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review

Aminopeptidases of the Genus *Streptomyces**

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Summary

This review surveys aminopeptidases from various Streptomyces species, which have been studied so far. Leucine preferring, basic amino acids preferring, proline specific, dipeptidyl, tripeptidyl and antibiotic altering aminopeptidases are described. Their physico-chemical characteristics, catalytic properties and available structure elements are compared with the data for aminopeptidases from other bacteria. Possible physiological role of these enzymes is deduced, and prospects for their utilization are presented.

Key words: Streptomyces, aminopeptidase, physiological role, utilization

Introduction

Hydrolysis of peptide bond, catalyzed by a balanced network of peptidases of different properties, is one of the major processes in growing and starving bacteria. Using external and internal sources, endopeptidases and exopeptidases by the combined action provide amino acids necessary for the new protein synthesis. Besides this nutritional role, these enzymes, directly or indirectly, perform a number of other functions important for the cell. They remove non-functional proteins and toxic peptides, regulate physiological processes by inactivating respective enzymes or by providing regulatory levels of certain amino acids, and also modify newly synthesized proteins for the purpose of their maturation, activation or transport.

Streptomycetes, filamentous Gram-positive bacteria, have been in the focus of scientific and industrial interest ever since their capacity to synthesize various substances with antibiotic activity was recognized. More recently the interest has even increased as they were found to be suitable hosts for the recombinant proteins production. Natural habitat and complexity of morphological transformations in the life cycle of these bacteria, impose a necessity of having an elaborate extracellular and intracellular proteolytic system. Synthesis of secondary metabolites seems to be connected with activity of peptidases as well *(1–4)*. Their undesirable effects are manifested during heterologous protein synthesis in *Streptomyces* species *(5,6)*. Nevertheless, the knowledge of streptomycetes' endo- and exopeptidases leaves a lot to be desired, this being particularly true for aminopeptidases. Aminopeptidases (APs) of various *Streptomyces* spp. studied so far are described in this review.

Classification of Aminopeptidases

Aminopeptidases are exopeptidases that degrade oligopeptides and proteins by splitting off amino acid, dipeptide or tripeptide from their NH_2-end. Due to the lack of specificity for a defined substrate, peptidases could not be classified solely by the reaction they catalyze, but additional criteria, like chemical nature of the catalytic site and the structural relationship, have to be used. According to Rawlings and Barrett *(7)*, a peptidase can be designated by a letter denoting catalytic site (S, C, A, M and U for serine, cysteine, aspartic, metallo or unknown) followed by a number assigned arbitrarily to a family to which it belongs. Families encompass a group of enzymes that share similar amino acid sequence at least in the catalytically active domain, and are evolutionary related. Aminopeptidases are named by amino acid forming the scissile bond and classified according to their localization and the described criteria.

* Dedicated to Professor Pavao Mildner for his 80th birdthday

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Table 1. Aminopeptidases from *Streptomyces* species

Streptomyces sp.	Name‡	Specificity	Status	M_m/kDa	pI	pH opt.	Type	Ref.
Extracellular								
S. sioyaensis	Leu-AP	Leu*	partially purified			8–9	M	*(9)*
S. fradiae	Leu-AP	Leu*	partially purified			10	M	*(10,11)*
S. peptidofaciens	Leu-AP	Leu (Phe)	crystalyzed	20	7.4	8	M	*(12,13)*
S. griseus	AP	Leu (Met, Phe)	homogeneous, crystalyzed	30	>7	8–9.7	M28	*(14–24)*
S. rimosus	Leu-AP	Leu (Phe, Met)	homogeneous	27	7.3	8–8.2	M	*(25)*
S. lividans	Leu-AP	Leu	homogeneous	34		8	M	*(5,26)*
S. lividans 66	Tap	N-tripeptide (AlaProAla-)	homogeneous	55		8–9	S	*(27,28)*
S. albidoflavus SM F301	MTP	Leu*	purified	18(SDS)		8	M	*(29)*
S. plicatus	IminoP (Pro-AP)	Pro	homogeneous	120(2)	4.7	8–9	C	*(30)*
	AP P	X-Pro	detected					*(30)*
Streptomyces sp. *WM 23*	DPP II	N-dipeptide (AlaPhe-)	homogeneous	37(SDS)	4.7	7	S	*(31)*
S. macromomyceticus	macromomycin	Phe (broad)	homogeneous	13.8	5.4			*(32)*
S. globisporus	antibiotic C-1027	Phe	homogeneous	15	3.6			*(33,34)*
Intracellular								
S. rimosus	Arg(Lys)-pref.-AP	broad (Arg)	homogeneous	83	4.4	7.1–7.8	M	*(35)*
	Pro-AP	Pro (Hyp)	homogeneous	228(8)	4.9	7.5–8.0		*(36)*
	AP P	X-Pro	purified	~120(2)		8	M	*(36)*
S. lividans 66	PepP (AP P)	X-Pro	partially purified	108(2)				*(37)*
	(Pro-AP)	Pro	detected					*(26,37)*
	PepN (AP N)	broad (Leu,Lys)	partially purified	95			M	*(38,39)*
	PepG (Gly-AP)	Gly(Ser)	partially purified					*(39)*
S. morookaensis	puromycin-hydrolyzing	broad	homogeneous	66		8		*(40)*

* Only few substrates were tested.
‡ As given by the authors. In parentheses is a corresponding systematic name.
Tap = tripeptidyl-peptidase
DPP II = dipeptidyl-peptidase II

Screening of streptomycetes for aminopeptidase activity revealed their excretion by many *Streptomyces* species *(8)*. However only a few of them were studied to a significant extent (Table 1). The number of described intracellular APs is even smaller. As far as specificity is concerned leucyl, broad specificity, proline, prolyl and glycyl aminopeptidase, one dipeptidyl and one tripeptidyl aminopeptidase (peptidase) were found. Majority of APs belong to metallopeptidases, whereas structural data are sufficient only for the *S. griseus* AP to be tentatively associated with one of the proposed peptidase families *(41)*.

Aminopeptidases that Prefer Leucine

Properties

Among extracellular APs the most frequent appear to be APs that prefer leucine, but also cleave peptide bonds of other amino acids, apparently with selectivity depending on the enzyme source (Table 1). Detailed comparison of specificity of APs of different species is hindered, though, by limited number of substrates tested with some enzymes, and owing to the application of different parameters for their comparison. Mostly the reaction rate was used and only sporadically data on k_{cat}/K_m were given. *S. lividans* leucyl AP was claimed to be strictly specific, as APs from *S. peptidofaciens*, *S. griseus* and *S. rimosus* besides leucine accept well methionine and phenylalanine residues. Substrates having acidic amino acid and glycine at P_1 position and proline at position P_1' are poorly or not at all cleaved by these enzymes.

Extracellular APs preferring leucine share other characteristics. They are small size monomers (molecular mass in the range 18 to 34 kDa), have pI close to neutrality, optimally are active at pH between 8 and 10, and they are stable even at higher temperatures (some up to 70 °C). With respect to the size they resemble extracellular enzymes of the same type from other bacteria *(42–45)*, but differ from polymeric mammalian *(46,47)* and intracellular bacterial APs *(48–50)*. As far as ionic nature is concerned, among bacterial APs there are acidic *(42,43)* and neutral enzymes *(49)*. Optimal activity in alkaline media is common to extracellular bacterial APs *(51)*, whereas thermostability is not. Similar tolerance to hi-

gher temperature have APs from bacteria *Aeromonas proteolytica (42)*, *Alteromonas* B207 *(52)* and *Bacillus stearothermophilus (48)*.

All *Streptomyces* leucine-preferring APs are metallopeptidases as demonstrated by inhibition with *o*-phenanthroline and EDTA and reactivation with divalent cations. Their most interesting feature is their response to the presence of calcium. It stabilizes and activates these enzymes, whereas activation by Ca^{2+} was not observed with APs from other sources. Comparing the effects of different chelating agents on *S. griseus* AP and restoration of apo-enzyme activity with different metal cations, the research group from the University of Tel Aviv *(18,19,24)* has shown that Ca^{2+} modulates AP activity, but it alone is not sufficient to support it. This is not in agreement with the report of Wu and Lin *(20)*, but their total removal of other metal ions is not convincing. Ben-Meir *et al. (19)*, have determined that the enzyme contains 2 atoms of zinc per molecule of protein, and that zinc is responsible for the catalysis. Activity of demetalized enzyme was restored with Zn^{2+}, Mn^{2+} and Co^{2+}, with the last one even to the higher level than with Zn^{2+} itself, especially when inferior substrates were used. With this finding activation by cobalt observed for AP from *S. sioyaensis*, *S. lividans* and *S. albidoflavus* could be ascribed to reactivation of the enzyme partially demetalized during isolation.

When Ca^{2+} was added to the reconstituted AP, additional increase of activity was obtained, leading to the conclusion that it binds outside of the active site occupied by zinc. This was confirmed by enzyme structure elucidation *(22)*.

Structure of Streptomyces griseus AP

S. griseus AP is one of the four metallo APs (bovine lens Leu-AP *(47)*, *Escherichia coli* Met-AP *(53)*, *Aeromonas proteolytica* Leu-AP *(45)*), whose crystal structure has been solved *(22)*. Based on primary structure *(21)* and X-ray analysis of apo- and holo-enzyme, overall structure and coordination of zinc atoms is determined. Both metal ions are localized in a single specificity pocket 0.36 nm apart from each other. They have five-coordination sphere, one being ligated to His85 and Asp160 carboxylate oxygen, another to His247 and Glu132 carboxylate oxygen, and both to the bridging Asp97 residue and to the nonprotein oxygen from phosphate molecule that was recognized in the prepared crystals.

S. griseus AP is most similar to *A. proteolytica* AP by its overall geometry and by the motif of the zinc binding site (HDEDH), also found in aminopeptidase Y from *Saccharomyces cerevisiae (54)*. Instead of phosphate *A. proteolytica* enzyme has water to bridge zinc ions. The sequence homology is only 24 %, though. These two enzymes do not bear resemblance to *E. coli* Met-AP that has cobalt ions in the active site and quite different primary and tertiary structure. On the other hand, mammalian Leu-AP from bovine lens contains two zinc atoms per subunit, similarly located as in *A. proteolytica* and *S. griseus* AP, and shares some secondary structure characteristics with both enzymes. However, zinc coordination is different. It uses carboxylate oxygen atoms of Asp255, Asp332 and bridging Glu334, and the carbonyl oxygen of Asp332 as ligands for one zinc ion, and amino group of Lys250 and carboxylate oxygen atoms of Asp273, Glu334 and Asp255 (weak) for the other, more tightly bound one. In the active site additional lysine and arginine residues are present. With these data *S. griseus* AP could be placed to the family which has been proposed for *A. proteolytica* AP, *i.e.* M28 *(41)*.

The unique feature of *S. griseus* AP is the possession of Ca^{2+} binding site. It is remote to the active site by a distance of 2.2–2.5 nm and most probably consists of Asp266, Asp262, Asp3 and Ile4. In the absence of Ca^{2+} the space is occupied by water molecules causing very little change of the enzyme structure. Explanation how calcium ions activate this AP could not be deduced from the structure alone. Greenblat *et al. (22)* and Papir *et al. (24)* have suggested that it causes changes in electrostatic potential distribution over the molecule and stabilizes a distinct conformation most probably of the enzyme-substrate complex. Final explanation would require the understanding of the catalytic mechanism, which has not been solved yet.

Mechanism of action

The mechanism of catalytic action of leucine-preferring APs was thoroughly studied with bovine lens enzyme. Crystallography of its complexes with inhibitors or transition state analogue has revealed that both zinc ions are involved in binding of substrate and its hydrolysis. Data are consistent with: (a) one zinc coordination to P_1 amino group and the other to scissile carbon carbonyl oxygen of the substrate, (b) the activation of water molecule that acts as nucleophile from its bridging position, (c) polarization of substrate carbonyl group, and (d) stabilization of the noncovalent transition state. Catalysis role is assigned also to Arg336 *via* active site water molecules, which in the form of bihydroxide ions function as a general base *(55–57)*.

Different nature of zinc ligands and their arrangement in the active site of both *A. proteolytica* and *S. griseus* APs imply, that the catalytic mechanism for these enzymes might be different from the mechanism of bovine lens AP.

Structural, kinetic and spectroscopic data on *A. proteolytica* AP *(58–60)* afforded conclusion that dinuclearity is included in its catalysis as well. The metal ions communicate electronically and both are needed for the enzyme full activity, but their individual roles are not identical. The proposed mechanism of catalytic action *(58,59)* starts with binding of substrate's NH_2-group to Tyr255, and carbonyl group to the first (catalytic) metal ion of the water-bridged bimetal active site. Upon substrate binding, water molecule becomes bound to a single metal (zinc1) ion and hydrogen bonded to Glu151. Glu151 deprotonates terminal water, and metal-bound hydroxide performs nucleophilic attack on activated scissile carbonyl carbon, while Glu151 forms hydrogen bond with the substrate penultimate nitrogen. The formed complex is stabilized by the second zinc ion, thus contributing to the catalytic process. (Fig. 1)

Data supporting *S. griseus* AP reaction mechanism are still insufficient, but some features can be recognized by analogy with similar enzymes. As changes of AP

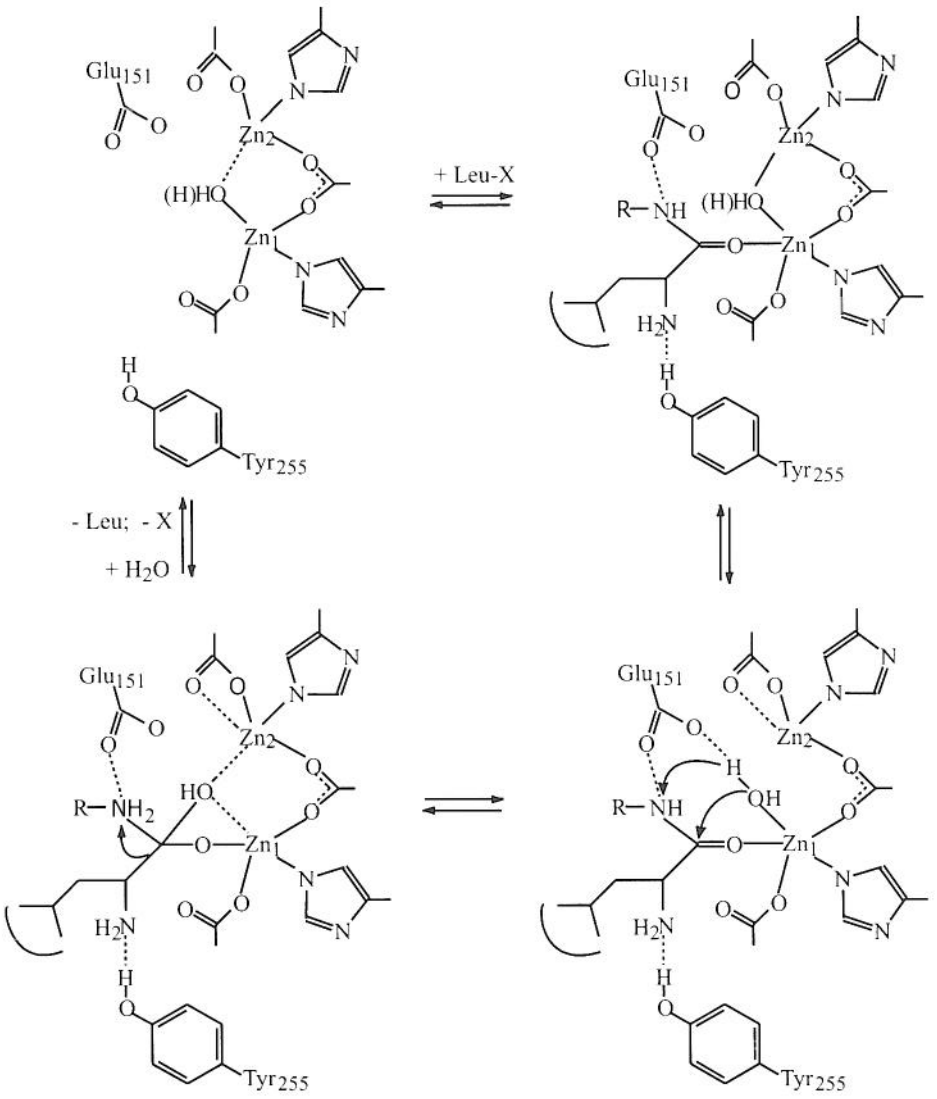

Fig. 1. Catalytic mechanism of leucine-preferring AP from *A. proteolytica* according to Bennett and Holz *(58)*

structure upon metal binding to apo-enzyme could not be observed *(22)*, it may be postulated that metal ions do not play a structural role, but participate in substrate binding and reaction catalysis. Their sequential introduction into the active site resulted in full activity only after both metal ions were in place *(23)*, demonstrating that *S. griseus* AP involves dinuclear mechanism like bovine lens and *A. proteolytica* AP. Proton NMR spectroscopy studies of *S. griseus* and *A. proteolytica* AP having copper(II) ions instead of zinc [CuCu(E)] in the active site *(23,60)* have revealed magnetic interaction between the two metal ions, which enables their mutual influence in the course of the catalytic process. NMR spectra also confirmed the structural similarity of dinuclear metal coordination centers of the two enzymes. Further, the rate-limiting step of the hydrolytic reaction catalyzed by *S. griseus* and *A. proteolytica* AP is product formation. Substrate binding (ES complex formation) is an energetically favorable process, whereas ES complex conversion to activated complex ($ES^{\ddagger}$) is characterized by rather large positive free energy of activation, positive enthalpy and negative entropy *(20,59)*. This indicates similar bond formation/breaking and conformation changes during catalysis in both cases.

Therefore, it is reasonable to assume that *S. griseus* leucine-preferring AP employs the same reaction mechanism as *A. proteolytica* enzyme. However, the activation of the former AP by Ca^{2+} ions, unique to streptomycetes APs, still remains to be elucidated. Leucine-preferring APs from other *Streptomyces* species that have properties similar to *S. griseus* AP might also have the similar structure and mode of action.

Aminopeptidases that Prefer Basic Amino Acids

Another group of broad specificity APs with ubiquitous occurrence in bacteria are basic amino-acids-preferring APs also designated as AP N *(51)*. They are intracellular metallopeptidases of high affinity for basic or bulky neutral amino acid residue at N-terminal position of the substrate. There are indications that such enzymes are present in many *Streptomyces* spp *(38)*, but only two are described to some extent. One was purified from *S.*

rimosus mycelia and its properties were determined *(35)*, while the other is identified and produced in *S. lividans* transformed by its own *pepN* gene *(38)*. Both APs are monomers, larger than extracellular APs and in size comparable to AP N from lactic acid bacteria *(51)*. *S. rimosus* enzyme is an acidic protein like AP N from *Lactobacillus casei*, *Lactococcus lactis* ssp. *cremoris* HP, *Lactobacillus delbrückii* ssp. *lactic* and *Lactobacillus helveticus* SBT 2171 *(51,61–64)*. Unlike extracellular leucine-preferring APs, these enzymes have lower tolerance to higher temperatures, optimally are active at neutral pH and Ca^{2+} does not modulate their activity. Instead, they are activated by Co^{2+}. Cobalt and zinc ions most effectively restored activity of the enzyme inhibited with chelating agents. *S. rimosus* AP was also inhibited by thiol-blocking reagents but not to the extent of *Lc. lactis* ssp. *cremoris* HP and *Lb. helveticus* SBT 2171 AP, thus resembling *Lb. casei* and insensitive *Lb. delbrückii* AP. Specificity characteristics of *S. rimosus* AP are: preference for basic amino acid at both P_1 and P_1' position, lack of tolerance for acidic amino acids and proline at P_1' position, and affinity increase with peptide chain length. It has unusually low K_m (2–6 μM) for its best substrates, comparable to that of AP from *Lb. helveticus* SBT 2171 for lysyl-*p*-nitroanalide as substrate *(64)*.

Comparison of amino acid sequence of *S. lividans* AP N, deduced from its gene analysis, with primary structures of AP N from lactobacteria, revealed the presence of HELAH motif and glutamic acid, 19 residues downstream, in all of them. Sequence of other amino acids in this whole region was highly conserved as well *(38,63,65,66)*. The HELAH + E pattern, also present in man, rabbit, rat and *Sacch. cerevisiae* AP N, comprises Zn-binding and catalytic site characteristic for M1 family of peptidases. Considering the whole molecule, APs of lactic acid bacteria share 49–72 % amino acid identity, while AP from *S. lividans* and *Lc. lactis* have 18 % identical amino acids. All this indicates that classification of streptomycetes AP N to the M1 family can be expected, but it will require more comparative data on structure and properties of these enzymes.

Aminopeptidases Specific for Proline

APs of broad specificity do not readily hydrolyze peptide bonds in which proline is involved. Therefore, to bring protein hydrolysis to completion and also to provide proline for protein synthesis, and possibly cell osmoregulation *(67)*, streptomycetes are likely to possess enzymes specific for this amino acid. A tandem appearance of prolyl AP (proline iminopeptidase) and aminopeptidase P (proline AP) were observed in three *Streptomyces* spp. (Table 1). However, only prolyl AP from *S. plicatus* culture filtrate *(30)* and prolyl AP from mycelium of *S. rimosus (36)* were purified to homogeneity. Though one is extracellular and the other intracellular enzyme both are acidic proteins of oligomeric structure, specific for amino-terminal proline. *S. rimosus* enzyme also accepts the substrate with hydroxyproline at P_1 position, whereas corresponding data for *S. plicatus* AP are not available.

According to their properties and structure the bacterial prolyl APs have been divided into two postulated groups *(68)*. If judged by the size and specificity, *Streptomyces* prolyl APs would belong to the group represented by *Aeromonas sobria (68,69)* and *Hafnia alvei (70)*. They differ from the small monomeric APs from *Bacillus coagulans (69)*, *Flavobacterium meningosepticum (71)*, *Serratia marcescens (72)* and *Xanthomonas campestris* pv. *citri (73)*. In spite of inhibition by cysteine peptidase inhibitors all these enzymes are serine peptidases. The explanation for sensitivity towards thiol reagents came from the three-dimensional structure of *Xanthomonas* AP *(73)*. It revealed a presence of cysteine residue close to the catalytic triad (Ser110, Asp266, His294) characteristic for serine peptidases. Based on its sensitivity to inibitors *S. plicatus* AP was classified as cysteine peptidase. *S. rimosus* AP had ambiguous response to inhibitors. By analogy with other prolyl APs both *Streptomyces* enzymes most probably are serine peptidases as well. Would they belong to S33 family as proposed for bacterial prolyl APs, is hard to predict at present.

Intracellular APs specific for penultimate proline residue have been partially purified from *S. lividans (37)* and *S. rimosus (36)*. Both enzymes are dimers of similar molecular weight and belong to metallopeptidases like *E. coli (74)* and *Lactococcus lactis (75)* AP P. In *S. lividans* two highly homologous genes for AP P were found *(39)*. The most similar to the predicted AP P sequences was the sequence of *E. coli* enzyme *(74)*.

Aminopeptidases with Other Specificities

Dipeptidyl AP

Dipeptidyl peptidases (DPP) are enzymes splitting dipeptides from NH_2-end of oligopeptides, presumably important for biologically active peptides processing in mammals. Their counterparts have been found in a number of bacteria *(51)*, one of them being *Streptomyces* sp. WM-23 *(31)*. DPP isolated from its culture filtrate was capable of removing N-terminal Ala-Phe, Ala-Ala, Val-Ala and Phe-Val dipeptide from various substrates including B-chain of insulin. Its activity was abolished by serine peptidase inhibitors, but resistant to amastatin and bestatin. The enzyme was considered to be DPP II, the only one of that type found in bacteria so far. However, the isolation of DPPs having unusual specificities from *Pseudomonas* sp., indicated that the classification system for mammalian DPPs might not always be applicable to the bacterial enzymes *(76)*.

Tripeptidyl AP

Production of heterologous proteins by *S. lividans* was often accompanied by their amino terminal truncation. This triggered a search for the responsible enzyme and led to the discovery of extracellular tripeptidyl peptidase of this organism *(27,28)*. It is a true aminopeptidase requiring free amino group at N-terminal, sensitive to serine protease inhibitors. Analysis of the corresponding gene revealed homology in the region of the putative active site serine to some members of the lipase family that have conserved the GXSXG motif. To my best knowledge this is the only tripeptidyl aminopeptidase described in bacteria.

APs with unusual activities

Inactivation of antibiotics by target organisms or by their producers is a widely present process accomplished through various mechanisms. Involvement of APs in antibiotic detoxification is best documented for bleomycin hydrolase, the enzyme that can hydrolyze both the carboxyamide bond in antitumor glycopeptide antibiotic bleomycin, and the peptide bond in substrates characteristic for aminopeptidases. The enzyme has been found in mammals, yeast and lactic bacteria, and belongs to oligomeric cysteine APs of broad specificity *(77–81)*. Streptomycetes, being antibiotic producers themselves, also synthesize APs that affect antibiotics. Two types of activities were recognized: inactivation of antibiotic, and participation in its activity. From cells of *S. morookaensis* an enzyme that inactivates puromycin was isolated *(40)*. It hydrolyzes the amide bond between O-methyl-L-tyrosine and aminonucleoside moiety, and has strict AP activity as well. By its N-terminal sequence it does not resemble any other peptidase.

Streptomycetes produce a group of antitumor antibiotics composed of a rather small (10–15 kDa) apoprotein part and non-protein chromophore. Its members macromomycin and antibiotic C-1027 were shown to be aminopeptidases preferring phenylalanine *(32,34)*. Only the holo-antibiotic had AP activity, as chromophor itself was cytotoxic. Inhibition of C-1027 by amastatin or monoclonal antibodies directed to an epitope on the protein part reduced the cytotoxic effect as well *(34,82)*. This clearly illustrates that AP contributes not only to the stability of the chromophore but also to the antitumor activity. It was postulated that the enzyme serves to deliver the chromophore to the cell DNA, but the mechanism of this process is not quite clear.

Physiological Role of *Streptomyces* Aminopeptidases

Direct evidence for the physiological role of APs in streptomycetes is very limited. However, it can be expected that in some part it is the same as in other bacteria *(51)*. Thus, the main function of extracellular APs would be the processing of peptides from the environment for the bacterial nitrogen nutrition. This is corroborated by the ability of mutants lacking individual aminopeptidase to grow normally on rich media, by their slower growth in minimal media and growth impairment of mutants with multiple deletions of AP genes *(28,83,84)*. Intracellular APs hydrolyze exogenous polypeptides that enter the cell, and also those formed by intracellular proteins catabolism. For the complete breakdown combined action of APs of different specificity is necessary. In *S. lividans* and *S. rimosus* the presence of various APs has been shown (Table 1). The lack of AP activity is accompanied with intracellular accumulation of peptides and decrease of free amino acids pool *(84)*.

Within the cell, there is a dynamic equilibrium of protein synthesis and catabolism, with expressed degradation selectivity and dependence on growth phase. This makes proteolysis an essential part of the mechanism for cellular function regulation, that acts on the post-translational level. Connection of proteinases activity with morphological differentiation of *Streptomyces* spp. is well documented. Formation of aerial mycelium and sporulation was shown to be regulated by the fine interplay of proteinases and their inhibitors action *(29, 85–87)*. Thus, in *Streptomyces exfoliatus* SMF13 leupeptin is produced during substrate mycelial growth and upon the start of the aerial mycelium formation it is degraded by leupeptin-inactivating enzyme. This allows trypsin-like proteinase to hydrolyze proteins from the substrate mycelia and provide materials necessary for aerial growth. The same system is operative in submerged cultures and the production of its components is regulated by mycelial growth rate.

The role of aminopeptidases was documented in *S. albidoflavus* SMF301, the strain capable to sporulate in submerged and solid culture *(29)*. Both type cultures of this microorganism, during spores germination and first mycelium formation, produced chymotrypsin-like proteinase. This was followed by production of trypsin-like proteinase during the formation of aerial and thickened mycelium, respectively. Concomitantly to the trypsin-like proteinase synthesis, production of leucyl AP (metallopeptidase) started and continued into the spore-formation phase. Addition of AP inhibitors to the culture medium completely suppressed submerged and aerial mycelium spores formation, without affecting mycelium growth. These data show that aminopeptidase(s) participate in the process of sporulation. Finding that *S. rimosus* secretes low molecular inhibitor specific for its leucyl AP, might indicate that spores formation is also under the control of the autogenous inhibitor *(88)*.

Potential Utilization of *Streptomyces* Aminopeptidases

Taxonomy

Bacterial taxonomy and some industry regulations often require differentiation of the closely related species and strains. For that purpose, in addition to the conventional taxonomic criteria, certain more specific parameters have to be used. Structure determination of particular proteins and electrophoretic zymogram patterns were proved applicable *(89–91)*. APs analysis has been applied to discern strains of the genus *Frankia*, which is closely related to streptomycetes *(91)*. Based on their variety in *Streptomyces* species APs could be good candidates for intra- and inter-species differentiation of these bacteria. At this point application of pyroglutamyl AP for structure determination of N-protected peptides should be mentioned *(92)*.

Hydrolytic reactions

APs are established as catalysts in several industrial processes and potentially can be used in many others. During cheese manufacture APs secreted by lactic acid bacteria participate in cheese ripening and flavor development. Accordingly, when AP isolated from *Aeromonas caviae* was introduced into the process together with neutral proteinase, Gouda cheese maturation period was shortened and flavor of the product was improved *(93)*. Cloning of prolyl AP gene into *Lc. lactic* for the same purpose was also proposed *(94)*.

Treatment with proteinases during food processing and production of protein hydrolysates are often accompanied by the development of bitter taste. Bitterness comes from liberated peptides containing hydrophobic amino acids at the N-terminal region. The procedure of choice for their removal is hydrolysis with APs. Broad specificity and proline specific APs are the most effective for that purpose *(95)*. Leucine-preferring AP from streptomycetes would be a suitable enzyme for such a technological process.

Organic synthesis

Oligopeptides are emerging as ever more important products for pharmaceutical, agrochemical and food industry. Being metabolic regulators, immunomodulators, neuromodulators, enzyme inhibitors, receptor antagonists, antibiotics and antiviral agents, many of them have a prospect of becoming new drugs. On the other hand, their flavors and ability to elicit various taste sensations make them valuable food additives and substitutes *(96)*. Peptides are synthesized mostly chemically, but to overcome some production difficulties and high cost, and to conform to the new health and safety regulations, alternative methods, like recombinant DNA technology and enzymatic synthesis, are examined and employed. The use of enzymes has notable advantages like: mild non-hazardous reaction conditions, possibility to omit group protection and deprotection step, and stereospecific catalysis that gives enantiomerically pure products. Proteolytic enzymes, mainly endopeptidases, have been successfully used in a number of synthetic processes, some of them carried out at the industrial scale (aspartame, malyl-tyrosine, amino acids) *(96)*. APs have been applied for stereoselective hydrolysis of racemic mixtures of amino acid amides and their derivatives. AP from *Pseudomonas putida* catalyzed preparation of optically pure α-H amino acids, as *Mycobacterium neoaurum* amidase was effective with α,α-disubstituted amino acid amides. In combination with racemase and non-stereospecific AP the process can be directed towards L or D enantiomer and designed to go to completion *(97,98)*.

Staphylococcus chromogenes aspartyl AP was shown to be useful for the introduction of malic acid residue at the N-terminus of probably all amino acids except proline and glycine, and also for the deprotection of malylamino group. The same enzyme was able to catalyze synthesis of aspartame *(99,100)*. Promising results were obtained with prolyl AP from *Bacillus subtilis* in synthesis of peptides containing proline *(101)*. Peptide bonds with proline are present in many biologically active peptides, but they are the most difficult ones to form *in vitro*.

Rather inventive application of APs was described by Whitsell and collaborators *(102)*. They have used mammalian APs to trim layers of helical polypeptide chains perpendicularly attached to the gold-coated surfaces, designed to be optical switches.

None of the known *Streptomyces* APs has been used in the described processes so far, which is certainly a reflection of inadequate attention paid to them. The importance and particularity of the *Steptomyces* genus, as well as the growing interest for APs in general, will certainly stimulate further study of these enzymes.

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Aminopeptidaze roda *Streptomyces*

Sažetak

Ovaj revijalni prikaz obuhvaća dosadašnja istraživanja aminopeptidaza iz različitih vrsta Streptomyces. Opisane su leucin aminopeptidaze, aminopeptidaze koje preferiraju bazične aminokiseline, prolin specifične, dipeptidil i tripeptidil aminopeptidaze te aminopeptidaze koje utječu na antibiotike. Njihove fizikalno-kemijske osobine, katalitička svojstva i elementi strukture uspoređeni su s podacima za aminopeptidaze drugih bakterija. Izvedene su neke pretpostavke o njihovoj fiziološkoj ulozi, te razmotrene mogućnosti primjene tih enzima.