An Alkaline d-Stereospecific Endopeptidase with β-Lactamase Activity from Bacillus cereus*

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We purified a novel extracellular d-stereospecific endopeptidase, alkaline d-peptidase (d-stereospecific peptide hydrolyase, EC 3.4.11.1.), to homogeneity from the culture broth of the soil bacterium Bacillus cereus strain DF4-B. The $M_r$ of the enzyme was 37,952, and it was composed of a single polypeptide chain. The optimal pH for activity was 10.3. The enzyme was strictly d-stereospecific toward oligopeptides composed of phenylalanine such as (D-Phe)$_n$ and (D-Phe)$_2$. The enzyme also acted to a lesser extent on (D-Phe)$_n$ (where Boc is tert-butoxycarbonyl), Boc-(D-Phe)$_2$, methyl ester, Boc-(D-Phe)$_2$ methyl ester, Boc-(D-Phe)$_2$ (D-Phe)$_2$, and others, but not upon their corresponding peptides composed of L-Phe (D-Ala)$_n$ ($n = 2–5$), (D-Val)$_n$ and (D-Leu)$_n$ and (D-Leu)$_2$. The mode of action of the enzyme was clarified with synthetic substrates ((D-Phe)$_n$-d-Tyr and (D-Phe)$_n$) and eight stereoisomers of (Phe)$_3$. The enzyme had $β$-lactamase activity toward ampicillin and penicillin G, although carboxypeptidase DD and $D$-aminopeptidase activities were undetectable. The gene coding for alkaline d-peptidase (adp) was cloned into plasmid pUC118, and a 1164-base pair open reading frame consisting of 388 codons was identified as the adp gene.

The predicted polypeptide was similar to carboxypeptidase DD from Streptomyces R61, penicillin-binding proteins from Streptomyces lactamdurans and Bacillus subtilis, and class C $β$-lactamases. Thus, the enzyme was categorized as a new “penicillin-recognizing enzyme.”

Some peptidases act on peptides containing $D$-amino acids. Soluble Streptomyces carboxypeptidase DD catalyzes not only the transpeptidation reaction on the peptide intermediate in peptidoglycan biosynthesis, but also the hydrolysis of $N^3,N^3$-diazetyl-L-lysyl-(D-Ala)$_n$ in water (1). A d-peptidase has been purified and characterized from an actinomycete, although it is not strictly specific toward peptides containing $D$-amino acids (2). In Enterococcus, the vanX gene product, (D-Ala)$_n$ hydrolase, plays a role in vancomycin resistance (3). The chemically synthesized “$d$-enzyme” of human immunodeficiency virus type 1, in which all of the amino acids were replaced with the corresponding $D$-amino acids, displays $d$-stereospecificity (4). We discovered $d$-aminopeptidase (EC 3.4.11.19) from Ochrobactrum anthropi and found that its primary structure is similar to the $β$-lactamases and penicillin-binding proteins (5, 6). The enzyme acts mostly on peptides with $D$-Ala at the $N$-terminus to yield $D$-amino acids and does not act on $D$-amino acid derivatives with bulkier substituents. We proposed that $d$-aminopeptidase is a new “penicillin-recognizing enzyme” (1), based on its primary structure, inhibition by $β$-lactam compounds, and the ability to catalyze peptide bond formation in organic solvents, although the enzyme does not show $β$-lactamase activity (6, 7).

In this paper, we describe the screening of soil microorganisms for $d$-stereospecific endopeptidases using a synthetic peptide (D-Phe)$_n$, characterization of the new enzyme alkaline d-peptidase (ADP), as well as cloning and sequencing of the adp gene from Bacillus cereus strain DF4-B.

EXPERIMENTAL PROCEDURES

Materials—DEAE-Toyopearl 650 M, Butyl-Toyopearl 650 M, and HPLC G-3000 SW and ODS-80Ts columns were purchased from Tosoh Corp. (Tokyo, Japan); Superdex 200 was from Pharmacia (Uppsala); and Cosmosil 5C18-MS from Nacalai Tesque (Kyoto, Japan). Membrane filters (Biolu Ultrafilter PM-30) and the Hollow Fiber cartridge system (Hollow Fiber Ultrafilter H10-P10) were obtained from Amicon, Inc. (Beverly, MA). Chemicals other than the peptides described below were from commercial sources and were used without further purification.

Synthesis of Substrates—Peptide substrates used to screen and test the substrate specificity were synthesized from $D$- and L-Phe. NH$_2$ and COOH termini were protected by Boc and methyl groups, respectively. Isobutyl chloroformate (9) and carbodiimide (10) condensed the monomer to a dimer and the dimer to a tetramer, respectively. The following peptide derivatives were synthesized: Boc-$n$-Phe, $n$-Phe tert-buty1 ester, (D-Phe)$_2$HCl, (D-Phe)$_2$, methyl ester-HCl, Boc-(D-Phe)$_2$ methyl ester, (D-Phe)$_2$HCl, Boc-(D-Phe)$_2$, methyl ester, Boc-(D-Phe)$_2$ tert-buty1 ester, (D-Phe)$_2$HCl, Boc-(D-Phe)$_2$, methyl ester-HCl, Boc-(D-Phe)$_2$ methyl ester, (D-Phe)$_2$HCl, Boc-(D-Phe)$_2$, methyl ester-HCl, (D-Val)$_n$-HCl, (D-Leu)$_n$-HCl, (D-Leu)$_2$-HCl, and (D-Leu)$_2$HCl. The details will be reported elsewhere.

Screening for (D-Phe)$_n$-degrading Microorganisms—We screened the ability of microorganisms to hydrolyze (D-Phe)$_n$ in LB medium (11) in enriched cultures at 30 °C. (D-Phe)$_n$ was dissolved in Me$_2$SO (10%, v/v) and then added to 2 ml of LB medium containing soil samples. The mixture was then aerobically shaken for 2 days. A loopful of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions.

Identification of the Microorganisms That Degraded (D-Phe)$_n$—A bacterial strain isolated from a soil of Kanagawa Prefecture, Japan completely degraded the substrate (D-Phe)$_n$ under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions. A small portion of the culture broth was transferred to the same medium and aerobically incubated under the same conditions.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL, GenBank, DDBJ, and NCBI Data Banks with accession number D86380.

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1 The abbreviations used are: ADP, alkaline d-peptidase; HPLC, high performance liquid chromatography; Boc, tert-butoxycarbonyl; kb, kilo-base pair(s); ORF, open reading frame.

2 Y. Asano, unpublished data.

This paper is available on line at http://www-jbc.stanford.edu/jbc/
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The supernatant was concentrated with an Amicon Hollow Fiber cartridge. Tris-HCl, pH 9.0, and MgSO\(_4\) and Me\(_2\)SO were omitted. The enzyme that catalyzes the formation of 2-nm. One unit of enzyme activity is defined as the amount of enzyme saturation) in 0.01M buffer. The enzyme was eluted by measuring the consumption of the substrate in a reaction mixture was added to 900 ml. After incubation at the same temperature, 100 ml of the enzyme solution in a total volume of 1 ml. After incubation at the same temperature, 100 ml of the reaction mixture was added to 900 ml of 0.1 M buffer containing 0.2 m NaCl. The active fractions were combined, dialyzed, and lyophilized.

Analytical Methods—\(^{-1}\)H NMR spectra were measured with a JEOL EX 400 apparatus. Protein was assayed, SDS-polyacrylamide gel electrophoresis, of the enzyme was estimated as described previously (6). Purified ADP (720 mg) was digested with lystyl-end peptidase (5.0 mg; Wako, Tokyo, Japan) at 30 °C for 16 h. The digest was monitored by HPLC on a reverse-phase column (ODS-80Ts) in a 10–80% linear gradient of acetonitrile containing 0.1% trifluoroacetic acid at a flow rate of 0.3 ml/min with continuous monitoring of the absorbance at 215 nm. To determine the NH\(_2\)-terminal amino acid sequence, the enzyme samples were covalently bound to Sequelon-arylamine and Sequelon-dissiorthiocyanate membranes and then analyzed with a Prosequencer 6625 automatic protein sequencer (Milligen/ Biosearch). The molecular mass of the enzyme was estimated with a PE-Sciex API III triple quadrupole mass spectrometer equipped with an ionSpray ion source in the positive ion mode (Sciex, Thornhill, Ontario, Canada).

Cloning of the \( \delta \)-Gene and Construction of \( p\)ADP1—\( B. \) cereus genomic DNA was isolated as described (6). \( E. \) coli cells were cultured in LB medium with 100 \( \mu \)g/ml ampicillin. Plasmids were purified using the QIAGEN plasmid purification kit. \( B. \) cereus genomic DNAs were partially digested with \( Mbo \)I and fractionated by sucrose density gradient ultracentrifugation (5–25%); 100,000 \( \times \) g, 16 h). DNA fragments of ~3–6 kb were purified and ligated into BamHI-digested and dephosphorylated pUC118 by T4 ligase. Ampicillin-resistant transformants expressing ADP activity were directly selected by monitoring halo formation from (p-\( \delta \)-Pepe), because the host E. coli JM109 cells did not show ADP activity. One of ~17,000 transformants exhibited ADP activity, and it harbored a plasmid designated pBDP2 with a 5-kb DNA insert. For subcloning, pBDP2 was digested with EcoRI and SacI, and the resulting 1.3-kb fragment was introduced into the EcoRI-\( \delta \) sites of pUC118 to yield pBDP2.

\( B. \) cereus genomic cDNAs were completely digested with EcoRI and SacI and then size-fractionated by sucrose density gradient ultracentrifugation to give DNA fragments of ~1.8 kb. These fragments were ligated by T4 ligase into pUC118 that had been digested with EcoRI and SacI and dephosphorylated and then introduced into E. coli JM109. Ampicillin-resistant transformants were screened with the DNA insert of pBDP2 as a probe by colony hybridization. One clone (designated pADP1) with an ~1.8-kb DNA insert was selected for further analysis. DNA Hybridization and Sequencing—Colony hybridization and Southern blot hybridization. B. cereus genomic DNA fragments were labeled using an \( \alpha \)-\( ^{32} \)P-dCTP or with an automatic gene sequencer (ALF red sequencers, Pharmacia).

RESULTS

Screening for the (p-\( \delta \)-Pepe) degrading Peptidase Producer and Its Growth Rates—\( B. \) cereus was isolated from a soil sample and considered as a likely source of the enzyme. When the strain was cultured in 400 ml of nutrient broth, the enzyme activity was detected in the culture broth, and its formation was associated with the growth of the microorganism. We harvested the supernatant at 48 h to avoid lowering the specific activity of the enzyme by cell lysis.

Purification of Alkaline \( \delta \)-Peptidase and Molecular Weight—Since the enzyme was constitutive, the substrate (p-\( \delta \)-Pepe) was omitted from the medium in large-scale culture. The enzyme was isolated from the supernatant of 140 liters of culture broth was electrophoretically pure. A summary of the purification procedure for the enzyme is shown in Table I. The enzyme was purified ~300-fold with an 8% yield.

The enzyme was judged to be homogeneous by SDS-polyacrylamide gel electrophoresis and HPLC on a TSK G-3000 SW column, as each of these procedures yielded a single band or a single peak. Fig. 1 (A and B) shows the results of SDS-polyacrylamide gel electrophoresis and the estimation of the \( M_r \) by HPLC, respectively. The \( M_r \) of the subunit calculated was...
TABLE I

Purification of ADP from the culture broth of B. cereus strain DF4-B

| Step               | Total protein mg | Total activity units | Specific activity units/mg | Yield % |
|--------------------|------------------|----------------------|-----------------------------|--------|
| Culture broth      | 22,400           | 9280                 | 0.414                       | 100    |
| Crude enzyme       | 15,600           | 6350                 | 0.407                       | 68     |
| Ammonium sulfate   | 9960             | 4860                 | 0.488                       | 52     |
| DEAE-Toyopearl (1st) | 4240       | 4830                 | 1.13                        | 52     |
| Butyl-Toyopearl (1st) | 2260       | 3730                 | 1.42                        | 40     |
| Butyl-Toyopearl (2nd) | 440         | 2230                 | 5.02                        | 24     |
| DEAE-Toyopearl (3rd) | 375         | 2200                 | 5.87                        | 24     |
| Superdex 200       | 16.8             | 1130                 | 67.3                        | 12     |
|                     | 6.15             | 768                  | 125                         | 8      |

Effect of pH and Temperature on the Enzyme Activity—The optimal pH for the activity of the enzyme was measured in several buffers at various pH values (final concentration of 0.1 M): potassium phosphate, pH 6.4–7.9; Tris-HCl, pH 7.5–9.0; ethanolamine HCl, pH 8.5–10.9; and glycine/NaCl/NaOH, pH 9.9–12.8. When the velocity of the observed maximal activity at pH 10.3 was taken as 100%, the relative activities at pH 7.1, 7.5, 7.9, 8.5, 9.0, 9.5, 9.9, 10.3, 11.0, 11.3, 11.8, and 12.3 were 7.9, 15, 30, 46, 79, 91, 93, 10, 94, 80, 76, and 10%, respectively. The enzyme activity was maximal at 45°C. About 60% activity remained after incubation at 43°C in 0.1 M potassium phosphate buffer, pH 8.0, for 10 min. No activity was lost between pH 5.0 and 10.0 after incubation at 30°C for 1 h in 0.05 M buffer at various pH values.

Substrate Specificity and Kinetic Properties—The substrate specificity of the enzyme was examined as shown in Table II. The enzyme was active toward (D-Phe)3 and (D-Phe)4, forming Boc-D-Phe, (D-Phe)2, and D-Phe. The enzyme was also active toward tripeptides with D-Tyr at the COOH or NH2 terminus and toward Boc-(D-Phe)2, (n = 2–4), forming Boc-D-Phe, (D-Phe)2, and D-Phe. The enzyme had esterase activity toward D-Phe methyl ester and (D-Phe)2 methyl ester. The products from Boc-(D-Phe)2 tert-butyl ester were Boc-D-Phe, D-Phe, and D-Phe tert-butyl ester. The enzyme was not active toward L-Phe methyl ester, (L-Phe)2 methyl ester, (L-Phe)4 methyl ester, (D-Val)2, (D-Leu)2, and (D-Ala)2 (n = 2–5). These properties indicate that the enzyme is an endopeptidase that acts β-stereospecifically upon peptides composed of aromatic D-amino acids. On the other hand, a dimer was formed when D-Phe methyl ester and D-Phe amide were the substrates. Eight stereoisomers of the phenylalanine trimer were synthesized, and their effectiveness as substrates for the enzyme was tested. The enzyme recognized the configuration of the second D-Phe of tripeptides and catalyzed the hydrolysis of the second peptide bond from the NH2 terminus. The calculated Vmax/Km values for the peptides containing L-Phe were lower than those for (D-Phe)3. The enzyme also showed β-lactamase activity toward ampicillin and penicillin G. The calculated Vmax values of the enzyme for β-lactam compounds were about the same as those for (D-Phe)3 and (D-Phe)4, while the Km values were several hundred times larger. On the other hand, carboxypeptidase DD (13) and D-aminopeptidase (6) activities were undetectable.

The following compounds were inert as substrates: (L-Phe)4, (L-Phe)3, D-Phe-L-Phe-D-Phe, L-Phe-(L-Phe)2, (L-Phe)2-D-Phe, (L-Phe)2-D-Phe, L-Phe-D-Phe, L-Phe methyl ester, L-Phe amide, Boc-(L-Phe)2, Boc-(L-Phe)2 methyl ester, L-Phe p-nitroanilide, p-Ala p-nitroanilide, p-phenylglycine amide, (D-Ala)2, (D-Ala)2, (D-Ala)2, (D-Val)2, (D-Leu)2, and DL-Ala-DL-Phe.

Effect of Metal Ions and Inhibitors—We investigated the effect of metal ions on the enzyme activity. The enzyme (30 units/ml) was dialyzed with 0.01 M potassium phosphate buffer, pH 7.0, containing 10 mM EDTA for 48 h and then diluted with the same buffer 20 times. Thereafter, 5 mM concentrations of cations were added in place of MgSO4, and the enzyme activity was assayed by the standard procedure. The enzyme activity was enhanced by Mg2+ (138%), Mo3+ (130%), and Ba2+ (123%).
We also measured the enzyme activity after incubation at 30 °C for 30 min with various compounds (at 5 mM unless otherwise noted). The activity was inhibited 94% by phenylmethylsulfonyl fluoride, 76% by Ag⁺, 74% by Fe³⁺, and 32% by Hg²⁺. The activity was not lost upon incubation with the following agents: Na⁺, K⁺, Ba²⁺, Mg²⁺, Mn²⁺, Sn²⁺, Pb²⁺, Ca²⁺, Ni²⁺, Co²⁺, Cd²⁺, Cu²⁺, Zn²⁺, Al³⁺, Fe³⁺, Cr³⁺, 5,5'-dithiobis(2-nitrobenzoic acid), hydroxyamine, N-ethylmaleimide, EDTA, EGTA, 8-oxyquinoline, 2,2'-dipyridyl, o-phenanthroline, Tiron (1,2-dihydroxybenzene-3,5-disulfonic acid), NaF, sodium azide, KCN, monooiodacetate, 2-mercaptoethanol, dithiothreitol, di-penicil-
lamine, d-cycloserine, and p-chloromercuribenzoate. These re-
sults indicate that the enzyme is a serine peptidase.

**Timecourse of (D-Phe)₄ Hydrolysis and Mode of Action of the Enzyme—**

We measured the time course of the (D-Phe)₄ degradation. As shown in Fig. 2, (D-Phe)₄ was hydrolyzed to (D-Phe)₂ and D-Phe. No (D-Phe)₃ was detected. These results coincide with the kinetic properties of the enzyme described above. The mode of action of the enzyme was examined with the synthetic substrates D-Tyr-(D-Phe)₂ and (D-Phe)₂-D-Tyr as shown in Fig. 3. When D-Tyr-(D-Phe)₂ was the substrate, D-Phe was released first, and then D-Tyr was slowly formed. When (D-Phe)₂-D-Tyr was used as the substrate, D-Tyr was released first, and then D-Phe was slowly formed. In both reactions, the second peptide bond from the NH₂ terminus of the substrate was hydrolyzed first. These results show that the enzyme acts as a D-stereospe-
cific dipeptidyl endopeptidase.

**Cloning and Nucleotide Sequencing of the adp Gene—**

**Table II**

| Substrate    | Relative activity | \( K_m \) | \( V_{max} \) | \( V_{max}/K_m \) |
|--------------|------------------|-----------|--------------|-----------------|
| (D-Phe)₆     | 1.8*             | 0.398     | 199          | 500             |
| (D-Phe)₄     | 100*             | 0.127     | 130          | 1020            |
| (D-Phe)₃     | 90*              | 50.1      | 13.7         | 0.270           |
| (D-Phe)₂     | <0.1             |           |             |                 |
| (D-Phe)₁     | 14.9*            | 0.522     | 30.6         | 59.0            |
| (D-Phe)₂     | 119*             | 0.455     | 154          | 346             |
| (D-Phe)₁     | 28.1             | 1.63      | 66.0         | 41.0            |
| (D-Phe)₂     | 83.6*            |           |             |                 |
| (D-Phe)₁     | 83.6*            |           |             |                 |
| (D-Phe)₁     | 15*              | 1.8*      | 73.1         | 3.58            |
| (D-Phe)₂     | 0.1              | 0.1*      | 48.9         | 5.11            |
| (D-Phe)₂     | 4.2*             |           |             |                 |
| (D-Phe)₂     | 1.8*             | 0.8*      |             |                 |
| (D-Phe)₂     | 3.2*             | 1.1*      |             |                 |
| (D-Phe)₂     | 7.0*             |           |             |                 |
| (D-Phe)₂     | 1.2*             | 0.3*      |             |                 |
| (D-Phe)₂     | 0.5*             | 0.2*      |             |                 |
| (D-Phe)₂     | 0.7*             | 0.9*      |             |                 |
| (D-Phe)₂     | 1.4*             |           |             |                 |
| Ampicillin   | 8.9*             | 73.1      | 262          |                 |
| Penicillin G | 9.7              | 48.9      | 250          |                 |

\* Formation of (D-Phe)₂.
\* Formation of (D-Phe)₁.
\* Formation of D-Phe.
\* Formation of Boc-D-Phe.
\* Formation of Boc-(D-Phe).
JM109 transformants expressing ampicillin resistance and ADP activity were screened by halo formation on LB agar containing (D-Phe)₄. Plasmid pBDP22 with a 1.3-kb insert conferred ADP activity in E. coli and was found to contain a lacZ-adp gene fusion, which encodes a b-galactosidase (1–12 amino acids)-ADP(250GAT to the COOH terminus) hybrid protein, by sequence analysis. Genomic Southern hybridization using the DNA insert of pBDP22 as a probe indicated that the 1.8-kb EcoRI-SalI fragment contained the full-length adp gene.

One clone (pADP1) that transformed E. coli into expressing ADP and that carried a 1.8-kb insert was further analyzed. Transcription in the plasmids appeared to be governed by an extant promoter of the adp gene because ADP activity was expressed when the direction of the transcription of the insert was opposite that of the original plasmid with pUC119. The nucleotide sequence of the 1.8-kb EcoRI-SalI fragment revealed a single open reading frame (ORF) that probably initiates at the 106ATG codon preceded by a potential ribosome-binding site (91GGAG). Translation of the ORF encoded a predicted protein of 388 amino acids with an M₉ of 42,033, with an amino acid sequence identical to those obtained by NH₂-terminal amino acid sequencing of the six peptides prepared from purified ADP as shown in Fig. 4. Considering that ADP was secreted in the culture broth and that the M₉ of the predicted ORF (42,033) was larger than those estimated by SDS-polyacrylamide gel electrophoresis and HPLC, ADP is synthesized with a signal peptide. In fact, the predicted ORF exhibits a positively charged NH₂ terminus, followed by a hydrophobic stretch with a high leucine content. This domain closely resembles those of the signal peptides of exported proteins in Bacillus species (14). The NH₂-terminal amino acid was suggested to be serine (223AGT) based on the mass spectrometry results (M₉ 37,952) with purified ADP. This ORF (223AGT to 1267AAG) encodes a protein with a calculated M₉ of 37,926, which is in agreement with those estimated by other methods. The observed difference of 26 mass units between the M₉ deduced from the primary structure and that calculated by mass spectrometry was probably caused by the formation of an oxazolidinone ring at the NH₂-terminal Ser. However, the exact molecular structure of the NH₂ terminus is not clear.

Deduced Primary Sequence and Similarities to Other Proteins—Alignment by the SWISS-PROT and NBRF-PIR data bases using the BLAST, FASTA, and DNASIS programs showed that the deduced primary structure of ADP is similar to alkaline D-Peptidase from B. cereus

Fig. 4. Nucleotide sequence and predicted amino acid sequence of the adp gene. An ORF of 1164 base pairs (388 amino acids) is shown with the deduced amino acid sequence. Underlined sequences were confirmed by the protein sequence.
**Alkaline D-Peptidase from B. cereus**

The first amino acid sequence is that of ADP from *B. cereus* (sequence size = 350 amino acids) (this study). The second amino acid sequence (DD) is that of Streptomyces R61 (sequence size = 381 amino acids) (13), identical residue; ; similarity of functional group. Ser1 of the NH2-terminal amino acid sequence of ADP corresponds to Ser3 of the enzyme was determined by mass spectrometry. The observed Ser was 37,952. Serine (223AGT), the signal peptide of ADP (39 amino acids) is identical over 346 amino acids) (13), penicillin-binding protein; BLA, β-lactamase; DAP, β-aminopeptidase.

**FIG. 5.** Comparison of the amino acid sequences of ADP from *B. cereus* and carboxypeptidase DD from *Streptomyces* R61. The first amino acid sequence is that of ADP from *B. cereus* (sequence size = 350 amino acids) (this study). The second amino acid sequence (DD) is that of Streptomyces R61 (sequence size = 381 amino acids) (13), identical residue; ; similarity of functional group. Ser1 of the NH2-terminal amino acid sequence of ADP corresponds to Ser3 of the enzyme was determined by mass spectrometry. The observed Ser was 37,952. Serine (223AGT), the signal peptide of ADP (39 amino acids) is identical over 346 amino acids) (13), penicillin-binding protein; BLA, β-lactamase; DAP, β-aminopeptidase.

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