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Outline of Research Result

Peptide Synthesis by *Streptomyces* Aminopeptidase

Tadashi HATANAKA

Research Institute for Biological Sciences (RIBS), Okayama

Research aims

Some dipeptides exhibit biological activity; for example, aspartyl-phenylalanine methyl ester (AspPhe-OMe) is a high-intensity sweetener, tyrosyl-arginine (TyrArg) is an opioid dipeptide (1), and valyl-tyrosine (ValTyr) inhibits an angiotensin I-converting enzyme's activity (2). In addition, some amino acids such as tyrosine (Tyr), which is considered as an essential amino acid for some premature infants, have a low solubility. Because these immiscible amino acids become soluble by ligation to another amino acid, alanyl-tyrosine (AlaTyr) is a potential source of tyrosine (3, 4).

Several metallopeptidases, such as thermolysin, synthesize peptides in organic solvents (5). Thermolysin prefers chemically N-protected peptides as substrates for hydrolysis, and therefore N-protected amino acids are used as acyl donors in dipeptide synthesis by thermolysin. Hence, deprotection is required to obtain a biologically active dipeptide. In addition, chemically N-protected amino acids are more expensive than free amino acids and their esters. Thus, from an economical point of view, N-protected amino acids are undesirable for use in the chemoenzymatic synthesis of biologically active dipeptides.

Recently, we have identified a thermostable aminopeptidase, which contains cocatalytic metallo-active sites, secreted by *Streptomyces septatus* TH-2 (SSAP), and succeeded in overproducing it using recombinant *E. coli* (6). SSAP has a broad specificity toward peptides (7); however, this enzyme cannot hydrolyze N-protected peptides. Thus, we postulate that SSAP synthesizes a wide variety of dipeptides using non-N-protected amino acids if SSAP has a function of reverse reaction.

Methods

SSAP was expressed using *E. coli* and purified as following our previous investigation (6). Dipeptide synthesis by SSAP was performed as follows: 20 μl of SSAP solution (1 mg/ml) was suspended in 10 μl of 1 M Tris-HCl (pH 8.0) containing 200 mM amino acid. This suspension was placed in a 1.5-ml microtube, frozen by immersion in liquid nitrogen, and lyophilized using FreeZone Freeze-Dry Systems Model 7679520 (Labconco) at a vacuum of 0.3 hPa for 2 h. This lyophilized enzyme was used for dipeptide synthesis. The activity remained at 78% after lyophilization. The reaction was initiated by adding 100 μl of 98% methanol (MeOH) containing 50 mM aminoacyl-OMe to the lyophilized enzyme. Then, the reaction was continued with vigorous shaking (300 rpm) at 25°C for an appropriate time (20 min～50 h). Because SSAP could not synthesize dipeptides in ＞99.8% MeOH solution, the reaction was terminated by adding 1.9 ml of 100% MeOH. After centrifugation, the reaction mixture was analyzed by thin layer chromatography or high-performance liquid chromatography.

Results

S*ubstrate specificity*. In our previous study, we found that SSAP could hydrolyze a wide variety of dipeptides containing hydrophobic residues (7). Thus, we postulate that SSAP can synthesize a wide variety of dipeptides containing hydrophobic residues. To investigate the synthesis of various dipeptides by SSAP, we examined acyl donor specificity by reacting Phe-OMe with 20 different free amino acids for 3 h, and acyl acceptor specificity by reacting free phenylalanine with 17 different kinds of aminoacyl-OMe for 3 h. As shown in Fig. 1, a high reaction rate is biased toward synthesis using acyl donors and acceptors that have bulky side chains. In addition, there was no synthesis when free cysteine was used as an acyl donor, and His-OMe and Pro-OMe were used as acyl acceptors.

Synthesis of biologically active dipeptides by SSAP. We further examined the application of SSAP to the production of several biologically active dipeptides, such as AspPhe-OMe, ValTyr-OMe, AlaTyr-OMe and Ala-3,4-dihydroxyphenylalanine(DOPA) -OMe. We surmised that other insoluble amino acids, such as DOPA, a therapeutic agent for Parkinson's disease, may increase their solubilities by conversion to dipeptides. Because DOPA has a similar structure to tyrosine, we predict that DOPA-OMe is a good acyl acceptor. All the dipeptides were synthesized by SSAP in 98% MeOH. In these dipeptide syntheses, the conversion rates of free amino acids to dipeptides were above 25% (data not shown). We further tried to synthesize ester-formed TyrArg using free tyrosine and Arg-OMe; however, this opioid dipeptide could not be obtained (data not shown). We surmise that tyrosine and Arg-OMe cannot behave as a good acyl donor and a good acyl acceptor, respectively.

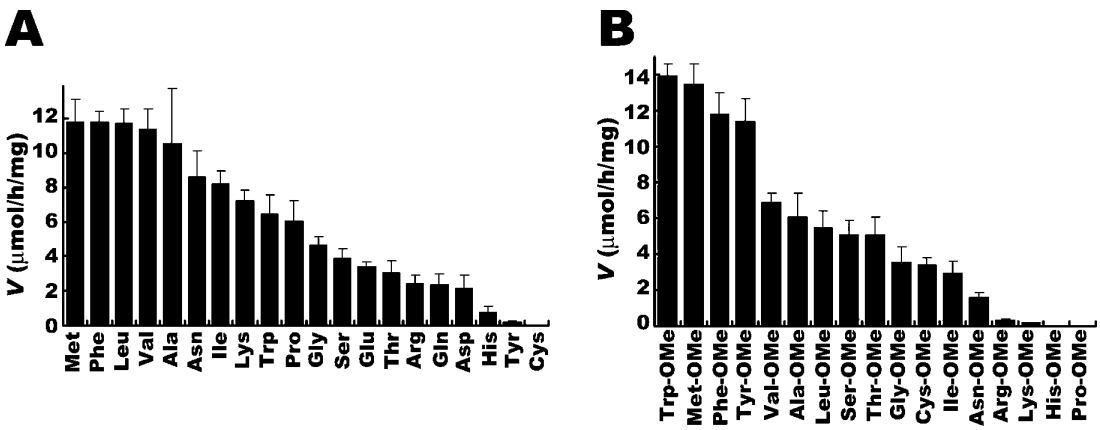

Fig. 1. Substrate specificity of dipeptide synthesis by SSAP.
A: Specificity of SSAP toward acyl donor. B: Specificity of SSAP toward acyl acceptor.

Conclusion

In this study, we first demonstrated that SSAP can synthesize dipeptides using a free amino acid (acyl donor) and aminoacyl-OMe (acyl acceptor) in 98% MeOH. Further, we showed that SSAP can be applied to the synthesis of several biologically active peptides.

References

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