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# Carboxypeptidase from *Streptomyces bikiniensis*: Primary Structure, Isolation, and Properties

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**Abstract**—A metallocarboxypeptidase produced by *Streptomyces bikiniensis* 27 strain (VKPM Ac-1783) (CPSb) was purified and characterized. The enzyme cleaves both basic and hydrophobic C-terminal amino acid residues from synthetic peptides, that is, it possesses specificity of mammalian carboxypeptidases A and B. The enzyme also hydrolyzes peptides bearing glutamic acid at the C-end. CPSb exhibits its maximal activity at pH 7.0-7.6 and 55°C. The nucleotide sequence encoding the mature CPSb in *S. bikiniensis* 27 (VKPM Ac-1783) genome (Accession No. GU362077) was determined. It is shown that the primary structure of the mature enzyme has a moderate degree of identity with orthologs from *Streptomyces griseus* (79% identity) and *Streptomyces avermitilis* (85% identity).

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Assimilation of proteins in the intestines of animals requires their cleavage to amino acids by the action of endo- and exopeptidases of various specificities. Two enzymes with carboxypeptidase activity, carboxypeptidases A and B (CPA and CPB), are present in human pancreatic fluid. These metallocarboxypeptidases belong to the A/B subfamily of the M14 peptidases family [1, 2]. CPB cleaves basic amino acid residues from the C-end of peptides, whereas CPA cleaves aromatic and branched aliphatic residues [1, 2]. CPA and CPB cooperate and exhibit wide total specificity, although "inconvenient" amino acid residues still remain, e.g. glutamic and aspartic acid residues. CPB is unable to cleave these residues, and CPA cleaves them very slowly [3]. An enzyme able to cleave glutamic acid residues from the C-end of peptides was found in the alimentary canal of insects [4]. Moreover, a carboxypeptidase specifically cleaving glutamic acid was isolated from the midgut of *Helicoverpa armigera* [5]. Also, carboxypeptidases specific to glutamate have been found in several pseudomonad strains [6-8].

Biosynthesis of digestive enzymes is violated in various gastrointestinal diseases; this results in decreased assimilation of proteins. In this case substitution therapy is widely used: enzyme preparations of various origins compensate the lack of the patient's own digestive enzymes [9]. Although most drugs thus used are based on pancreatin (copying of the natural situation) [10], proteolytic enzymes of other origin can also be used. Catalytic properties of such enzymes can differ from those of pancreas components. In particular, it is of interest to use carboxypeptidase of wide specificity combining the properties of CPA and CPB and also able to cleave some "inconvenient" amino acid residues, e.g. Glu and Asp. Such carboxypeptidase is absent among animal carboxypeptidases similar to CPA (e.g. $CPA_3$ [11] and $CPA_5$ [12]) or CPB (e.g. CPU [13] and CPN [14]).

Carboxypeptidases of microorganisms are more promising. For example, serine carboxypeptidases produced by fungi of the *Aspergillus* genus, e.g. *Aspergillus saitoi*, cleaves basic, neutral, and acidic amino acids and even proline from the C-end of the substrates [15-17]. Carboxypeptidase T (CPT) from *Thermoactynomyces vulgaris* [18, 19], CPSG composing the proteolytic complex of *Streptomyces griseus* [20-22], and carboxypeptidases from *Sulfolobus solfataricus* archaea [23] also demonstrate wide specificity.

In this work we isolated and studied the physicochemical and catalytic properties of a carboxypeptidase

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from *S. bikiniensis* (CPSb) and also cloned the corresponding gene from the genome of this microorganism.

## MATERIALS AND METHODS

**Cultivation of microorganisms.** *Streptomyces bikiniensis* 27 strain (VKPM Ac-1783) was used in this work. The microorganism was grown in 750-ml flasks containing 30 ml of fermentation medium of the following composition (g/liter): bacteriological peptone from Sigma (USA), 50; maize extract, 5.0; glucose, 10.0; chalk, 0.5; NaCl, 5.0; pH 7.0-7.2. Flasks were placed on a rotary shaker (220 rpm) and cultivated for 96 h at 30°C. Then the micellae was precipitated by centrifugation of the culture liquid for 20 min at 15,000*g*.

**Isolation of CPSb.** After centrifugation, $(NH_4)_2SO_4$ was added to 450 ml of the culture liquid to 80% saturation. The resulting suspension was stored overnight at 4°C, then the precipitate was separated by centrifugation (20 min at 15,000*g*) and dissolved in 40 ml of 10 mM Tris-HCl, pH 8.2, containing 10 mM $CaCl_2$ (buffer A). The solution was then centrifuged as described above, and the supernatant was dialyzed overnight against the same buffer.

The dialysate was applied onto a column with bacitracin-Silochrom (synthesized in our laboratory according to [24]) equilibrated with buffer A and then eluted with 1 M NaCl in the same buffer. To separate trypsin admixture, the eluate was applied onto a column with benzamidine-Sepharose from GE Healthcare (Sweden) equilibrated with buffer A containing 1 M NaCl. The carboxypeptidase remained in the non-absorbed fraction. This fraction was dialyzed overnight against 5 mM Tris-HCl, pH 7.0, containing 5 mM $CaCl_2$ (buffer B). The dialyzate was applied onto a HiTrap SP column (GE Healthcare) equilibrated with buffer B. The protein was eluted with a gradient of NaCl concentration (0-1 M) in buffer B. Fraction with enzymatic activity was applied onto a column with Arg-Sepharose (GE Healthcare) equilibrated with 50 mM Tris-HCl, pH 7.0, containing 100 mM NaCl and 1 mM $CaCl_2$. The enzyme was eluted with the same buffer with salt concentration increased to 0.5 M. All procedures were performed at 4°C.

**Estimation of carboxypeptidase activity.** Carboxypeptidase activity of various fractions obtained in the course of enzyme isolation was estimated by hydrolysis of Dnp-Ala-Ala-Arg (synthesized in our laboratory according to [25], hereafter called substrate (I)). Protein solution (1-20 µl) was added to 180-200 µl of 50 mM Tris-HCl, pH 7.6, containing 1 mM $CaCl_2$ (buffer C). Substrate solution (200 µl) at concentration 0.5 mg/ml was added to this mixture. The reaction time and temperature were 5-20 min and 37°C. The reaction was stopped by addition of 50% acetic acid (40 µl). A column with SP-Sephadex C-25 equilibrated with 1 M acetic acid was used to separate the reaction product from non-hydrolyzed substrate. The reaction product was eluted with 1 M acetic acid. Carboxypeptidase activity (*A*) was estimated using molar extinction coefficient of the Dnp group (15 $mM^{-1} \cdot cm^{-1}$). The amount of enzyme catalyzing conversion of 1 µmol of substrate in 1 min was taken as the activity unit.

**SDS-PAGE.** Electrophoresis was performed according to Laemmli [26] in a slab of 12.5% polyacrylamide gel at 150 V. The following molecular mass standards from Sigma-Aldrich (USA) were used: bovine serum albumin (66 kDa), ovalbumin (45 kDa), phosphoglycerol aldehyde dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), and soybean trypsin inhibitor (20.1 kDa).

**Mass-spectroscopy.** Enzyme sample (4.8 µg in 3 ml) was subjected to HPLC on a C4 column (4.6 × 250 mm) from Vydac (USA) in 25-60% acetonitrile gradient for 40 min. The mass spectrum of the compound with the protein peak was recorded using a MALDI Ultimate mass spectrometer from Bruker (Germany).

**Determination of N-terminal amino acid sequence.** Purified carboxypeptidase (3.5 µg in 2.2 ml of solution) was chromatographed on a Vydac C4 column (4.6 × 250 mm) as described above. The compound composing the protein peak was sequenced using a Procise Sequencing System automatic sequencer (model 492) from Applied Biosystems (USA).

**Dependence of CPSb activity on pH** was studied using substrate (I). The following buffers (50 mM) were used: Na acetate, pH 4.4-5.5; bis(tris-(hydroxymethyl)-methylamino)propane-HCl from BIOMOL (Germany), pH 5.5-9.5; Tris-HCl from DiaM (Russia), pH 7.0-7.9; and 30 mM universal buffer (boric and citric acids, $KH_2PO_4$, diethylbarbituric acid from Reanal (Hungary)), pH 4.5-9.2. All buffers contained 1 mM $CaCl_2$. Enzyme (20 µl, in case of universal buffer – 50 µl) was added to 180 µl (in case of universal buffer – 150 µl) of buffer with the corresponding pH value. Then 200 µl of substrate solution (0.5 mg/ml) was added to this mixture. The reaction was run for 10 min at 37°C.

**Determination of CPSb temperature optimum.** Substrate (I) (200 µl, 0.5 mg/ml) was added to 190 µl of buffer C. The mixture was heated for 2 min in water bath to the specified temperature. Then 10 µl of enzyme was added, and the reaction was run for 10 min at the same temperature.

**Study of thermal stability of CPSb.** Enzyme (100 µl) was added to 150 µl of buffer C and incubated at 37 or 50°C. Aliquots (50 µl) of this mixture were taken after 15, 30, 45, 60, and 120 min and added to a mixture of buffer (150 µl) and substrate (I) (200 µl) preincubated for 2 min at 37°C. The reaction was run for 10 min (for study of thermal stability at 37°C) or 15 min (for study of thermal stability at 55°C) at 37°C. In both cases, in order to obtain the "zero point", 20 µl of enzyme solution was added to a mixture of buffer (180 µl) and substrate (200 µl) heated

for 2 min at 37°C. The reaction was run as described above. The residual activity is expressed as percentage of activity at the "zero point".

**Effect of inhibitors on CPSb activity.** An aliquot of the enzyme (20 µl) was added to 180 µl of buffer C containing one of the inhibitors, and the mixture was incubated for 60 min at room temperature. Then 200 µl of substrate (I) at concentration 0.5 mg/ml was added, and the resulting solution was incubated for 10 min at 37°C. In each case enzyme activity was expressed as percent of that in the absence of the inhibitor. All inhibitors used were from Sigma.

**Determination of catalytic characteristics of hydrolysis of peptide substrates.** The following N-substituted peptide substrates synthesized in our laboratory were used: Dnp-Ala-Ala-Arg (I), Z-Ala-Ala-Arg (II), Z-Ala-Ala-Leu (III), and Z-Ala-Ala-Glu (IV). If substrate (I) was hydrolyzed, 10 µl of $6.81 \cdot 10^{-8}$ M enzyme was added to 400 µl of buffer C containing substrate at various concentrations (from 0.052 to 5.2 mM). Reactions were run for 5-30 min at 37°C. The amount of product was determined as described earlier. If substrates (II)-(IV) were hydrolyzed, 2.8 ml of buffer C containing substrate at various concentrations (0.025-0.4 mM for (II) and (III) and 0.2-4 mM for (IV)) were placed in a thermostable (37°C) cuvette of a Shimadzu UV-mini 1240 spectrophotometer (Japan). After 5 min, 200 µl of enzyme solution at concentration $2.7 \cdot 10^{-8}$ M ($1.7 \cdot 10^{-7}$ M for substrate (IV)) was added. Product concentration was determined via decrease in optical absorption at 225 nm using $\varepsilon_{225} = 376$ $M^{-1} \cdot cm^{-1}$. The reaction rates were measured thrice for 4-7 concentrations in the given range. Results were processed by nonlinear regression using Origin software (Origin Lab Corporation, USA).

**Cloning of the CPSb gene.** Using bik6a (gggatccgtcaggtgcagtactgcgcttccttgccga) and bik7a (gaattCACCAGCACGCCCGCGAGCACCTGAC) with genome DNA of *S. bikiniensis* 27 strain (VKPM Ac-1783), we amplified a DNA fragment inside the site corresponding to mature CPSb. Based on the resulting sequence, bik3b (cctgcagccccacttgtagttccagttgcggtt) and bik5b (gaattcGGCGGCTTCTACCCGCCCGACGAGGT) primers were synthesized; their elongation results in replication of flanking DNA sequences outside the fragment. Genome DNA of *S. bikiniensis* VKPM Ac-1783 was subjected to incomplete restriction by *Taq*I restrictase according to the producer's protocol (Fermentas, Lithuania). The products of restriction were converted into circular form by ligation at low concentrations (0.005 mg/ml) using DNA ligase from Fermentas in a standard buffer for DNA ligase using the producer's conditions (Fermentas). The products of subsequent PCR amplification of circular ligation products (inverted PCR) were sequenced; this allowed us to determine the sequence of the DNA fragment corresponding to mature CPSb.

We searched for protein sequences orthologous to that experimentally established using "BLAST search for short, nearly extract matches" software and NCBI GenBank. BLASTN and BLASTP softwares were used for alignment nucleotide and amino acid sequences.

## RESULTS AND DISCUSSION

**Isolation of carboxypeptidase from *S. bikiniensis* 27 strain (VKPM Ac-1783).** The data on isolation of the enzyme from culture liquid obtained on *S. bikiniensis* growth are presented in Table 1. The specific activity of CPSb increased 180-fold in the course of the isolation (since it was impossible to determine protein in the initial centrifugate accurately, the specific activity of the precipitate obtained with $(NH_4)_2SO_4$ was used for comparison). The yield of the enzyme was 3.2% of its initial content in the culture liquid.

Purity of the preparation was checked by SDS-PAGE (Fig. 1). There is only one protein band with molecular mass 36 kDa. The presence of only one protein peak was shown also by HPLC of the CPSb preparation

**Table 1.** Isolation of carboxypeptidase produced by *S. bikiniensis* 27 strain (VKPM Ac-1783)

| Isolation stage | Volume, ml | Protein, mg/ml | Specific activity, U/mg | Activity yield, % |
|---|---|---|---|---|
| Centrifugate of culture liquid | 600 | | | 100 |
| Dissolved precipitate after $(NH_4)_2SO_4$ | 57 | 0.57 | 0.73 | 78.6 |
| Bacitracin-Silochrom | 60 | 0.12 | 2.67 | 64.0 |
| Benzamidine-Sepharose | 120 | 0.047 | 2.3 | 46.3 |
| HiTrap SP | 15.5 | 0.039 | 7.05 | 14.2 |
| D-Arg-Sepharose | 7.5 | 0.001 | 129 | 3.2 |

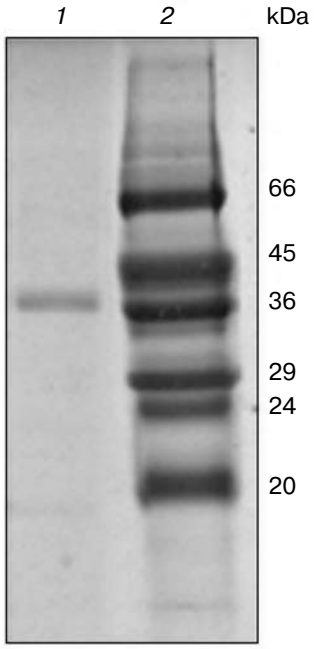


**Fig. 1.** Electrophoregram of purified CPSb (0.2 μg). Lanes: *1*) CPSb; *2*) molecular mass markers.

on a Vydac C4 column; its molecular mass determined by mass spectroscopy was 36.699 kDa.

**N-Terminal sequence of CPSb.** The N-terminal amino acid sequence of CPSb (Asp-Phe-Pro-Ser-Ala-Asp-Ser-Arg-Tyr-His-Asn-Tyr-Ala-Glu-Thr) appeared to be essentially similar to the primary structure of carboxypeptidases from *S. griseus* and *Streptomyces avermitilis* MA-4680 in the sites between residues 124 and 138. Residue 124 begins the sequence of the mature enzyme in the mentioned proteins. This indicates that processing of CPSb and carboxypeptidases of other actinomyces occurs at the same site.

**Characteristics of primary structure of CPSb established from nucleotide sequence of the corresponding gene.** Based on the N-terminal sequence of CPSb, we identified orthologs of this protein, carboxypeptidases from *S. avermitilis* and *S. griseus*, in the Primary Structure Bank. However, comparison of the corresponding genes showed that the degree of identity of two DNA sequences in the conservative region of mature enzyme is 82%, but it is significantly lower in other regions – only 50%. That is why we chose a two-stage strategy for cloning the *CPSb* gene. First, we obtained fragments of conservative regions of this gene by amplification. Based on these fragments, we planned specific primers allowing cloning of flanking gene sequences according to the inverted PCR strategy (see "Materials and Methods").

It was finally demonstrated that the gene fragment encoding mature CPSb contains 990 nucleotide pairs (np) (GenBank GU3620077). Comparison of nucleotide sequence with analogous regions of orthologous genes from the genomes of *S. griseus* and *S. avermitilis* gives 81 and 87% identity, respectively. The primary structure of CPSb is presented in Fig. 2. It contains 329 amino acid residues (a.a.), and its molecular mass is 37,006.7 Da. Comparison of the protein sequence with that of orthologs gives 79% identity for the enzyme from *S. griseus* (YP_001824303) and 85% identity for the carboxypeptidase from *S. avermitilis* (NP_824442). These values are close to the degree of identity of the latter two enzymes (75%) and are significantly higher than the degree of identity between CPSb and carboxypeptidases CPT (CAA40219), CPA (CAA83955), and CPB (ACA96505): 61, 29, and 31%, respectively.

Amino acid residues composing the active site of the *S. griseus* enzyme are also present in corresponding places in the CPSb sequence. First of all, these are Glu400 (Glu278 in CPSb sequence, nucleophile) and Tyr378 (Tyr256, proton donor) residues and His327 (His205), Glu195 (Glu73), and His192 (His70) residues binding a zinc ion.

So, CPSb is a new enzyme close but not identical to two known carboxypeptidases from *Streptomyces* species.

**Determination of pH optimum of CPSb activity.** The data presented in Fig. 3 indicate that CPSb is active in the pH range 6.0-9.5 with maximal activity at pH 7.0-7.6 (depending on buffer content). This is close to the value

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1   DFPSADSRYHNYAETNAAIDQRLSAYPMGWMSQRVICTYYHGRNILALEVSGCVATDKNE 60
61  PEVLRPFHQHAREHLTVEMALYLLRELGAGYCSDSRVTNMVNCREIWIVPDLNPDGGEYD 120
121 IATGSYRSWRKNRQPDSGSSYVGTDLNRNWDYKWGCCGGSSGTKSSETYRGTAPESAPEV 180
181 KVVADFVRSRVIGGKQQIKASIDFHTYSELVLWPFGWTTADTAPGMTQDDRNAFAAVGKK 240
241 MAASNGYTPEQSSDLYVTDGSIDDYLWGAQRIFAYTFEMYPTSSWNGGFYPPDEVIERET 300
301 ARNRDAVLQLLENADCMYRSIGKEAQYCT 329
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**Fig. 2.** Amino acid sequence of mature carboxypeptidase from *S. bikiniensis* 27 strain (VKPM Ac-1783) determined from the nucleotide sequence of its gene.

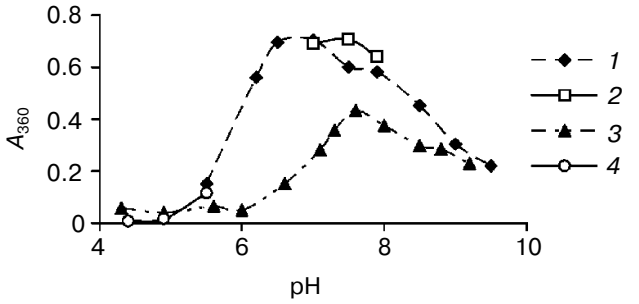


**Fig. 3.** CPSb activity versus pH. Activity was estimated in the following buffers: *1*) 1,3-bis(tris-(hydroxymethyl)-methyl-amino)propane-HCl; *2*) Tris-HCl; *3*) universal buffer system; *4*) Na-acetate.

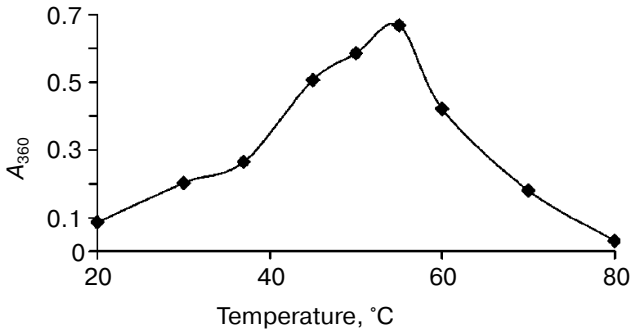


**Fig. 4.** Temperature dependence of CPSb activity.

obtained for CPSG (7.3-8.2 depending on substrate) [20-22].

Enzyme activity varies depending on the buffer. Although in experiments with universal buffer 2.5-fold more enzyme was used than with the other buffers, $A_{360}$ values in the pH range 6.0-8.5 were significantly lower (Fig. 3). Polyvalent anions present in universal buffer seem to inhibit CPSb, e.g. due to binding of $Ca^{2+}$. However, buffer content negligibly influences the pH optimum.

**Temperature optimum and thermal stability of CPSb.** As shown in Fig. 4, CPSb is active in the temperature range 30-70°C with the optimum at 55°C. This is a difference between CPSb and CPSG, whose activity increases up to 70°C [22]. CPSb is rapidly inactivated at 55°C. Residual activity after incubation for 15 and 45 min is 22.4 and 5.3%, respectively (Fig. 5). However, the enzyme is relatively stable at 37°C (Fig. 5), the residual activity after incubation for 120 min being 80%.

**Inhibitor analysis of CPSb.** The data presented in Table 2 indicate that phenylmethylsulfonyl fluoride, an inhibitor of serine proteinases, does not inactivate CPSb. However, chelators of metal ions inhibit the enzyme activity: *ortho*-phenanthroline – completely, and EDTA and EGTA – significantly. It follows from these results that the carboxypeptidase from *S. bikiniensis* is a metalloenzyme. Iodoacetamide significantly suppresses the carboxypeptidase activity, and this indicates the presence of a sulfhydryl group that affects CPSb activity. But *p*-chloromercuribenzoate, also a sulfhydryl group agent, does not inhibit CPSb. This is possibly due to steric hindrance, but the contradictory data prevent a firm conclusion that CPSb is an SH-dependent enzyme. Of bivalent ions of heavy metals, only $Cu^{2+}$ and $Hg^{2+}$ inhibit CPSb, whereas $Mn^{2+}$, $Mg^{2+}$, and $Co^{2+}$ have practically no effect on its activity. According to its sensitivity to the studied inhibitors, CPSb is much like CPSG [20, 21].

**Catalytic properties of CPSb.** Experiments with substrates (I) and (II) demonstrate that CPSb efficiently cleaves basic amino acid residue (Arg) from the C-end of N-substituted synthetic peptides. Values of $K_m$, $k_{cat}$, and $k_{cat}/K_m$ are 0.16 mM, 48.8 $sec^{-1}$, and $3.05 \cdot 10^5$ $M^{-1} \cdot sec^{-1}$ for substrate (I) and 0.05 mM, 52.9 $sec^{-1}$, and $10.6 \cdot 10^5$ $M^{-1} \cdot sec^{-1}$ for substrate (II), respectively (Table 3). As for efficiency of substrate (I) hydrolysis, CPSb is almost equal to mammalian CPB ($k_{cat}/K_m = 5 \cdot 10^5$ $M^{-1} \cdot sec^{-1}$) and significantly more efficient than CPT ($k_{cat}/K_m = 3.3 \cdot 10^3$ $M^{-1} \cdot sec^{-1}$) (Table 3). CPSb more efficiently hydrolyzes substrate (II) than substrate (I); this indicates that the nature of protective group of the substrate influences hydrolysis.

CPSb cleaves hydrophobic amino acid residue (Leu) from the C-end of peptides with the same efficiency. For hydrolysis of substrate (III), $K_m$, $k_{cat}$, and $k_{cat}/K_m$ values are 0.03 mM, 57.2 $sec^{-1}$, and $19.1 \cdot 10^5$ $M^{-1} \cdot sec^{-1}$, respectively (Table 3). The value of $k_{cat}/K_m$ for CPSb is comparable with that for CPA ($29 \cdot 10^5$ $M^{-1} \cdot sec^{-1}$) and larger than that for CPT ($2.2 \cdot 10^5$ $M^{-1} \cdot sec^{-1}$).

Finally, CPSb cleaves acidic amino acid residue (Glu) from the C-end of N-substituted peptide. For hydrolysis of substrate (IV), $K_m$, $k_{cat}$, and $k_{cat}/K_m$ values

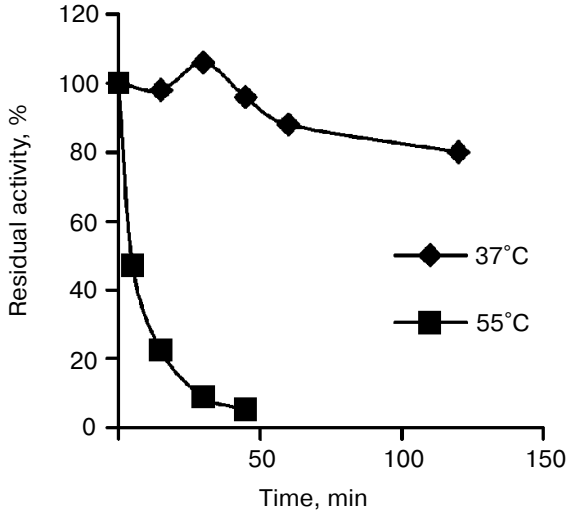


**Fig. 5.** Thermal stability of CPSb.

**Table 2.** Effect of inhibitors on CPSb activity

| Inhibitor | Concentration, mM | Residual activity, % |
|---|---|---|
| Phenylmethylsulfonyl fluoride | 5 | 95 |
| *ortho*-Phenanthroline | 2.5 | 3.9 |
| EDTA | 10 | 55.2 |
| EGTA | 10 | 24 |
| Iodoacetamide | 1 | 3.9 |
| *p*-Chloromercuribenzoate | 1 | 83 |
| $Hg(CH_3COO)_2$ | 5 | 9.3 |
| $Cu(CH_3COO)_2$ | 5 | 11.5 |
| $CoCl_2$ | 2 | 93.7 |
| $MnCl_2$ | 2 | 91.7 |
| $MgCl_2$ | 20 | 86.6 |

are $7.6 \cdot 10^{-4}$ M, 5.3 $sec^{-1}$, and $6.97 \cdot 10^3$ $M^{-1} \cdot sec^{-1}$, respectively (Table 3). Cleavage of this peptide is two orders of magnitude less efficient than that of substrates (I)-(III). However, cleavage of substrate (IV) by CPSb is comparable with that of CPT, and the degree of this cleavage indicates that CPSb specificity covers also cleavage of acidic C-terminal amino acid residues.

It is difficult to compare catalytic properties of CPSb and carboxypeptidase from *Sulfolobus solfataricus* because their substrates are different. However, for hydrolysis of Z-Gly-Gly-Phe, $k_{cat}/K_m$ value of the enzyme from the archaean is one order of magnitude lower than that of CPA [23]. Moreover, this enzyme cleaves aliphatic amino acid residues significantly less efficiently than aromatic ones.

Carboxypeptidase from *T. vulgaris* (CPT) and CPSb cleave acidic amino acid residues with approximately equal efficiency (Table 3). However, in contrast to CPSb, CPT cleaves basic amino acid residues two orders of magnitude slower than hydrophobic ones [27]. Besides this, absolute efficiency of hydrolysis of Dnp-Ala-Ala-Arg and Z-Ala-Ala-Leu by CPT is significantly lower than that by CPSb and pancreatic enzymes.

CPSb is very similar to CPSG in substrate specificity. When the latter hydrolyzes such substrates as Z-Glu-Leu, Bz-Gly-Arg, and Bz-Gly-Lys, $k_{cat}/K_m$ values are 41, 7.5, and 82.6 $M^{-1} \cdot sec^{-1}$, respectively [22]. The rates of hydrolysis of hippuryl-Arg and hippuryl-Phe by this enzyme are similar [20]. Unfortunately, we failed to find any data on the ability of this enzyme to cleave acidic amino acid residues from peptides.

In conclusion, we isolated and characterized an extracellular metallocarboxypeptidase produced by *S.*

**Table 3.** Kinetic parameters of carboxypeptidases from various sources (data on CPA [19], CPB [19], and CPT [27] are taken from the literature)

| Substrate | Kinetic parameter | CPA | CPB | CPT | CPSb |
|---|---|---|---|---|---|
| Dnp-Ala-Ala-Arg (I) | $K_m$, mM | | 0.07 | 4.4 | 0.16 |
| | $k_{cat}$, $sec^{-1}$ | | 35 | 14.6 | 48.8 |
| | $k_{cat}/K_m$, $M^{-1} \cdot sec^{-1}$ | | $5 \cdot 10^5$ | $3.3 \cdot 10^3$ | $3.05 \cdot 10^5$ |
| Z-Ala-Ala-Arg (II) | $K_m$, mM | | | | 0.05 |
| | $k_{cat}$, $sec^{-1}$ | | | | 52.9 |
| | $k_{cat}/K_m$, $M^{-1} \cdot sec^{-1}$ | | | | $10.6 \cdot 10^5$ |
| Z-Ala-Ala-Leu (III) | $K_m$, mM | 0.012 | | 0.046 | 0.03 |
| | $k_{cat}$, $sec^{-1}$ | 35 | | 10.3 | 57.2 |
| | $k_{cat}/K_m$, $M^{-1} \cdot sec^{-1}$ | $29 \cdot 10^5$ | | $2.2 \cdot 10^5$ | $19.1 \cdot 10^5$ |
| Z-Ala-Ala-Glu (IV) | $K_m$, mM | 0 | | | 0.76 |
| | $k_{cat}$, $sec^{-1}$ | | | | 5.3 |
| | $k_{cat}/K_m$, $M^{-1} \cdot sec^{-1}$ | | | $6.1 \cdot 10^3$ | $6.97 \cdot 10^3$ |

*bikiniensis* 27 (VKPM Ac-1783) strain. This enzyme hydrolyzes substrates of pancreatic carboxypeptidases (CPA and CPB) with high efficiency and possesses wide substrate specificity, cleaving not only hydrophobic and positively charged amino acid residues but also Glu. By this criterion CPSb differs from other known carboxypeptidases including CPT and CPSG. This property and also thermal stability and the character of pH dependence of the enzyme activity recommend CPSb for use in substitution therapy.

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