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A STUDY OF AMINOPEPTIDASES FROM LACTIC STREPTOCOCCI

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry at Massey University.

RICHARD JOHN LLOYD

1989

ABSTRACT

Two aminopeptidase enzymes from the proteolytic system of *Streptococcus lactis* 4760 have been studied.

An X-Prolyl dipeptidyl aminopeptidase has been purified and characterised. The enzyme has a native molecular weight of approximately 150 kDa determined by gel filtration, and a subunit molecular weight of 83 000, determined by denaturing polyacrylamide gel electrophoresis, showing the native enzyme to be a dimer. It is inhibited by phenyl methyl sulphonyl fluoride and is active over a pH range of 6 - 9. A range of X-Prolyl-amido methyl coumarin (X-Pro-AMC) derivatives with different aminoacyl residues in the X position have been used to define the steady state kinetic parameters. The Km and k_{cat} values obtained with all of the X-Pro-AMC substrates tested were similar, with the exception of Glu-Pro-AMC, which gave a somewhat higher Km value. The action of the enzyme in degrading small peptides has been studied. It was found to be capable of removing X-Proline residues from peptides, except where two proline residues are situated in consecutive positions.

A Lysyl-aminopeptidase has been partially purified and its characteristics studied. This enzyme has been shown to have a native molecular weight of approximately 78 000. It hydrolyses lysyl-, arginyl-, and leucyl-amido methyl coumarin derivatives, but has little or no activity with other aminoacyl-AMC substrates. It also catalyses the removal of lysine and arginine residues from the amino-terminus of short peptides. The partially purified aminopeptidase preparation also has endopeptidase activity which is probably due to contamination by a separate enzyme.

The individual and combined effects of these two enzymes on β-casein-derived oligopeptides (produced by proteolytic action of the *S.lactis* proteinase) have been studied. These results indicate that these enzymes may be important in degradation of some casein-derived peptides during cheese ripening, while other peptides are resistant to hydrolysis.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr. G.G. Pritchard most sincerely, for his invaluable guidance and continuous encouragement throughout the course of this study.

I am most grateful to Dr. D.R.K. Harding and Dr. N. Haggarty of the Massey University Separation Science Unit for their advice on synthesis of fluorogenic substrates and affinity resins, and to Mr. D.J. Poll for help with HPLC work.

I wish to thank Dr. G.G. Midwinter and Mr. J. Reid for the peptide sequence and amino acid composition analyses, and Dr. C.H. Moore for practical help and advice.

I also wish to thank the staff of the Biochemical Processing Centre, DSIR, for preparation of the large scale bacterial cultures, the N.Z Dairy Research Institute for supplying the bacterial strains, and Miss J. Egan and Mrs J. Lloyd for proof reading this thesis.

LIST OF ABBREVIATIONS

AH-SEPHAROSE	aminohexyl-Sepharose
AMC	7-amino-4-methyl coumarin
BOC	t-butyl oxy carbonyl
CBZ	benzyl chloroformate
DEAE	diethylaminoethyl
EDC	1-ethyl-3-(3-dimethyl amino propyl) carbodiimide
EDTA	ethylenediaminetetra-acetic acid
FITC	fluorescein isothiocyanate
MES	2-(N-morpholino-) ethane sulphonic acid
NADH	nicotinamideadeninedinucleotide
PAGE	polyacrylamide gel electrophoresis
pNA	p-nitroanalide
RP HPLC	reverse phase- high performance liquid chromatography
RSM	reconstituted skim milk medium
SDS	sodium dodecyl sulphate
TEMED	NNN'N'-tetramethylethylene-diamine
TES	(N-tris[hydroxymethyl] methyl-2-amino ethane sulphonic acid)
TRIS	tris-(hydroxymethyl-) aminomethane
TCA	trichloroacetic acid
TFA	trifluoroacetic acid

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CHAPTER 1 - INTRODUCTION

1.1 IMPORTANCE OF PROTEOLYTIC ACTIVITY OF STARTER BACTERIA

The group N (or lactic) streptococci, consisting of the species *Streptococcus lactis, S. cremoris* and *S.diacetylactis* together with the closely related thermophilic bacteria *S.thermophilus, Lactobacillus bulgaris* and *L.helveticus* are of major importance to the dairy industry as cheese starter bacteria. Individual strains, or combinations of different strains, ferment the lactose in milk by the Embden-Meyerhof pathway to pyruvate, $NADH/H^+$ and ATP. This process provides the major source of ATP for bacterial growth. To regenerate NAD^+ the pyruvate is reduced to lactic acid, and consequently the milk pH is lowered to 4.5 to 5.0, preventing the growth of other undesirable microorganisms.

Lactic streptococci are nutritionally fastidious organisms being unable to synthesise most of the amino acids they require. They may grow to high cell densities of approximately 500μg (dry weight) bacteria per ml (or about 10^9 colony forming units per ml) in milk, requiring the synthesis of approximately 260μg bacterial protein per ml. The concentrations of free amino acids in milk are well below the minimum required for synthesis of this amount of protein (Thomas and Mills 1981). Therefore amino acids must be provided by degradation of milk proteins, primarily casein. (Table 1.1)

The process of casein hydrolysis during cheese manufacture has recently become of interest to the dairy industry since the texture and taste developed during cheese ripening is, at least in part, related to the size and composition of peptides resulting from proteolysis. Casein molecules contain a high proportion of hydrophobic residues (for example leucyl, prolyl, phenylalanyl residues). Bitterness in cheese has been attributed to peptides with a high proportion of these amino acids. (Visser *et al.*, 1983).

The degradation of milk proteins during milk fermentation and subsequent ripening of cheese is due to the combined action of rennet, a proteolytic enzyme added as a coagulant, and the proteolytic enzymes of the starter bacteria. Studies of the respective roles of these (O'Keeffe *et al.*, 1976, 1978) suggest that the main role of the starter enzymes is the slow degradation of the β-casein and of the polypeptides generated from α-casein hydrolysis by rennet.

The lactic streptococci possess two distinct groups of proteolytic enzymes which together degrade casein to small peptides and free amino acids.

a) Proteinases Located on or near the cell surface which degrade one or more of the casein components to large oligopeptides.

b) Peptidases Located in the cell wall, attached to the cell membrane and/or present within the cell which degrade the oligopeptides generated by the proteinases.

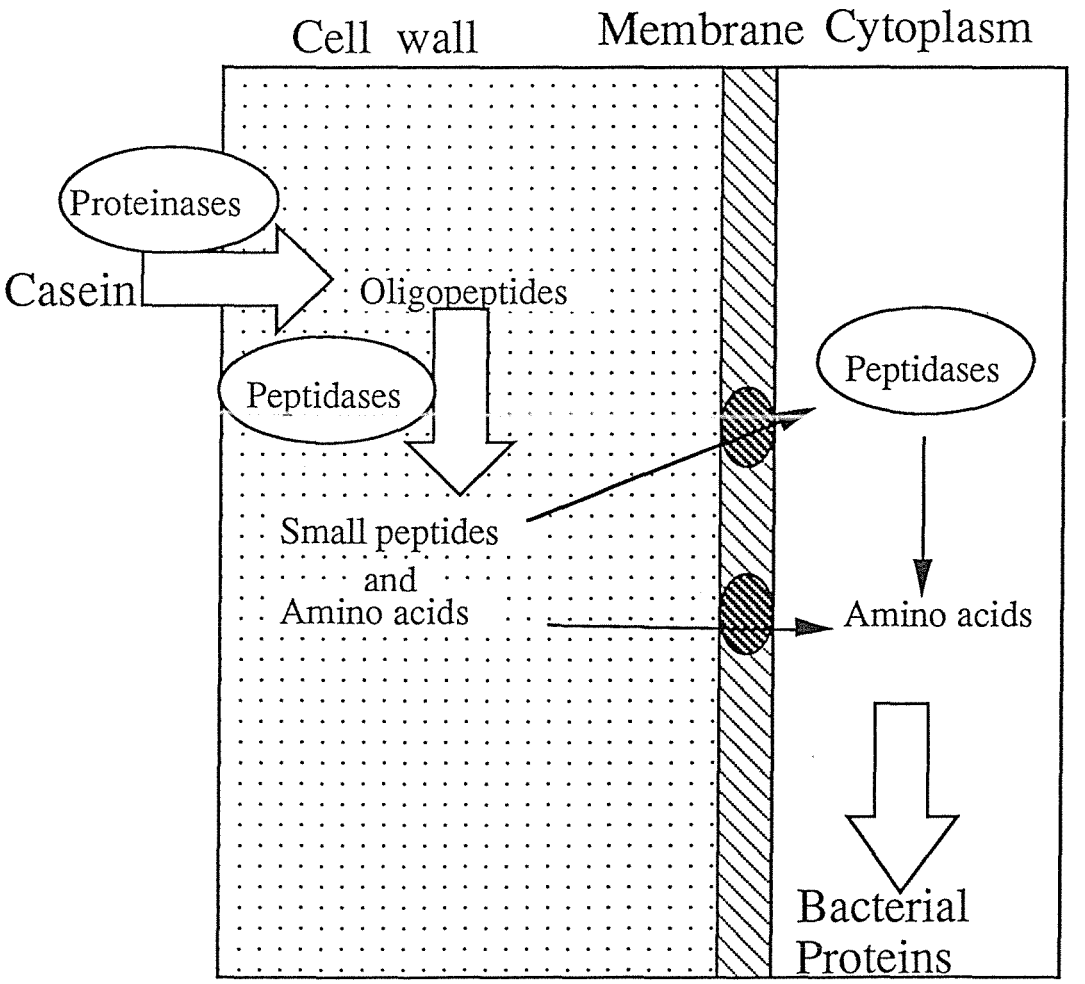

FIG 1.1 Proposed utilisation of casein for synthesis of bacterial proteins by lactic streptococci.

AMINO ACID	REQUIREMENT FOR GROWTH [a]		MINIMUM	CONCENTRATION
	S.cremoris	*S.lactis*	REQUIRED (µg/ml)	IN MILK (µg/ml)
Asp	-	-	29.4	5.0
Thr	-	-	14.9	1.3
Ser	+-	-	12.0	3.7
Glu	+	+	40.1	35.9
Pro	+	-	8.8	0.8
Gly	+-	-	11.9	5.3
Ala	+-	-	19.2	3.5
Cys	-	-	ND	nd
Val	+	+	14.7	2.6
Met	+	+	6.5	nd
Ile	+	+	12.5	0.8
Leu	+	+	21.5	1.2
Tyr	+-	-	10.4	ND
Phe	+	+-	15.8	ND
Lys	+-	-	23.1	4.1
His	+	+	5.9	2.8
Trp	+-	-	ND	ND
Arg	+-	+-	13.1	1.6

TABLE 1.1 *Amino acid requirements of lactic streptococci and the minimum concentrations required for bacterial protein synthesis compared with the levels of amino acids present in milk. (Adapted from Thomas and Pritchard, 1987)*

[a] + = required by all strains tested :+- = required by some strains tested : - = not required.
ND = Not determined
nd = not detectable

1.2 PROTEINASES

Proteinases have the ability to catalyse hydrolysis of intact proteins producing peptides. In the strains of lactic streptococci studied to date, proteinases primarily responsible for the degradation of extracellular proteins remain bound to the cell wall. (Thomas and Mills, 1981). The only recorded exception is the strain *S.cremoris* ML1 which grows in milk, but lacks any detectable cell bound proteinase activity, suggesting the possibility of proteinase liberation into the medium (Exterkate 1976).

The most widely studied strains of *S.cremoris* have a single cell wall proteinase encoded on a plasmid (the proteinase or lactose plasmid). The gene coding for the proteinase from *S.cremoris* Wg2 has been sequenced, and the amino acid sequence deduced from the nucleotide sequence (Kok *et al.*, 1988a). The sequence bears some homology to subtilisin around its active site but is a much larger protein. Several proteolytically active components of differing molecular weights have been reported (Hugenholz *et al.*, 1984; Cliffe and Law, 1985) but these are probably a result of proteinase autoproteolysis (Kok *et al.*, 1988b).

Two clearly distinct types of proteolytic activity have been identified by studying the patterns of degradation of the different caseins by *S.cremoris*. One type designated AM1 degrades both α_{s1} and β-casein, whereas the HP type of proteinase only degrades β-casein (Visser *et al.*, 1986). Whether the proteinases from all strains of lactic streptococci show similar specificity for α and β-casein is uncertain. However, marked preference for β-casein has been found with proteinases from several other *S.cremoris* and *S.lactis* strains, and preference for α and κ-caseins found with the proteinases of some lactobacilli.

The peptide products of β-casein hydrolysed by a purified cell wall proteinase from *S.lactis* 763 have recently been investigated (Monnet *et al.*, 1986). Figure 1. 2 identifies the cleavage points producing five oligopeptides from the C-terminal region which range in size from two to sixteen amino acids.

A largely identical cleavage pattern was found for β-casein hydrolysis by the proteolytic enzymes from *S.cremoris* HP (Visser *et al.*, 1988) although some minor differences in the cleavage pattern were found. A third study (Ng, 1989) of the β-casein cleavage pattern carried out in this laboratory using the protease from *S.lactis* 4760 (the strain used for much of the work reported in this thesis) confirmed the findings of Visser *et al.* (1988). Figure 1. 2 summarizes the results of these three studies which indicate a very consistant cleavage pattern for the proteinases from three different strains. It has yet to be established whether this cleavage pattern represents the process of β-casein hydrolysis during growth of these starter bacteria utilising micellar casein in milk. However these studies give an indication of the possible nature of the oligopeptide substrates for the second group of enzymes involved in proteolysis - the peptidase.

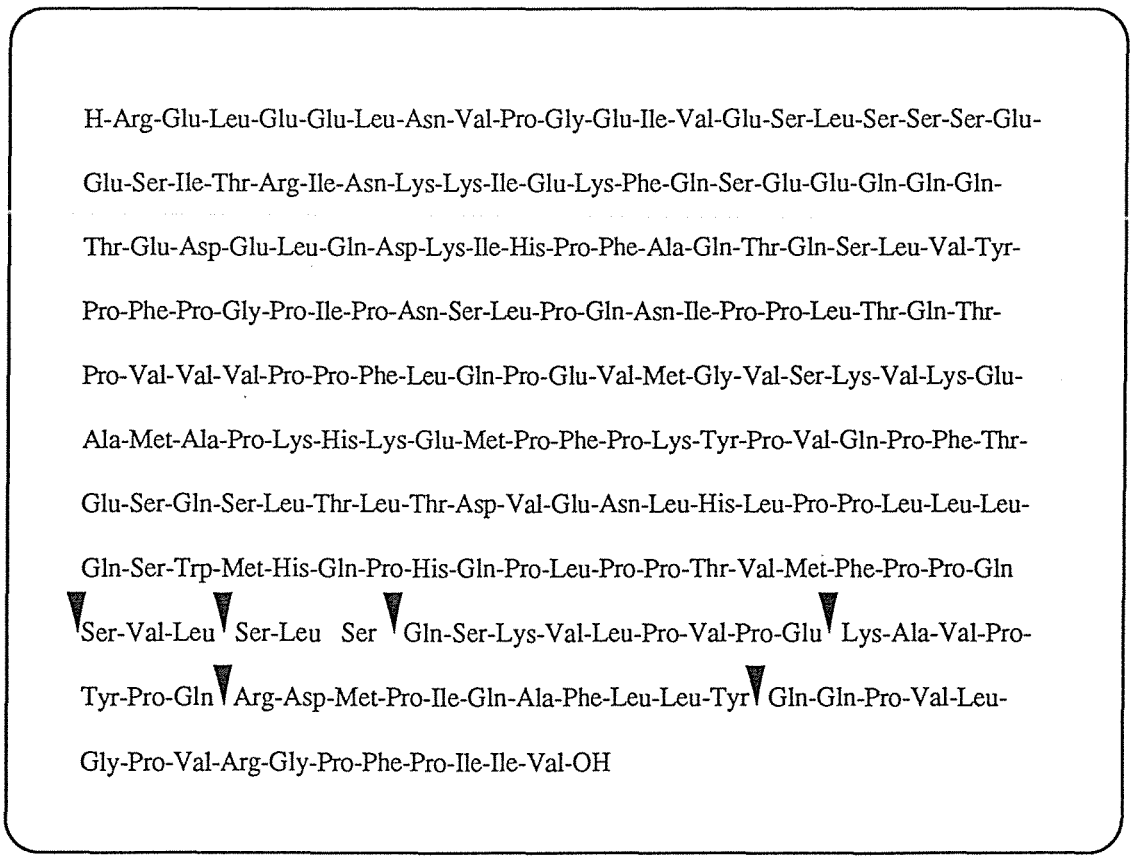

FIG.1.2 *Sequence of β-casein showing the peptides formed after incubation with proteinase enzyme.*

1.3 PEPTIDASES

These enzymes degrade oligopeptides to small peptides and amino acids (Fig 1.1). Although there have been numerous published studies on peptidases from lactic streptococci, there are no systematic studies of the complete peptidase complement from any one strain, and therefore the synergistic interactions between them leading to the hydrolysis of proteinase-generated oligopeptides is not understood.

1.3.1 NOMENCLATURE AND CLASSIFICATION

Peptidase nomenclature in the literature is quite variable. The following is a summary of the peptidase classification used in this thesis.

Peptidases may be classified as endopeptidases or exopeptidases. Endopeptidases cleave bonds distant from the ends of a polypeptide. In addition to the proteinases referred to above, other endopeptidases catalysing the hydrolysis of oligopeptide products of proteinase action may be present in lactic streptococci. Exopeptidases, on the other hand, cleave bonds only near (one or two residues from) the ends, and can be assigned, at least to their major classes, on the basis of substrate specificity. Exopeptidases may be further subclassed into the aminopeptidases (including di -and tri-peptidases) which hydrolyse amino acids from the N-terminal end of a peptide, and the carboxypeptidases which hydrolyse amino acids from the C-terminus. A survey of the published literature reveals the presence of several types of peptidase in lactic streptococci. No carboxypeptidase activity has been reported in any of the strains of lactic streptococci studied, hence the following types may be classified as aminopeptidases.

Peptidase	Bond specificity (*)
a) A"general" aminopeptidase	X--*--Y.....
b) A proline iminopeptidase	Pro--*--Y.....
c) Aminopeptidase P	X--*--Pro.....
d) X-Prolyl dipeptidyl peptidase	X-Pro--*--Y.....
e) A "general" dipeptidase	X--*--Y
f) An imino dipeptidase	Pro--*--X
g) An imido dipeptidase	X--*--Pro
h) A "general" tripeptidase *	X--*--Y-Z or X-Y--*--Z

*TABLE 1.2 A list of aminopeptidase types isolated from lactic streptococci. *The mechanism of action of the tripeptidase has not been studied so the bond specificity pattern is uncertain.*

1.3.2 THE "GENERAL" AMINOPEPTIDASE.

The designation "general" aminopeptidase is given to an enzyme which is active in cleaving aminoacyl derivatives of various chromogenic or fluorogenic substrates with relatively broad specificity for the amino acid. The most widely used substrates are the β-napthylamide and p-nitroanalide (pNA) derivatives, although more recently fluorogenic substrates such as 7-amido 4-methyl coumarin (AMC) derivatives have been introduced (Kato *et al.*, 1978). There are numerous reports of this enzyme in lactic streptococci. It was found to be present in DEAE column fractions distinct from those containing di- and tri-peptidases in a wide range of *S.lactis* and *S.cremoris* strains (Kaminogawa *et al.*, 1984). Aminopeptidases specific for leucyl, glycyl and glutamyl N-terminal amino acids have been found in the soluble fraction of *S.cremoris* after ultrasonic disruption of lysosyme-treated cells (Exterkate, 1984). These aminopeptidase activities, when isolated, appear to be associated with proteins which show different mobilities in gel electrophoresis. A membrane bound aminopeptidase with specificity for glutamyl or aspartyl N-terminal residues has also been reported (Exterkate *et al.*, 1986). In this case however, a free γ–carboxyl group rather than the amino group of the N-terminal amino acid appears to be essential for catalysis. The specific nature of these enzymes contrasts with the wide specificity of the "general" aminopeptidase reported by Kaminogawa *et al*. (1984) although it was not established in this work that the DEAE column fractions contained a single type of enzyme.

A peptidase of molecular weight 36 000 has been purified from the cell wall of *S.cremoris* AC1 which shows aminopeptidase activity, effectively hydrolysing lysyl- p-nitroanalide and, to a lesser extent, leucyl-, alanyl-, and alanyl-alanyl-p-nitroanalides (Geis *et al.,* 1985). This enzyme was found to be active over a pH range of 5.5 to 8 with a maximum at about 7, and was inhibited severely by 1mM EDTA.

"General" aminopeptidases have been purified from two other species of lactic acid bacteria, *L.acidophilus* R26 (Machuga and Ives 1984) and from *L.lactis* (Eggimann and Bachmann 1980).The peptidase from *L.acidophilaus* R26 was responsible for all of the N-terminal exopeptidase and amidase activities observed in crude extracts. The native enzyme, which was found to be a tetramer of molecular weight 156 000, contained four tightly bound Zn atoms. The catalytically inactive native Zn metalloenzyme was capable of being activated by either Zn^{2+}, Co^{2+}, Ni^{2+} or Mn^{2+}.This peptidase is however clearly distinct from the enzyme reported by Kaminogawa *et al,* since it is active against a wide variety of L-amino acid, peptide, amide and p-nitroanalide derivatives including di-, tri- and tetrapeptides.

During the preparation of this thesis, a paper describing the purification and characterisation of an aminopeptidase from *S.cremoris* AM2 was published (Neviani *et al.*, 1989). This intracellular enzyme is reported to be a hexamer of 300 000 molecular weight composed of identical 50 kDa subunits. The authors claim, using a range of β-napthalamide substituted amino acids and di- and tripeptides, that the enzyme had a broad specificity of aminopeptidase activity, but no endopeptidase activity. It was also shown that this enzyme was not a metallopeptidase, but activity was dependant on a thiol group. This distinguishes it from other aminopeptidases detected in mesophilic streptococci or lactobacilli, these generally being metalloenzymes.

1.3.3 "GENERAL" DIPEPTIDASE

There have been numerous studies on dipeptide utilisation and dipeptidase activity from lactic streptococci .

A highly purified dipeptidase from *S.cremoris* H61 has been shown to catalyse hydrolysis of a wide range of dipeptides except those containing proline or glycine as the N-terminal amino acid (Hwang *et al.,* 1981). The substrates of this enzyme can be classified into groups according to their kinetic properties. The Km of this enzyme for various dipeptides were divided into three groups. The first group comprised mainly neutral dipeptides such as Leu-Gly, Leu-Leu and Leu-Ala which had a low Km in the range 4.0 - 6.0mM. Group two consisted of dipeptides with aromatic amino acids at either N or C terminal positions, such as Leu-Phe, Phe-Ala and Leu-Tyr which had very low Km values of 1.0 - 2.4mM. The

third group comprised dipeptides with acidic or basic amino acids at the N terminal position, for example, His-Ala and Glu-Val. This group had high Km values of 10 - 20mM. The optimum pH for this enzyme was found to be pH 8.0.

A second highly purified dipeptidase purified from *S.cremoris* Wg2 has been reported (Van Boven *et al.,* 1988). This enzyme had a molecular weight of 49 000 and specifically hydrolysed a range of dipeptides. It clearly differs from the dipeptidase purified from *S.cremoris* H61, which had a molecular weight of 100 000 (Hwang *et al.,* 1981). Furthermore, the turnover numbers for substrates such as leucyl-leucine or alanyl-alanine were significantly different for the two enzymes. Moreover the metal dependence of both enzymes was distinctly different. For example, 1mM Co^{2+} inhibits the enzyme from *S.cremoris* Wg2, but activates the *S.cremoris* H61 enzyme. There are several clear differences between the types of substrate the two enzymes hydrolyse. There are however also certain similarities between the two enzymes, such as their broad specificity , the requirement of metal ions for activity and the inhibition of the enzymes by reducing agents.

Dipeptidases purified from *S.thermophilus* (Rabier *et al.,* 1973) and *S.diacetylactis* (Desmazeaud *et al.*., 1977) show some similarities to the enzyme from *S.cremoris* Wg2, having a molecular weight of 50 000 - 51 000 and being metalloenzymes with a broad substrate specificity.

Electrophoretic separation of the exopeptidases and the use of a range of dipeptide substrates for activity staining of the gels (Kolstad and Law, 1985) also suggests the presence of only a small number of dipeptidases of broad specificity.

1.3.4 PROLINE AMINOPEPTIDASES

The high proline content of casein has prompted several groups to look specifically for peptidases acting on proline-containing substrates. The five types listed in Table 1.2 have been demonstrated in various lactic streptococci, although there have been no studies demonstrating the presence of all five activities in a single strain. An imido dipeptidase (prolidase) which is highly specific for X-prolyl dipeptides, which may have been removed from the N terminus of a larger peptide, has been purified and characterised from *S. cremoris* H61 (Kaminogawa *et al.,* 1984). An aminopeptidase P which specifically cleaves the amino terminal amino acid when the penultimate residue is proline, and an imino dipeptidase which is specific for proline-X dipeptides have been distinguished in cell-free extracts in a range of lactobacilli and streptococci (Hickey *et al.,* 1983).

Casey and Meyer (1985) have studied the iminopeptidase and dipeptidyl peptidase activity from several lactic acid bacteria by disc electrophoresis. In species such as *L.casei* subsp. *rhamnosus*, the relative activities were found to be about equal and the relative mobilities of the two enzymes were quite separate, but in some species, for example, *S.cremoris* and *S.thermophilus*, the two activities were indistinguishable by electrophoresis . It is conceivable that the iminopeptidase activity in the latter examples is a result of a residual activity of the dipeptidyl peptidase for the iminopeptide. This is supported by the observation that in *S.thermophilus* the dipeptidyl peptidase activity is about five hundred fold greater than that of the proline iminopeptidase.

Meyer and Jordi (1987) have charactised the dipeptidyl peptidase from *L.lactis* and *S.thermophilus*. The enzyme, in both cases, is a serine-type peptidase which has specificity for a wide range of proline containing di- and tri-peptide pNA or AMC derivatives, but no activity with proline-AMC and therefore does not act as an iminopeptidase. It was shown, however, that the *S.thermophilus* enzyme had some endopeptidase activity with the substrate N-succ-gly-pro-AMC. Both enzymes had a molecular weight of 165 000 daltons, an isoelectric point near pH 4.5, and are dimers in their native form. The pH optimum of the enzyme isolated from *L .lactis* was 7.0, whereas the enzyme from *S.thermophilus* possessed a broad pH optimum between 6.5 and 8.2 using Gly-Pro-AMC as a substrate. At pH values lower than 5.0, both enzymes were found to be unstable. However, although of similar molecular weight, the structure of the two dipepeptidyl peptidases differs since the *L.lactis* enzyme is more sensitive to SH blocking agents such as iodoacetate and p-chloromercuribenzoate, while the *S.thermophilus* enzyme is more sensitive to PMSF, a serine protease inhibitor. Both enzymes were only slightly sensitive to EDTA. Even after

incubation with EDTA for 20 hours, activity was not totally lost, and re-addition of bivalent cations did not restore initial activity. To date, a similar enzyme has not been reported in *S.lactis* or *S.cremoris*.

During the course of writing this thesis, a paper describing the purification of an X-Prolyl dipeptidyl peptidase from the cell wall proteolytic system of *S.cremoris* has been published (Kiefer-Partsch *et al.,* 1989). This serine peptidase was reported to have a subunit molecular weight of about 90 000, a native molecular weight of 160 000 - 180 000 and a broad pH optimum between 6.5 and 8. However little work was done to characterise the enzyme further, the extent of testing its substrate specificity being limited to Gly-Pro-pNA and Ala-Pro-pNA.

1.3.5 TRIPEPTIDASES

Existence of tripeptidase activity, distinct from dipeptidase and aminopeptidase activity, has been clearly established by the studies of Kaminogawa *et al.,* (1984) and Kolstad and Law (1985) but to date no tripeptidase has been purified from lactic streptococci.

1.3.6 ENDOPEPTIDASES

Two distinct endopeptidases which may contribute to degradation of casein fragments have been found in the cell free extract of *S.cremoris* H61 (Yan *et al.,* 1987 a and b). One of the endopeptidases termed LEP 2 was purified to homogeneity and shown to be a metallo-endopeptidase of the serine protease type with a molecular weight of 80 000 and a specificity for the peptide bonds involving the amino groups of hydrophobic residues, and has been shown to cleave the N-terminal twenty three-residue oligopeptide from αs1 casein. The involvement of this enzyme in the degradation of β-casein oligopeptide fragments is as yet unclear. From its broad substrate specificity, and its ability to recognise the size of its substrate, it may play a role in providing peptides of reduced size. However its cytoplasmic location might suggest that LEP 2 participates in the cleavage of signal peptides which are highly hydrophobic and of the correct size to be substrates for the enzyme.

A second metallo-endopeptidase termed LEP1 was purified to homogeneity from the same strain (Yan *et al.,* 1987 b) and was found to be a monomeric enzyme with a molecular weight of 98 000. This enzyme showed no detectable hydrolysing activity for milk caseins. LEP1 showed affinity towards a range of peptide bonds found in casein derived peptides and some peptide hormones showing that the substrate specificity of LEP1 was dependent not only on the subsite sequence of substrates but also on the spatial construction of the

substrates. In these respects LEP1 is similar to LEP 2, however the maximum substrate size which could be hydrolysed by LEP1 was smaller than the largest substrate hydrolysed by LEP 2. Both enzymes are clearly different to the cell wall proteinase discussed in section 1.2.

1.4 CELLULAR LOCATION OF PEPTIDASES

There is an increasing body of evidence for the existence of distinct peptidases in different subcellular locations of lactic streptococci. Kolstad and Law (1985) have isolated electrophoretically distinct peptidases from the cell wall and compared them with intracellular fractions using di- and tri-peptide substrates. Contamination of the cell wall fraction by leakage of intracellular enzymes was assessed to be less than three percent by aldolase and lactate dehydrogenase activites. Peptidases located in the cell wall or associated with the cell membrane appeared to differ slightly in their substrate specificity from intracellular peptidases but it is arguable whether these differences are sufficient to substantiate the claim by Kolstad and Law that these are distinct enzymes (Thomas and Pritchard, 1987). However inhibition studies of the lysosyme-released cell wall fraction, and cytoplasmic fractions, has supported the view that the dipeptidases in the cell wall fraction are distinct from the intracellular enzymes (Law 1979). The cell wall-located peptides from *S.cremoris* 1196 and *S.lactis* 763, in contrast to intracellular enzymes were inhibited by mercaptoethanol,and inhibition of cell wall dipeptidases by EDTA was reversed by calcium but not cobalt or manganese, whereas with intracellular peptidases cobalt and manganese were specifically required.

No aminopeptidase activity was detected by Kolstad and Law (1985) in the cell wall fraction but since the dipeptidase recovered from the growth medium by Law (1979) appears to be the same as one of the intracellular dipeptidases, the absence of other peptidase activities, such as aminopeptidase activity, in the growth medium raises the question of the basis for the apparently selective "leakage" of specific peptidases (Kolstad and Law,1985). Exterkate (1984) found that intact cells do possess aminopeptidase activity, as assayed by amino acid-pNA derivatives. However the ability of the pNA derivatives to penetrate the cell membrane and therefore assay intracellular aminopeptidases has not been studied. In support of Exterkate though, an apparently cell wall-bound aminopeptidase of molecular weight 36 000 has been isolated from *S.cremoris* AC1 by repeated washing of intact cells with Tris buffer (Geis *et al.*, 1985). No lysis or protoplasting was observed by microscopy, and no significant contamination of the preparation with cytoplasmic material was detected, confirming that this enzyme is located at the outer boundary of the bacterial cell wall. The penetration of substrates in this instance was not an issue, since the enzyme was completely separated from the cell. Thus, although the existence of different intracellular and cell wall-bound peptidases has been implied, more work is needed to characterise these differences.

1.5 OBJECTIVES OF THE PRESENT PROJECT

The main aim of the present study was to purify as far as possible two aminopeptidases from lactic streptococci and to study their properties and possible role in the degradation of casein-derived oligopeptides.

1) An X-Pro dipeptidyl peptidase. This enzyme has been purified and characterised from *L. lactis* and *S.thermophilus* (Meyer and Jordi 1987) but not from mesophilic lactic streptococci. Their study of the specificity of the enzyme was restricted to X-proline dipeptide derivatives so the ability of this enzyme to hydrolyse proline-containing peptides has not been examined.

2) A "general" aminopeptidase. There are no published studies of a purified "general" aminopeptidase from lactic streptococci even though many studies have reported the presence of an enzyme (or enzymes) catalysing the hydrolysis of a range of aminoacyl-pNA or AMC derivatives. The ability of this enzyme to catalyse the sequential hydrolysis of amino acids from oligopeptides has not been studied nor is there any information on its substrate specificity.

The specific objectives are as follows:

a) The purification and study of a dipeptidyl peptidase and the partial purification of a "general" aminopeptidase from a strain of *S.lactis* * (*S.lactis* H1) . This will involve the assessment of potential affinity purification techniques and the development of gel electrophoresis activity staining procedures.

b) Synthesis of a range of fluorogenic substrates and a study of the specificity of the purified enzymes using these substrates .

c) An investigation of the ability of these enzymes to degrade large oligopeptides and a comparison of their bond specificity for oligopeptides and AMC derivatives.

d) A comparison of the dipeptidyl peptidase purified from *S.lactis* H1 with that from an *S.cremoris* strain.

*The lactic streptococci have recently been placed in a new genus *Lactococcus,* with the single species having two sub-species, *L.lactis* subsp.*lactis,* and *L.lactis* subsp.*cremoris*. While this new nomenclature is currently gaining wide acceptance in the literature, the old designations are still in use and are retained in this thesis.