 M Tris buffer (pH 8) containing 30% sucrose and lysozyme at 0.3 mg/ml. After incubating for 3 h at 37°C (7), the spheroplasts were recovered by centrifugation at 10,000 × *g* for 20 min. The supernatant, called the cell wall extract, was recovered and filtered through a 0.22-μm-pore-size filter.

The spheroplasts were suspended in the same buffer without sucrose. After the spheroplasts burst, the suspension was centrifuged at 20,000 × *g* for 30 min to separate the cell extract from membranes and cell wall debris.

The degree of lysis during the formation and bursting of the spheroplasts was determined by measuring A_{600} and by assaying lactate dehydrogenase (22), expressed as a percentage of the total cellular lactate dehydrogenase activity determined after lysis with lysozyme in Tris buffer without sucrose.

Purification of enzyme. Nucleic acids in the cell extract were hydrolyzed by adding RNase and DNase (21). The solution was then precipitated in the presence of 50 mM $MnSO_4$ and centrifuged at 20,000 × *g* for 30 min.

(i) First ion-exchange chromatography. Fast protein liquid chromatography was done with a Mono Q HR 10/10 column (Pharmacia, Uppsala, Sweden). The column was equilibrated with 20 mM Tris hydrochloride (pH 7.5). The extract (150 mg of proteins) was injected into the column and eluted with a linear NaCl gradient of 0 to 0.6 M in 1 h with a flow rate of 3 ml/min, and 3-ml fractions were collected. Fractions active against His-Phe-β-NA were recovered and dialyzed against 20 mM Tris hydrochloride (pH 7.5).

(ii) Second ion-exchange chromatography. The second ion-exchange chromatography was done as described in the first step, but with a linear NaCl gradient of 0.4 to 0.5 M in 60 min. During purification, active fractions were stored at −18°C in the presence of stabilizers (10% glycerol, 100 mM ammonium sulfate, 1 mM dithiothreitol).

Characterization of aminopeptidase. (i) Electrophoresis. The purified fraction was analyzed by polyacrylamide gel electrophoresis (PAGE) in 10% acrylamide gels with and without sodium dodecyl sulfate (SDS) (13), followed by staining with Coomassie blue. SDS-PAGE was used to determine molecular sizes with the following markers: phosphorylase *b* (94 kilodaltons [kDa]), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). Migration was in Tris-glycine-hydrochloride buffer (pH 8.8) for 2 h at 4°C and 30 V/cm.

After PAGE in the absence of SDS, enzyme activity was detected directly in the gel by the method of Miller and McKinnon (18). The gel was incubated at 25°C for 30 min with the following mixture: 1 mg of His-Phe-β-NA in 0.1 ml of dimethylformamide and 10 mg of fast Garnet GBC (Sigma) in 5 ml of 0.2 M Tris hydrochloride (pH 7.5). Hydrolysis of the substrate was reflected by the appearance of a red color at the level of the enzyme band.

(ii) Gel filtration. The molecular size of the active enzyme was estimated by filtration through Ultrogel AcA 34 (IBF, Villeneuve-la-Garenne, France) in a column (90 by 1 cm) with 0.15 M NaCl in 20 mM Tris hydrochloride (pH 7.5) at a flow rate of 12 ml/h. The column was calibrated with thyroglobulin (670 kDa), ferritin (440 kDa), immunoglobulin G (158 kDa), ovalbumin (42.7 kDa), myoglobin (17 kDa), and vitamin B_{12} (1.35 kDa).

(iii) Amino acid analysis. The purified enzyme was hydrolyzed with 6 N HCl under vacuum at 110°C for 24, 48, and 96

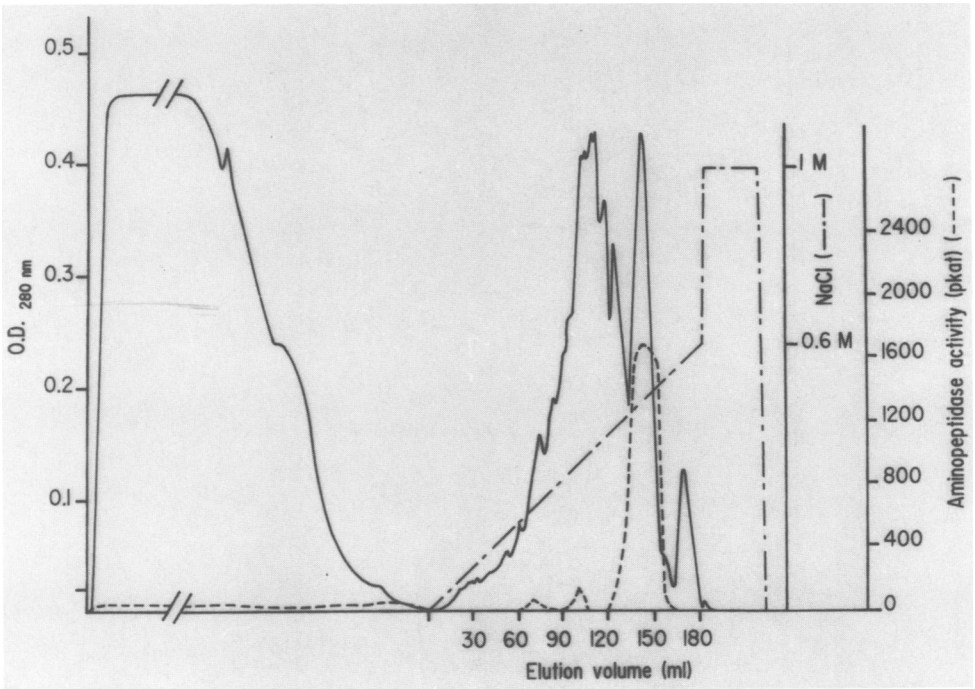

FIG. 1. First ion-exchange chromatogram for purification of aminopeptidase from *L. lactis* subsp. *cremoris* AM2. Proteins (150 mg) from the cell extract were injected onto a Mono Q HR 10/10 column. A_{280} (—) was recorded. Aminopeptidase activity was assayed with His-Phe-β-NA. The volume of the nonadsorbed fraction was 200 ml. O.D., Optical density.

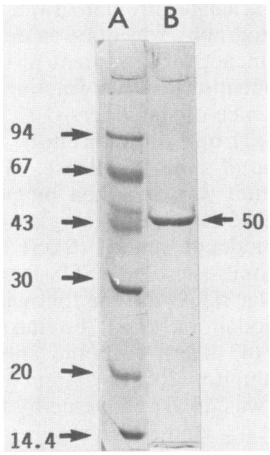

FIG. 2. SDS-PAGE of purified aminopeptidase (lane B). Lane A represents standard proteins. Bands were visualized by Coomassie blue staining. Molecular sizes are indicated in kilodaltons.

h. Tryptophan was determined after hydrolysis with mercaptoethanesulfonic acid. For cysteine, performic oxidation preceded the hydrolysis. Amino acids were quantitatively assayed with an LC 5000 amino acid analyzer (Biotronik, Munich, Federal Republic of Germany).

(iv) Determination of N-terminal sequence. An Edman recurrent degradation was done with an automatic amino acid sequencer (Applied Biosystems, San Jose, Calif.). The sample was first desalted by reversed-phase high-pressure liquid chromatography on a 7-μm 300 A Aquapore RP 300 column (30 by 4.6 mm; Brownlee, Santa Clara, Calif.) Elution was with a linear gradient from 100% solvent A (0.115% trifluoroacetic acid) to 100% solvent B (0.1% trifluoroacetic acid in acetonitrile–2-propanol–water, 37.5:37.5:30, vol/vol/vol) in 50 min at 40°C and at a flow rate of 1 ml/min.

(v) Protein assay. The quantity of protein in the extracts was determined by the method of Bradford (2), with lysozyme as the standard. For purified extracts, a more precise assay was done on amino acids after hydrolysis at 110°C for 24 h, using a Biotronik LC 5000 amino acid analyzer.

(vi) Antiaminopeptidase serum. The antiserum to the enzyme was raised in rabbits after immunization by the following protocol. A 100-μg sample of purified enzyme was injected intradermally at several points with complete Freund adjuvant. A monthly booster injection of 50 μg was then given in incomplete Freund adjuvant. The rabbits were bled 5, 10, and 15 days after each booster injection. Serum was prepared from blood by centrifugation at 10,000 × *g* for 10 min.

(vii) Immunoblotting. After PAGE of samples containing the crude extract or purified enzyme, the proteins separated on the gel were transferred to a sheet of nitrocellulose with an LKB semi-dry transfer apparatus by the technique of Towbin et al. (25). The proteins were first stained with Ponceau red to verify that transfer had effectively been accomplished. The nitrocellulose membrane was then incubated with rabbit antiaminopeptidase serum diluted 1/500, after first saturating with 5% skimmed milk. It was then incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin serum (Bio-Rad Laboratories, Richmond, Calif.) and diluted 1/1,000. The enzyme was visualized with

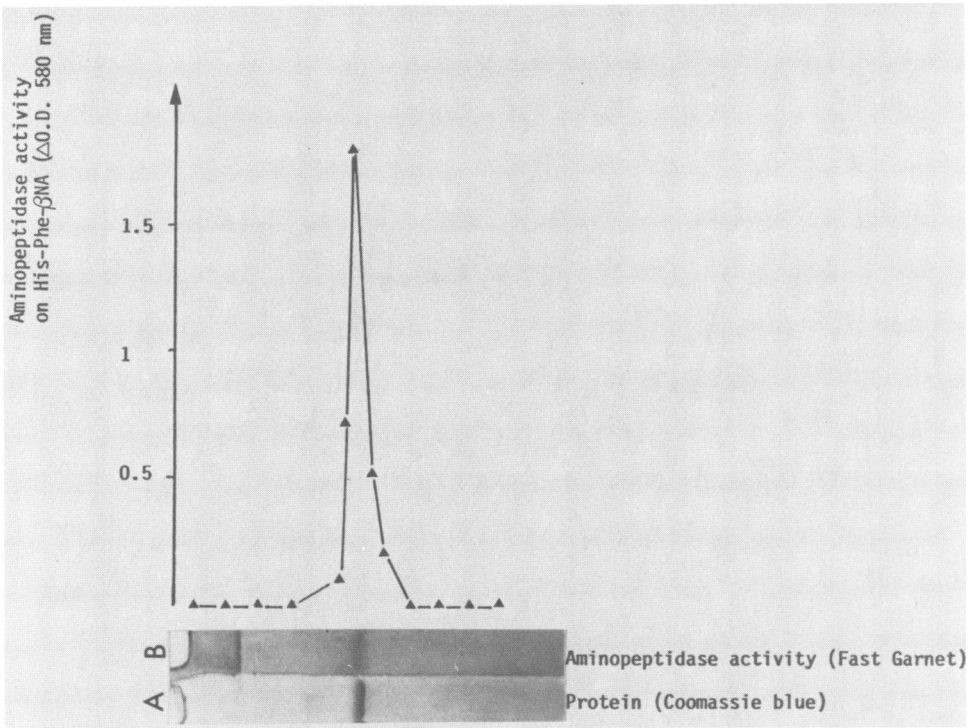

FIG. 3. PAGE of purified aminopeptidase in absence of SDS. Lane A, Coomassie blue staining. Lane B, Aminopeptidase activity detected directly in the gel with His-Phe-β-NA as the substrate. The graph represents aminopeptidase activity detected by incubating 2-mm gel slices with the substrate His-Phe-β-NA and measuring the quantity of naphthylamine liberated (optical density [O.D.] at 580 nm). See Materials and Methods for details.

TABLE 1. Purification of an aminopeptidase from *L. lactis* subsp. *cremoris* AM2

Purification step	Total activity[a] (nkat)	Yield (%)	Total protein (mg)	Sp act (pkat/mg)	Purification (fold)
Spheroplasts	210	100	12,000	17.5	1
DNase	65	31	5,000	13.0	0.7
Ion exchange I	32	15	65	492.0	28
Ion exchange 2	19	9	8	2,375.0	136

[a] Determined with the substrate His-Phe-β-NA.

4-chloro-1-naphthol (Bio-Rad): 60 mg of substrate was dissolved in 20 ml of methanol mixed with 100 ml of 20 mM Tris hydrochloride (pH 7.5) containing 0.5 M NaCl and 60 μl of 30% H_2O_2.

RESULTS

Purification of aminopeptidase. There was little lysis of the spheroplasts (estimated at less than 4% by lactate dehydrogenase assay) during their preparation. Activity from the cell wall fraction, i.e., released when the spheroplasts were formed, was only 15% of the total activity of lysed cells. After the spheroplasts were burst, 75% of the total cell activity was recovered. Cell wall and cell extracts were also active with His-β-NA and Phe-β-NA. When mutanolysin was used as a lytic agent to prepare spheroplasts, there was a clear-cut increase in the lysis of spheroplasts during their formation (determined by assaying lactate dehydrogenase and A_{600}), as well as an increase in the peptidase activity from the cell wall fraction. These observations suggest that the major peptidase activity, present in the soluble fraction of ruptured spheroplasts, was intracellular. The enzyme in this fraction was the one we purified.

The first ion-exchange chromatography led to the separation of four fractions active against His-Phe-β-NA: one nonadsorbed fraction and three eluting at 0.23, 0.33, and 0.47 M NaCl, accounting for 35, 1, 3, and 46% of total peptidase activity injected, respectively (Fig. 1). The second ion-exchange chromatography yielded two peaks, of which that eluting at 0.45 M NaCl was active. PAGE analysis of this purified fraction in the presence or absence of SDS revealed one protein band after staining with Coomassie blue (Fig. 2 and 3). In the absence of SDS, one red band with the same electrophoretic mobility as the purified fraction was observed when enzymatic activity was revealed directly on the gel (Fig. 3).

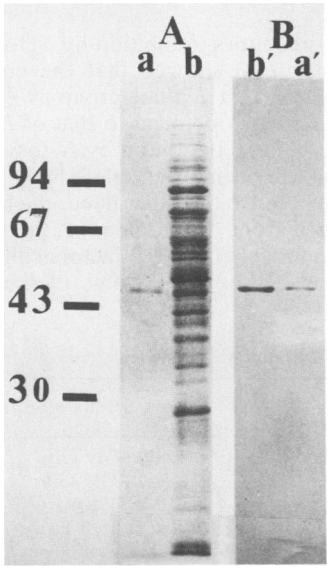

FIG. 4. (A) SDS-PAGE of purified aminopeptidase (lanes a and a') and crude extract (lanes b and b') from *L. lactis* subsp. *cremoris* AM2. (A) Coomassie blue staining. (B) Immunoblotting with a polyclonal antiaminopeptidase serum. Molecular sizes of standard proteins are indicated in kilodaltons. See Materials and Methods for details.

TABLE 2. Inhibition of the aminopeptidase

Inhibitor	Concn (mM)	Residual activity (%)
Iodoacetamide	1	0
Iodoacetic acid	1	0
p-Chloromercuric benzoic acid	1	0
n-Ethylmaleimide	1	30
Diisopropyl fluorophosphate	1	100
Phenylmethylsulfonyl fluoride	1	100
EDTA	10	100
1,10-Phenanthroline	1	90
Dithiothreitol	1	100
Mercaptoethanol	1	100
N-tosyl-L-lysine chloromethyl ketone	1	16
Phosphoramidon[a]		100

[a] Used at a molar ratio of enzyme/inhibitor of 1/200.

Activities of the extracts were measured with His-Phe-β-NA as the substrate at the different steps in the purification (Table 1). The purification factor of the enzyme was 136 and the yield was 9%.

Antisera against this purified fraction were obtained, and immunoblottings were done with the purified fraction and a total cell extract. In this case, only one band was revealed, with an electrophoretic mobility identical to that of the aminopeptidase (Fig. 4). These results show that the enzyme was pure and that the antiserum obtained was monospecific, since there was no cross-reaction between this antiserum and other proteins in the crude extract.

Characterization of aminopeptidase. Molecular size was estimated to be 300 kDa by gel filtration and 50 kDa by SDS-PAGE with or without β-mercaptoethanol. These data suggest that the native aminopeptidase is a hexamer composed of six identical subunits. Since electrophoresis in the presence of SDS with or without β-mercaptoethanol resulted in identical profiles (data not shown), the subunits are not linked by disulfide bridges. The optimal activity of the purified peptidase was at pH 7 and 40°C with approximately 15% activity at pH 5. After storage for 24 h at 0°C without the stabilizers used during purification and in different buffers (acetate and sodium phosphate buffers), stability was found to be optimal between pH 6 and 6.5.

Enzyme activity was measured in the presence of different classes of inhibitors (Table 2). Compounds acting on the SH groups of proteins inhibited the enzyme either totally (iodoacetamide, iodoacetic acid, *p*-chloromercuric benzoic acid) or partially (*N*-ethylmaleimide). Inhibitors of serine enzymes (diisopropyl fluorophosphate, phenylmethylsulfonyl fluoride) and of metalloenzymes (EDTA, 1,10-phenanthroline), as well as reducing agents (dithiothreitol, β-mercaptoethanol) had no effect on peptidase activity. *N*-Tosyl-L-lysine chloromethyl ketone, an inhibitor of certain serine proteases, inhibited the aminopeptidase. The

TABLE 3. Amino acid composition of the aminopeptidase

Amino acid	Quantity (nmol)	Molar ratio (%)	No. of residues[a]
Asx	16.3	11.7	54
Thr[b]	9.4	6.7	31
Ser[b]	9.4	6.7	31
Glx	19.3	13.9	64
Gly	9.7	6.9	32
Ala	10.2	7.3	34
Cys	1.5	1	5
Val[c]	8.2	5.9	27
Met	3.6	2.6	12
Ile[c]	2.9	2.1	10
Leu	10.6	7.6	35
Tyr	5.2	3.7	17
Phe	7.8	5.6	26
His	2.8	2.0	9
Trp	1.3	0.9	4
Lys	8.8	6.3	29
Arg	4.1	2.9	14
Pro	7.7	5.5	26

[a] Nearest integer of the number of residues of an enzyme subunit based on a molecular size of 50 kDa.
[b] Values obtained by extrapolation at time zero.
[c] Values obtained after 72 h of hydrolysis.

powerful metalloprotease inhibitor phosphoramidon (12) had no effect on the enzyme. Clear-cut inhibition of activity was also observed with Cu^{2+}, Zn^{2+}, and Co^{2+} at 0.1 mM, while Mg^{2+} and Mn^{2+} had no effect at the same concentration.

Amino acid composition and N-terminal structure. The aminopeptidase contained high levels of Asp-Asn and Glu-Gln and moderate quantities of hydrophobic amino acids (Table 3). The partial N-terminal sequence was determined on an active purified fraction and thus corresponds to the mature enzyme. It was determined up to the 19th residue: Thr-Val-Thr-Glu-?-Phe-Glu-Gln-Lys-Leu-Tyr-Glu-Asn-Phe-Ala-Gln-Asn-Thr-Lys.

Specificity. The activity of the purified peptidase was tested with several substrates. Specificity of aminopeptidase activity was broad with substrates of the type X-β-NA and X-Y-β-NA, except if one of the amino acids was proline (Table 4). Dipeptides and tripeptides were also hydrolyzed, except if they contained proline or if their N-terminal extremity was blocked. No carboxypeptidase activity was detected with the substrates CBZ-Gly-Tyr and CBZ-Phe-Phe. Hydrolysis of His-Phe-β-NA released free histidine and phenylalanine, and so the enzyme purified in this work was not a dipeptidylaminopeptidase, but an aminopeptidase. It is nevertheless possible that dipeptidylaminopeptidase activity was present in the cell extract and was eliminated during the first chromatography (Fig. 1).

Lineweaver-Burk representation showed that the K_m and the V_{max} of the aminopeptidase for His-β-NA were, respectively, 4.5 mM and 3,600 pkat/mg.

Hydrolysis of the B chain of insulin carried out with an enzyme/substrate ratio of 1/16 (wt/wt) showed that after 16 h of incubation at 40°C, the protein chain was weakly hydrolyzed—4.4% of phenylalanine (N-terminal residue of the B chain) and 3.1% of valine (penultimate residue) could be assayed. No endopeptidase activity could be attributed to the purified aminopeptidase.

DISCUSSION

The crude extract used to purify the aminopeptidase was obtained after lysing spheroplasts, suggesting that the enzyme was intracellular. The quantity of hydrophobic amino acids was not very high, consistent with it not being a membrane protein. This is true for most aminopeptidase activities in group N streptococci (11, 14). As a result of their localization, these aminopeptidases probably play an important role in supplying cells with free amino acids required for growth. The cell wall extract also contained aminopeptidase activity against His-Phe-β-NA, His-β-NA, and Phe-β-NA. Lysozyme resulted in little lysis of spheroplasts, and this activity could be due to an exocellular aminopeptidase(s). Exterkate (5) postulated the existence of an aminopeptidase localized at the cell wall-membrane interface of *L. lactis* subsp. *cremoris*. Geis et al. (8) purified an aminopeptidase from a cell wall extract obtained by incubating streptococci in calcium-free buffer.

The *L. lactis* subsp. *cremoris* AM2 aminopeptidase is characterized by its high molecular mass (300 kDa) and its hexameric structure of identical subunits not linked by disulfide bridges. Peptidases purified in the past (3, 14) were usually monomers of lower molecular weight, except for the membrane aminopeptidase described by Exterkate and de Veer (6).

The effect of inhibitors (inhibition by SH-group reagents, no effect of chelators) showed that the enzyme is not a metalloenzyme and that a thiol group is essential for its activity. Its behavior is identical to that of *Escherichia coli* aminopeptidase N (16). Inhibition by *N*-tosyl-L-lysine chloromethyl ketone had been observed with another thiol group enzyme, papain (29). On the other hand, the purified enzyme was distinguished from other aminopeptidases detected in mesophilic streptococci (3, 8) or lactobacilli (4, 15), which are generally metalloenzymes. Some of these aminopepti-

TABLE 4. Relative activity[a] of the aminopeptidase with different substrates

Substrate	Relative activity (%)	Substrate	Relative activity (%)	Substrate	Relative activity (%)
Ala-β-NA	100	His-Phe	100	Phe-Gly-Gly	100
Lys-β-NA	100	Leu-Leu	61	Leu-Gly-Gly	89
His-β-NA	100	Met-Ala	56	Met-Leu-Gly	85
Glu-β-NA	80	Leu-Gly	53	Leu-Leu-Leu	78
Phe-β-NA	75	Leu-Tyr	50	Ala-Leu-Gly	74
Leu-β-NA	70	Met-Pro	0		
Pro-β-NA	0	CBZ-Gly-Tyr	0		
His-Phe-β-NA	47	CBZ-Phe-Phe	0		
His-Pro-β-NA	0				
Gly-Pro-β-NA	0				

[a] Expressed as percentage of maximal activity measured with NA-substituted amino acids, dipeptides, or tripeptides.

dases are nevertheless inhibited by EDTA and *p*-chloromercuric benzoic acid (14).

The purified aminopeptidase had a broad specificity of action. Thus, it could hydrolyze β-NA derivatives, dipeptides, and tripeptides (Table 4). Its activity was high when a histidyl group occupied the N-terminal position (Table 4), in contrast to observations with the aminopeptidase of *Streptococcus thermophilus*, characterized by Rabier and Desmazeaud (21).

The enzyme appeared to have higher activity with short peptides than with longer chains. The N-terminal phenylalanine of the B chain of insulin (29 residues) was hardly attacked, while Phe-β-NA and the tripeptide Phe-Gly-Gly were hydrolyzed very well. Proteolytic activities toward varied substrates have previously been observed with the aminopeptidases of strains of *L. lactis* (3, 10) and *Lactobacillus lactis* (4).

The enzyme was shown to be pure by the use of antiaminopeptidase antibodies. Van Boven et al. (26) had used the same method to verify the purity of a dipeptidase from *L. lactis* subsp. *cremoris* Wg2, but the antibodies were directed against a cell extract and not a purified enzyme. The antiaminopeptidase antibodies used in our work led to the detection of the presence of the enzyme in the crude extract from *L. lactis* subsp. *cremoris* AM2.

One of the functions of lactic streptococcal peptidases is to supply the organism with indispensable amino acids. The peptides released from caseins by cell wall proteinases are rich in hydrophobic amino acids and proline (20, 28). The broad specificity of the aminopeptidase studied here should enable it to degrade these hydrophobic peptides. Its action must nevertheless be complemented by that of a peptidase(s) which can release prolyl residues. The X-prolyl dipeptidyl aminopeptidases demonstrated in lactic acid bacteria (17; J.-C. Gripon et al., unpublished data) should lead to the optimal intracellular utilization of peptides. Some of these peptides released during ripening by rennet or streptococcal cell wall proteinases are bitter, as a result of their high levels of hydrophobic amino acids. As peptidases are liberated from cells after lysis during ripening, the aminopeptidase in association with prolyl peptidase could help to reduce the levels of hydrophobic peptides and thus the bitterness. The differences between bitter and nonbitter strains could, as previously observed, be due to the specificity of cell wall proteinases (27) but also to the nature and quantity of peptidases synthesized.

It is thus a priori consistent that it would be of value to increase the production of this enzyme in cells by the use of a multicopy vector, thereby intensifying its action during ripening. For this reason, the aminopeptidase is currently being cloned in the laboratory.

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