St-PepA, a *Streptococcus thermophilus* aminopeptidase with high specificity for acidic residues

Francoise Rul, Jean-Claude Gripon and Véronique Monnet

Author for correspondence: Francoise Rul. Tel: +33 1 34 65 21 49. Fax: +33 1 34 65 21 63.
e-mail: rul@jouy.inra.fr

Station de Recherches
Laitières, Institut National
de la Recherche
Agronomique, 78352 Jouy-
en-Josas Cedex, France

The proteolytic system of lactic acid bacteria has been extensively studied over the past 10 years and peptidases from lactococci are now well known. The situation is, however, different for *Streptococcus thermophilus* from which only a few peptidases have been purified and characterized. The present work was conducted to characterize an aminopeptidase of *S. thermophilus* CNRZ 302, called St-PepA, with high specificity for acidic amino acids. St-PepA was purified by a three-step procedure from a spheroblast extract of *S. thermophilus* CNRZ 302. Its molecular mass was estimated to be 360 kDa by gel filtration and 45 kDa by SDS-PAGE, indicating that it had an octameric structure. Its activity against aspartate-*p*-nitroanilide was maximal at pH 8.5 and 62 °C and highly enhanced by Zn²⁺, Co²⁺ and Mg²⁺. St-PepA was inhibited by metal-chelating reagents and, to a lesser extent, by agents modifying sulfhydryl groups. It showed an activity towards *p*-nitroanilide derivatives, di- and tripeptides, and larger peptides such as fragment 43–58 of α_1 -casein. It had a high substrate specificity towards N-terminal acidic amino acid residues but it could also release serine and malic acid, the α -hydroxy acid homologue of aspartic acid. Kinetic studies revealed that the affinity of St-PepA was more than 18-fold higher for aspartic acid-*p*-nitroanilide ($K_m = 0.42$ mM) than for glutamic acid-*p*-nitroanilide ($K_m = 7.65$ mM) with a similar V_{max} for both substrates [about 40 $\mu\text{mol min}^{-1}$ (mg enzyme)⁻¹]. St-PepA is heat stable, with a maximal loss of activity of 15% after incubation for 120 min at 50 °C and 60 °C. It is likely to be involved in the nitrogen metabolism of *S. thermophilus* and in the development of the organoleptic characteristics of cheese and yoghurt.

Keywords: *Streptococcus thermophilus*, aminopeptidase A, glutamic and aspartic acids, metallopeptidase

INTRODUCTION

Lactic acid bacteria are widely used as starter cultures in industrial milk fermentations. They require an exogenous nitrogen source for optimal growth (Law & Kolstad, 1983; Thomas & Pritchard, 1987). Because milk is relatively poor in free amino acids and small peptides, nitrogen mostly comes from caseins. Thus, the optimal growth of lactic acid bacteria depends on their proteolytic system composed of proteinases and peptidases which can produce these amino acids and small peptides from milk caseins (Kok, 1990; Laan *et al.*, 1989). This proteolytic system is also important because it contributes to the

development of texture and flavour during cheese ripening.

The cell-envelope-associated proteinase and a variety of peptidases with different specificities have been described for the genus *Lactococcus* (for reviews see Monnet *et al.*, 1993; Tan *et al.*, 1993). Less information is available about the proteolytic enzymes of *Streptococcus thermophilus*, although it is widely used in the dairy industry. *S. thermophilus* exhibits low levels of proteinase activity and it develops in milk in association with lactobacilli species, the latter producing small peptides which are substrates for peptidases. Several of these peptidases have been described in *S. thermophilus*: a neutral intracellular endopeptidase was purified and characterized from *S. thermophilus* CNRZ 160 (Desmazeaud, 1974). A dipeptidase

Abbreviations: pNa, *p*-nitroanilide; β -Na, β -naphthylamide.

(Rabier & Desmazeaud, 1973), specific for dipeptides with a large amino acid residue at the N terminus, and a X-prolyl-dipeptidyl-aminopeptidase (Meyer & Jordi, 1987) were also described. In addition, two general aminopeptidases similar to those isolated from lactococci were purified from *S. thermophilus*: PepN (Tsakalidou & Kalantsopoulos, 1992; Midwinter & Pritchard, 1994; Rul *et al.*, 1994) and PepC, whose gene has recently been cloned (Chapot-Chartier *et al.*, 1994).

As *S. thermophilus* is widely used in the dairy industry in cheese making and yoghurt manufacturing, it is of interest to know more about its peptidasic system. After a screening of aminopeptidase activities, we detected four aminopeptidases in extract of *S. thermophilus* CNRZ 302 spheroblasts. Three are general aminopeptidases (Rul, 1994); the fourth, St-PepA, is an aminopeptidase with high specificity for acidic amino acids. To investigate further the potential role of St-PepA in nitrogen metabolism and organoleptic development of yoghurt and cheese, we purified it and determined its biochemical properties.

METHODS

Bacterial strain and culture conditions. *Streptococcus thermophilus* CNRZ 302 was obtained from the collection of the Station de Recherches Laitières (INRA, Jouy-en-Josas, France). It was grown at 37 °C in 5 l of M17 broth (Difco) containing 5 g lactose l⁻¹. Growth was assessed by measurement of OD₆₅₀.

Intracellular extract preparation. Cells were collected at the end of the exponential growth phase by centrifugation (6000 g, 15 min, 4 °C). The pellet was washed twice with 50 mM β -glycerophosphate and then incubated for 2 h at 30 °C in 50 mM triethanolamine (TEA) buffer (pH 7) containing 30% (w/v) sucrose, 0.1 mg lysozyme ml⁻¹ (Sigma) and 42 U mutanolysin ml⁻¹ (Sigma). The spheroplasts obtained were collected by centrifugation (12000 g, 30 min, 4 °C) and resuspended in the same buffer without sucrose. After the spheroplasts had burst, the supernatant was recovered by centrifugation (20000 g, 30 min, 4 °C).

Protein assay. Proteins were determined in the extracts and purified fractions by the method of Bradford (1976) with bovine serum albumin (Pierce) as standard.

Aminopeptidase activity assay. St-PepA activity was assayed against three types of substrates.

Firstly, against amino acid-*p*-nitroanilides (-pNa) and - β -naphthylamides (- β -Na). The purification of St-PepA was followed by monitoring the release of *p*-nitroaniline from Asp-pNa (Bachem) at 410 nm as described by Zevaco *et al.* (1990). In the case of β -Na derivatives, the reaction mixture contained 0.3 mM substrate in 50 mM Tris/HCl (pH 8) in a final volume of 1 ml and incubation was performed at 37 °C. The reaction was stopped by adding 0.5 ml of 1 mg Fast Garnet ml⁻¹. After 25 min incubation at 37 °C, the release of β -naphthylamine was monitored by measuring A_{550} . The unit of activity used was the katal (the quantity of enzyme releasing 1 mol *p*-nitroaniline or β -naphthylamine s⁻¹).

Secondly, with the purified aminopeptidase, against several di-, tri- and tetrapeptides (Sigma, or Bachem for Asp-Tyr, Asp-Tyr-Met and Asp-Tyr-Met-Gly). Asp-Phe-methyl ester (Asp-

Phe-OMe; Aspartame), Asp-Phe-ethyl ester (Asp-Tyr-OEt) and Mal-Tyr-ethyl ester (Mal-Tyr-OEt; Mal, maly) were provided by BioEurope. The enzyme fraction was incubated at 37 °C in the presence of 82 μ M substrate in 50 mM TEA (pH 8) and 1 mM CoCl₂. After 1 h incubation, the reaction was stopped by addition of 10% 1 M HCl. The free amino acids present in the hydrolysates of the different peptides were directly identified with a LC3000 amino acid analyser (Biotronik).

Thirdly, against oligopeptides. Angiotensin I and leucokinin were from Sigma. Fragment 43–58 of bovine α_{s1} -casein and fragment 115–124 of caprine α_{s1} -casein (with free or phosphorylated serine at the N terminus) were gifts from G. Brignon (INRA, Station de Recherches Laitières, Jouy-en-Josas, France). The enzymic reaction was achieved as described above with 1 h incubation (or 24 h for the α_{s1} -casein fragments). The samples were analysed by RP-HPLC using a C18 column in a trifluoroacetic acid (TFA)/acetonitrile solvent system (solvent A, 0.115% TFA; solvent B, 0.1% TFA, 60%, v/v, acetonitrile). A linear gradient of 20–80% solvent B was used over 15 min for leucokinin and angiotensin and over 20 min for fragment 43–58 of bovine α_{s1} -casein. A linear gradient of 10–50% solvent B was used over 30 min for fragment 115–124 of caprine α_{s1} -casein. The peptides collected were then identified with a sequencer (model 477A; Applied Biosystems).

Aminopeptidase purification. Nucleic acids in the cell extract were hydrolysed by adding RNase (2.75 mg per 100 ml extract; Sigma) and DNase (0.05 mg per 100 ml extract; Sigma) in the presence of 1 mM MgCl₂. Nucleic acids were then precipitated in the presence of 60 mM MnSO₄ and the solution was centrifuged at 10000 g for 30 min. The extract was then dialysed overnight against 10 mM TEA (pH 7) and filtered through a 0.45 μ m filter (type HVLP, Millipore).

The first ion exchange chromatography was done on a Q-Sepharose Fast Flow XK 26/40 column (2.6 $\times$ 15 cm; Pharmacia) with a FPLC system. The column was equilibrated with 50 mM TEA (pH 7). The extract (317 mg protein) was applied to the column and eluted with a linear NaCl gradient of 0–0.5 M over 5 h at a flow rate of 5 ml min⁻¹ and 10 ml fractions were collected. Fractions active against Asp-pNa were recovered and dialysed overnight against 10 mM TEA (pH 7.5).

The second ion-exchange chromatography was performed by FPLC on a MonoQ HR 10/10 column (1 $\times$ 10 cm; Pharmacia) equilibrated with 50 mM TEA (pH 7.5). The proteins were eluted with a linear NaCl gradient of 0–0.5 M over 4 h at a flow rate of 3 ml min⁻¹. Three millilitre fractions were collected and the fractions active against Asp-pNa were pooled.

The following hydrophobic interaction chromatography was done with an Alkyl Superose HR5/5 column (0.5 $\times$ 5 cm; Pharmacia). The column was equilibrated with 50 mM sodium phosphate buffer (pH 7.5) containing 1.7 M (NH₄)₂SO₄. This was added to the sample which was applied to the column and eluted with a linear gradient of 1.7–0 M (NH₄)₂SO₄ in 50 mM sodium phosphate (pH 7.5) over 3.5 h at a flow rate of 0.5 ml min⁻¹; 0.5 ml fractions were collected. Fractions active against Asp-pNa were recovered and dialysed against 10 mM sodium phosphate buffer.

Aminopeptidase characterization

Electrophoresis. The purified fraction was electrophoresed under denaturing and reducing conditions (SDS-PAGE) using 10% acrylamide gels (acrylamide/bisacrylamide, 29:1, v/v) according to Laemmli (1970). The proteins were stained with Coomassie Brilliant Blue R-250. The following markers were

used to determine the molecular mass of St-PepA: myosin (205 kDa), β -galactosidase (116.25 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa) and ovalbumin (45 kDa). Native electrophoresis (in the absence of SDS and β -mercaptoethanol) allowed the detection of the aminopeptidase activity under the following incubation conditions: 30 ml 0.2 M Tris buffer (pH 7.5) containing 10 mg Asp-pNa at room temperature. Hydrolysis of the substrate was reflected by the appearance of an unstable yellow colour at the level of the enzyme band.

Gel filtration. The molecular mass of St-PepA was estimated by gel filtration on a Superose 12 column (Pharmacia) in 0.15 M NaCl, 50 mM TEA (pH 7.5) at a flow rate of 0.25 ml min⁻¹. The column was calibrated with thyroglobulin (670 kDa), γ -globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and vitamin B-12 (1.35 kDa).

Effect of pH on aminopeptidase activity. The effect of pH on the aminopeptidase activity was determined on Asp-pNa at 37 °C over a pH range of 4–10 using the following 0.1 M buffers: sodium acetate pH 4–5.5; Bis-Tris, pH 6–7; TEA, pH 7.5–8; Tris, pH 8.5–9.5 and CAPS, pH 10.

Effect of temperature on aminopeptidase activity. The effect of temperature on the aminopeptidase activity was determined at temperatures ranging from 20 to 75 °C with Asp-pNa as a substrate. After incubation of the enzyme at the desired temperature in 50 mM TEA buffer (pH 8) for 5 min, substrate was added and the reaction mixtures were incubated for 0–20 min at 20–75 °C. The enzymic activity was then measured as described above.

To determine the thermostability of St-PepA activity, a solution of the purified aminopeptidase in 50 mM TEA buffer (pH 8) containing 1 mM MgCl₂, was incubated at 30, 40, 50 or 60 °C. After 20, 30, 60, 120 and 480 min, samples were taken from each incubation mixture and assayed for aminopeptidase activity using Asp-pNa as a substrate described above.

Effect of bivalent cations on the aminopeptidase activity. Bivalent cations were assayed firstly for their effect on aminopeptidase activity and secondly for their ability to restore aminopeptidase activity after inactivation by EDTA. The aminopeptidase was preincubated with various bivalent cations: CaCl₂, CuCl₂, CoCl₂, MgCl₂, MnCl₂ and ZnCl₂ for 20 min at 37 °C at final concentrations of 1 mM in 50 mM TEA buffer (pH 8). Its activity was then assayed at 37 °C for 20 min using Asp-pNa as a substrate. The rate of hydrolysis of Asp-pNa in the absence of metal ion was taken as 1. After inactivation with 1 mM EDTA, the aminopeptidase was incubated with each cation preparation for 20 min at 37 °C before the restored activity was measured as described above.

Effect of inhibitors on aminopeptidase activity. The aminopeptidase was preincubated at various concentrations of 4-(2-aminoethyl)-benzenesulfonyl fluoride (Pefablock-SC), iodoacetic acid, *p*-hydroxymercuribenzoate, EDTA, *o*-phenanthroline, bestatin, amastatin or DTT for 20 min at 37 °C in 50 mM TEA buffer (pH 7.5). Its activity was measured following the standard enzyme assay procedure and the rate of hydrolysis of Asp-pNa in the absence of any inhibitor was taken as 100%.

Determination of kinetic parameters. K_m and V_{max} values for Asp-pNa and Glu-pNa were determined from a Lineweaver–Burk plot generated from the initial reaction velocity obtained with substrate concentrations ranging from 0.5 to 15 mM and 95 ng purified enzyme ml⁻¹. Each assay was carried out at 37 °C in 50 mM TEA (pH 8), 1 mM MgCl₂, and the variation in A_{410} was monitored continuously for 10 min using a 931 Uvikon spectrophotometer (Kontron Instruments).

RESULTS

Aminopeptidase purification

St-PepA was purified about 130-fold from the cell-free extract in three chromatography steps, with an activity recovery of 2.7% as shown in Table 1. The last purification step (Alkyl Superose) resulted in a peak which showed a single protein band upon examination by SDS-PAGE (Fig. 1) and by PAGE; after non-denaturing PAGE, the enzyme activity was detected at the same position as the protein band.

Aminopeptidase properties

The molecular mass of St-PepA was estimated to be 360 kDa by gel filtration and 45 kDa by SDS-PAGE. These data suggest that St-PepA is an octamer, with subunits of 45 kDa. It was active over a pH range of 6.5–10 with maximal hydrolysis of Asp-pNa at pH 8.5; 75% of its activity remained at pH 10. At pH 8, with Asp-pNa as a substrate, the optimum temperature was 62 °C (Fig. 2). In fact St-PepA showed a rather high degree of thermal stability since more than 82% of the initial activity was retained after incubation at 30 or 40 °C for 8 h, or at 50 or 60 °C for 2 h (Fig. 3).

Activity was measured in the presence of different classes of inhibitors (Table 2) and different levels of inhibition were observed. Strong inhibition (62–90%) was observed with DTT and metal-chelating reagents such as *o*-phenanthroline or EDTA which suggests St-PepA is a metallopeptidase. Moderate inhibition (7–34%) was observed with inhibitors of aminopeptidases (amastatin and bestatin) and inhibitors of cysteine proteases (iodoacetic acid and *p*-hydroxymercuribenzoate). There was no inhibition at all with Pefablock, a serine protease inhibitor.

St-PepA was also inhibited by 1 mM CuCl₂, while incubation with 1 mM CaCl₂, ZnCl₂, CoCl₂ and MgCl₂ resulted in an increase of activity (> twofold with ZnCl₂, CoCl₂ and MgCl₂; Table 3). After treatment with 1 mM EDTA, activity was only recovered with the addition of Zn²⁺.

Substrate specificity

St-PepA's activity was tested with several substrates (Table 4). The enzyme had three properties as follows.

Firstly, it was observed to release only acidic amino acids (Glu and Asp), Asp being preferentially released, among all the amino acid derivatives (-pNa or - β -NA) tested (Arg, Phe, Lys, Pro, Asp, Glu).

Secondly, it hydrolysed di-, tri- and tetrapeptides with a N-terminal acidic amino acid but no preference for Asp or Glu was observed. The best Asp-X dipeptide substrates were those containing an aromatic residue (Tyr or Phe) at position P₁' (according to the nomenclature of Schechter & Berger, 1967). St-PepA could also hydrolyse dipeptides with N-terminal malic acid, the α -hydroxy acid homologue of Asp; the highest rate of hydrolysis was

Table 1. Purification of aminopeptidase St-PepA from *S. thermophilus* CNRZ 302

Purification step	Total protein (mg)	Total activity (nkat)	Specific activity (nkat mg ⁻¹)	Yield (%)	Purification (-fold)
Cell-free extract	317	72	0.23	100	1
Q-Sepharose	10.5	50	4.8	69	21
MonoQ	1.2	21	17.5	29	77
Alkyl Superose	0.068	2	29.4	2.7	130

Fig. 1. SDS-PAGE of different fractions obtained during the purification of aminopeptidase St-PepA from *S. thermophilus* CNRZ 302. Lanes: 1, molecular mass standards (kDa); 2, cell-free extract; 3 and 4, fractions from first and second anion-exchange chromatography, respectively; 5, fraction from hydrophobic interaction chromatography. **Fig. 2.** Effect of temperature on the activity of St-PepA of *S. thermophilus* CNRZ 302.

measured on Mal-Tyr-OEt. Serine was released from fragment 1–4 of adrenocorticotrophic hormone (SYSM) but both fragments 115–124 of caprine α_{s1} -casein with free or phosphorylated N-terminal serine were not

Fig. 3. Effect of temperature on the stability of St-PepA of *S. thermophilus* CNRZ 302. Samples containing 95 ng of purified enzyme in 50 mM TEA buffer (pH 8), were incubated at 30 (▲), 40 (●), 50 (■) and 60 °C (○) prior to assaying at 37 °C using Asp-pNa as substrate. Initial activity was taken as 100 %.**Table 2.** Effect of inhibitors on St-PepA activity of *S. thermophilus* CNRZ 302

Reagent	Final concentration (mM)	Inhibition (%)
Amastatin	0.01	10
Bestatin	0.01	7
Iodoacetic acid	0.1	12
<i>p</i> -Hydroxymercuribenzoate	1	34
Pefablock	1	0
EDTA	0.1	62
	1	91
<i>o</i> -Phenanthroline	1	90
DTT	1	70
Control		0

hydrolysed. Dipeptides with an additional group at the C terminus were better substrates than those without that additional group, as demonstrated with Asp-Phe, Asp-Phe-NH₂ and Asp-Phe-OMe (aspartame).

Finally, it was observed to hydrolyse larger peptides. N-Terminal Asp was slowly released from fragment 43–58 of bovine α_{s1} -casein (15 residues). Peptides with N-

Table 3. Effect of 1 mM bivalent cations on the aminopeptidase St-PepA activity of *S. thermophilus* CNRZ 302

Ion	Relative activity
Mg ²⁺	3.7
Zn ²⁺	3.0
Co ²⁺	2.2
Ca ²⁺	1.4
Mn ²⁺	0.8
Cu ²⁺	0.6
Control	1

terminal Asp-Pro or Asp-Arg (leucokinin, angiotensin) were not hydrolysed. Liberation of serine was observed from SYSM but not from larger peptides like fragment 115–124 (10 residues) of caprine α_{s1} -casein. These results show that St-PepA preferentially degrades small (< 5 residues) rather than large peptides.

Neither carboxypeptidase activity on carbobenzoxy-Glu-Tyr nor endopeptidase activity on leucokinin, angiotensin I, fragment 43–58 of bovine α_{s1} -casein or fragment 115–124 of caprine α_{s1} -casein were detected.

Kinetic parameters

Kinetic parameters were determined for the interaction of St-PepA with Asp-pNa and Glu-pNa as substrates. Lineweaver–Burk plots showed that K_m for Asp-pNa and Glu-pNa were of 0.42 mM and 7.65 mM, respectively,

thus indicating a better affinity of St-PepA for Asp-pNa. Maximal rates of hydrolysis were similar for both substrates (V_{max} of 41.7 and 38.5 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ for Asp-pNa and Glu-pNa, respectively).

DISCUSSION

Our work demonstrates the existence in *S. thermophilus* CNRZ 302 of an aminopeptidase, St-PepA, specific to peptide bonds in which a N-terminal acidic amino acid is involved.

All the aminopeptidases A described up to now are metalloenzymes and specifically liberate Glu and Asp residues. Only a few of them have been described in bacteria, the others being from human or animal origin. St-PepA differs from aminopeptidases A of human origin (Lalu *et al.*, 1984; Mizutani *et al.*, 1981; Nagatsu *et al.*, 1970; Yamada *et al.*, 1988) or of animal origin (Cheung & Cushman, 1971; Danielson *et al.*, 1980; Feracci *et al.*, 1981; Petrovic & Vitale, 1990; Tobe *et al.*, 1980) in many ways, including biochemical properties such as molecular mass, multimeric structure, optimal conditions of activity and the nature of the metal ion it requires. It is closer to the bacterial aminopeptidases A which have been purified from *Lactococcus lactis* (Bacon *et al.*, 1994; Niven, 1991; Exterkate & De Veer, 1987) and *Staphylococcus chromogenes* (Yoshpe-Besançon *et al.*, 1993). All of these have a multimeric structure, are activated by Zn²⁺ or Co²⁺ and are characterized by a rather alkaline optimal pH and a high optimal temperature (about 60 °C). In addition, St-PepA has other properties in common with lactococcal aminopeptidases A. Firstly, Zn²⁺ can restore the activity of St-PepA and PepA from *L. lactis* HP (Exterkate & De

Table 4. Substrate specificity of St-PepA from *S. thermophilus* CNRZ 302

The rate of hydrolysis of Asp-Tyr was taken as 100 %. OMe and -OEt, methyl ester and ethyl ester, respectively.

Substrate	Relative activity (%)	Substrate	Relative activity (%)
Asp-pNa	15	Glu-Val-Phe	139
Glu-pNa	5	Ser-Tyr-Ser-Met	70
Asp- β -Na	6	Bovine α_{s1} -casein	4
Glu- β -Na	0	(fragment 43–58)	
Asp-Gln	37	(DIGSESTEDQAMEDIK)	
Asp-Lys	13	P P	
Glu-Lys	34	Angiotensin I	0
Glu-Ser	100	(DRVYIHPFHL)	
Asp-Phe	52	Leucokinin	0
Asp-Phe-OMe	120	(DPAFNSWG)	
Asp-Phe-NH ₂	100	Caprine α_{s1} -casein	0
Asp-Tyr	100	(fragment 115–124)	
Mal-Tyr-OEt	266	(SAEEQLHSMK)	
Asp-Tyr-Met	100	P	
Asp-Tyr-Met-Gly	94		

Veer, 1987) after treatment with EDTA and, secondly, St-PepA and PepA from *L. lactis* HP (Exterkate & De Veer, 1987) and AM2 (Bacon *et al.*, 1994) exhibit especially good temperature stability.

St-PepA has a high substrate specificity towards acidic amino acid residues at the N terminus of peptides but it can also release serine. St-PepA shares with the aminopeptidase A from *S. chromogenes* (Yoshpe-Besançon *et al.*, 1993) the ability to liberate N-terminal malic acid, the α -hydroxy acid homologue of Asp. Thus, streptococcal and staphylococcal aminopeptidases A show no strict requirement for a free α -amino group, contrary to the aminopeptidase A from *L. lactis* subsp. *cremoris* AM2 (Bacon *et al.*, 1994).

The specificity of St-PepA and especially its capacity to liberate Glu is interesting for two reasons.

Firstly, because St-PepA is most probably important in the nitrogen metabolism of *S. thermophilus*. *S. thermophilus* generally does not possess a substantial extracellular proteolytic activity (Shahbal *et al.*, 1991) and peptides from caseins must be released by the cell-wall-associated proteinase of lactobacilli and by the rennet, in the case of cheese manufacturing. These peptides are rich in Asp and Glu as caseins are and could be transported into the cell; Desmazeaud & Hermier (1972) indeed suggested that *S. thermophilus* possesses an oligopeptide transport system. Moreover Glu is essential for *S. thermophilus* growth (Bracquart *et al.*, 1978) and none of the peptidases of *S. thermophilus* so far characterized could release acidic amino acids very efficiently (Midwinter & Pritchard, 1994; Rul, 1994; Tsakalidou & Kalantopoulos, 1992).

Secondly, because St-PepA could contribute to flavour development during cheese ripening by liberating free amino acids. Glutamic acid is the major free amino acid in Swiss-type cheese and Biede & Hammond (1979) suggested that it can play a role in cheese flavour because of its broth taste and its flavour-enhancing properties. Furthermore, it has been shown recently that Glu can contribute to lowering the bitterness of a trypsin hydrolysate of casein (Warmke & Belitz, 1993). St-PepA could also participate to the decrease of bitterness by hydrolysing, with other peptidases we have described (Rul, 1994), hydrophobic peptides responsible for bitterness.

Three aminopeptidases have now been purified from *S. thermophilus*: St-PepA and two general aminopeptidases, St-PepC and St-PepN. Biochemical studies and sequence data supported the evidence of a striking resemblance between the streptococcal aminopeptidases and PepC, PepN and PepA from lactococci. The aminopeptidases isolated from the two species indeed have similar biochemical properties and substrate specificities. Moreover, the N terminus sequences we obtained from the purified proteins St-PepN (Rul *et al.*, 1994) and St-PepA (data not shown) are 67% (19 residues) and 77% (13 residues) homologous, respectively, to the sequences of the corresponding lactococcal aminopeptidases PepN (Tan &

Konings, 1990) and PepA (F. Mülholland, personal communication). Finally, the gene encoding St-PepC was sequenced by our group (Chapot-Chartier *et al.*, 1994) and was found to be 82% similar to the gene *pepC* encoding PepC from *L. lactis*.

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