Shimizu, T., & Hatano, M. (1985) *Inorg. Chem. 24*, 2003–2009.
Shiro, Y., & Morishima, I. (1986) *Biochemistry 25*, 5844–5849.
Sievers, G. (1979) *Biochim. Biophys. Acta 579*, 181–190.
Sievers, G. (1981) *FEBS Lett. 127* 253–256.
Ugarova, N. N., Savitski, A. P., & Berezin, I. V. (1981) *Biochim. Biophys. Acta 662*, 210–219.

Primary Structure of Paim I, an α-Amylase Inhibitor from *Streptomyces corchorushii*, Determined by the Combination of Edman Degradation and Fast Atom Bombardment Mass Spectrometry

Kazuo Hirayama,*,‡ Rei Takahashi,‡ Satoko Akashi,‡ Ken-ichi Fukuhara,‡ Naoki Oouchi,‡ Asao Murai,‡ Motoo Arai,§ Sawao Murao,∥ Kazuo Tanaka,⊥ and Ittetsu Nojima⊥

Central Research Laboratories, Ajinomoto Company, Inc., Kawasaki-ku, Kawasaki, Kanagawa 210, Japan, Department of Agricultural Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 591, Japan, Department of Applied Microbial Technology, Kumamoto Institute of Technology, Kumamoto 860, Japan, and Analytical Instruments, Technical and Engineering Division, JEOL Ltd., Akishima 196, Japan

Received December 15, 1986; Revised Manuscript Received April 2, 1987

ABSTRACT: Paim I, a protein α-amylase inhibitor, inhibits animal α-amylases from pig, dog, cow, horse, etc. but has no activity against human salivary and pancreatic amylases. The primary structure of Paim I has been determined by Edman degradation and fast atom bombardment mass spectrometry (FABMS). This protein is a single-chain polypeptide of 73 amino acid residues with a calculated molecular weight from the sequence data of 7415.3 (monoisotopic molecular weight) and 7420.2 (average molecular weight). The sequencing strategy chosen for Paim I consists of four steps. First, the accurate molecular weights of the intact and tetra-S-carboxymethylated Paim I are determined by fast atom bombardment mass spectrometry. Second, the primary fragments generated by *Staphylococcus aureus* V8 protease are isolated by reversed-phase high-performance liquid chromatography. The molecular weights of these subpeptides are determined by FABMS. The peptides that must be sequenced are selected by the molecular weights of these subpeptides and the tetra-S-carboxymethylated Paim I. Third, these subpeptides and the whole protein are sequenced by automated Edman degradation. Finally, the primary structure of tetra-S-carboxymethylated Paim I is confirmed by the combination of tryptic, chymotryptic, and *S. aureus* V8 protease digestion and FABMS. The sequence of Paim I is compared with those of Haim II, Hoe-467A, Z-2685, and AI-3688 because they have different α-amylase inhibition spectra against mammalian α-amylases but belong to a family of related proteins.

Many protein amylase inhibitors, which inhibit mammalian α-amylases specifically but have no activity against plant and microbial α-amylases, are obtained from plants and microbes (Aschauer et al., 1983; Maeda et al., 1983; Marshall & Lauda, 1975; Murao et al., 1981). These phenomena indicate that animal α-amylases have specific site(s) for protein α-amylase inhibitors in addition to binding sites for substrates, i.e., α-1,4-glucan. Therefore, elucidation of the inhibition mechanism of these inhibitors might provide important information on the characteristics of animal α-amylases. These inhibitors should be useful not only for the elucidation of active sites of animal α-amylases but also for the investigation of protein–protein interaction. In addition, the measurement of serum and urine amylase has clinical significance in the diagnosis of diseases. For this purpose, an amylase inhibitor could be used in the determination of activities of amylase isozyme (O'Donnell et al., 1977). Immobilized amylase inhibitors are also effective for purification of amylases (Buonocore et al., 1975; Burrill et al., 1981).

During the screening of amylase inhibitors of microbial origin, we isolated an inhibitor that strongly inhibited pig pancreatic α-amylase. The α-amylase inhibitor was designated Paim I (pig pancreatic α-amylase inhibitor of microbes) (Murao et al., 1983, 1985). Paim I inhibited animal α-amylases from pig, dog, cow, horse, etc. but had no activity against human salivary and pancreatic amylases (Oouchi et al., 1985; Arai et al., 1985a). This is an important characteristic of Paim I. Thus, Paim I might be useful in investigating differences between human and other animal amylases.

This paper describes the spectrum of inhibition of Paim I as compared with Haim II (Arai et al., 1985a,b; Goto et al., 1983, 1985a,b; Murai et al., 1985; Murao et al., 1980a,b), which inhibits human salivary and pancreatic amylases. Knowledge of the exact molecular weight of the protein is very useful in determining the primary structure of the protein. We report molecular weights of Paim I, tetra-S-carboxymethylated Paim I, and endopeptidase fragments of tetra-S-carboxymethylated Paim I as determined by FABMS[1] and the primary

* Address correspondence to this author.
‡ Ajinomoto Co., Inc.
§ University of Osaka Prefecture.
∥ Kunamoto Institute of Technology.
⊥ JEOL Ltd.

0006-2960/87/0426-6483$01.50/0

structure of Paim I as determined by a combination of automated Edman degradation and FABMS (Samy et al., 1983).

Materials and Methods

Paim I was prepared in the same manner as in the previous study (Oouchi et al., 1985). Dithiothreitol and iodoacetic acid were obtained from Wako Pure Chemical Industries Ltd. *Staphylococcus aureus* V8 protease was purchased from Miles Laboratories Inc. Trypsin and chymotrypsin were from Sigma Chemical Co.

Amino acid compositions were determined with a Hitachi Model 835 amino acid analyzer after hydrolysis of the protein or peptide with 6 N HCl in evacuated ampules at 110 °C for 18 h. The NH_2-terminal amino acid was determined by reaction of the protein or the peptide with 5-(dimethylamino)-naphthalene-1-sulfonyl chloride (dansyl method) (Hartley, 1970; Weiner et al., 1972).

Automated Edman sequence analyses were performed on an Applied Biosystems 470A protein sequencer. Phenylthiohydantoin derivatives of amino acids were identified by HPLC.

Mass Spectrometry. Positive-ion FAB mass spectra were recorded with a JEOL JMS-HX 110 double-focusing mass spectrometer fitted with a 23-kG high-field magnet and operating at accelerating voltages at 10 kV. The atom emission gun current of 20 mA was maintained at 6 kV for the generation of the xenon fast atom beam. The mass spectra were recorded with a JMA-DA 5000 mass data system. Samples of the underivatized peptides and proteins were prepared by dissolving ca. 20-μg samples (50 μg in the case of proteins) in ca. 1.5 μL of 1:1:2 glycerol–thioglycerol–1 M HCl or 1:1:2 glycerol–thioglycerol–29% NH_4OH (tetra-S-carboxymethylated Paim I). This solution was deposited on a stainless steel sample stage for introduction into the mass spectrometer ion source on the end of an axially mounted sample introduction probe. Spectra were recorded at resolution 1000 (M_r >5001) or 5000 (M_r <5000) by scanning the magnetic field.

Tetra-S-carboxymethylated Paim I. Two micromoles of Paim I was dissolved in 3 mL of 0.5 M Tris-HCl buffer (pH 8.2) containing 8 M urea and 0.2% EDTA. After addition of 250 μmol of dithiothreitol, the mixture was gassed with nitrogen for 10 min before it was heated for 3 h at 50 °C and then allowed to stand overnight in the dark at room temperature. To this mixture was added 500 μmol of iodoacetic acid in 0.2 mL of 1 M NaOH. Alkylation was conducted at pH 8.0 or 8.5 by addition of 1 M NaOH. After 30 min, the mixture was dialyzed against 0.2 M acetic acid and lyophilized.

Enzymatic Digestion. The tetra-S-carboxymethylated Paim I (0.5 mg) was digested under the conditions of a 50:1 molar ratio of substrate to enzyme in 50 μL of 5 mM ammonium carbonate buffer (pH 8.8). The solution was incubated at 37 °C for 8 h (*S. aureus* V8 protease), 18 h (trypsin), or 30 min (chymotrypsin). The hydrolysis was stopped by freezing and lyophilizing the solution.

Results

Effects of Paim I on Various Amylases. The inhibitory activities of Paim I and Haim II are shown in Table I. Paim I inhibited mammalian α-amylases except for human and rabbit ones. Enzymatic properties of pig pancreatic α-amylase and human salivary α-amylase are very similar in optimum pH, activation by Cl^-, hydrolysis mode of starch, hydrolysis products, amino acid composition, amino acid sequence, etc. (Thoma et al., 1971; Kluh, 1981; Nakajima et al., 1986). In spite of these similarities, Paim I distinguished between pig and human α-amylases. Paim I did not inhibit cock pancreatic α-amylase, but Haim II inhibited it strongly. Therefore, Paim I had higher or narrower inhibitory specificity than Haim II.

Table I: Effect of Paim I on Various α-Amylases[a]

enzyme (origin)	pH	inhibition[b] Paim I	Haim II
pig pancreas	7.0	+++	+++
dog pancreas	7.0	+++	+++
cow pancreas	7.0	++	+++
horse pancreas	7.0	++	+++
sheep pancreas	7.0	+	ND[c]
rabbit pancreas	7.0	–	–
human pancreas	7.0	–	+++
human saliva	7.0	–	+++
human urine	7.0	–	+++
cock pancreas	7.0	–	+++
carp pancreas	7.0	+++	+++
Cambarus alimentary tract	7.0	–	–
earthworm	7.0	–	–
bacterial liquefying	5.5	–	–
bacterial saccharifying	5.5	–	–
malt	5.5	–	–

[a] Each enzyme solution (0.5 mL) was incubated with 0.5 mL of inhibitor solution (52 μg/mL) at 37 °C for 10 min, and the residual activity was assayed. [b] Degree of inhibition: +++, 80–100%; ++, 50–80%; +, 20–50%; –, 0–10%. [c] ND = not determined.

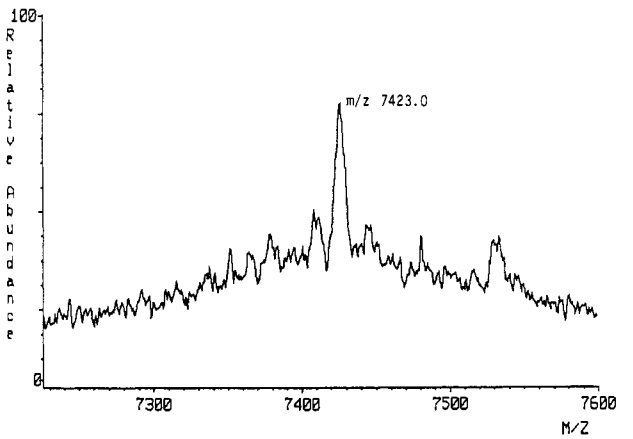

FIGURE 1: Fast atom bombardment mass spectrum of Paim I. The spectrum was recorded at resolution 1000 by scanning the magnetic field. The mass value was recorded at average mass.

Molecular Weights of Paim I and S-Carboxymethylated Paim I. The positive-ion FAB mass spectrum of Paim I is shown in Figure 1. Paim I and S-carboxymethylated Paim I gave protonated molecular ions of m/z 7423.0[2] and 7657.0[2] (Yergey et al., 1983), respectively. The mass difference of the protonated molecular ions, before and after carboxymethylation, corresponds to four carboxymethyl groups (mass 236.2). A monoisotopic molecular weight of tetra-S-carboxymethylated Paim I would be 7652.0, which is estimated from the protonated average molecular ion of m/z 7657.0. The mass difference between the observed average molecular weight of Paim I (7422.0) and the one calculated from the sequence data (7420.2) should be caused by the partially reduced disulfide bonds (Buko & Fraser, 1985; Yazdanparast et al., 1986).

[1] Abbreviations: FABMS, fast atom bombardment mass spectrometry; HPLC, high-performance liquid chromatography; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

[2] Spectra were recorded at resolution 1000 by scanning the magnetic field. Mass values were recorded at average mass.

Table II: Amino Acid Composition of Peptide S3-2

amino acid	amino acid analysis[a]	sequence
Thr	3.7	4
Glu	1.2	1
Gly	4.0	4
Ala	3.2	3
Val	2.2	2
Leu	1.2	1
Pro	3.2	3
CmCys	0.7	1
total	19.4	19

[a] This amino acid composition was calculated on the basis of 19 residues per mole of S3-2.

Isolation and Molecular Weight Determination of Endopeptidase Peptides. The larger peptides from tryptic and *S. aureus* V8 protease digests of tetra-S-carboxymethylated Paim I were purified by HPLC on a TSK gel ODS-120T column. The molecular weights of isolated peptides were determined by FABMS. N-Terminal amino acids of these peptides were determined by the dansyl method. Tryptic peptides T1 and T2 gave a monoisotopic molecular weight of 1852.8 (Ala:N-terminal amino acid) and an average molecular weight of 5820.5[2] (Tyr). *S. aureus* V8 protease fragments S1, S2, S3, and S4 gave molecular weights of 1946.8 (Gly), 1423.7 (Ala), 4317.1 (Ser), and 1427.7 (N-terminal amino acid could not be determined by the dansyl method). The calculated monoisotopic molecular weight of tetra-S-carboxymethylated Paim I from S1, S2, and S3 [1946.8 + 1423.7 + 4317.1 − (2 × 18) = 7651.6] agreed well with the estimated monoisotopic molecular weight of tetra-S-carboxymethylated Paim I by FABMS (7652.0). Peptides S1, S2, and S3 were chosen for sequencing by automated Edman degradation.

Sequence of S. aureus V8 Protease Peptides. Automated Edman degradation of tetra-S-carboxymethylated Paim I revealed the sequence up to the 28th cycle ending with valine-28. The amino acid sequence in this region is

```
1                                   10
Ala-Ser-Glu-Pro-Ala-Pro-Ala-CmCys-Val-Val-Met-Tyr-Glu-Ser-
                    20                                  28
Trp-Arg-Tyr-Thr-Thr-Ala-Ala-Asn-Asn-CmCys-Ala-Asp-Thr-Val-
```

Peptide S2 corresponds to Ala-1–Glu-13 in this sequence on the basis of its molecular weight, N-terminal amino acid, and *S. aureus* V8 protease specificity.

The Edman degradation of peptide S3 revealed the sequence up to the 25th cycle:

```
14                  20
Ser-Trp-Arg-Tyr-Thr-Thr-Ala-Ala-Asn-Asn-CmCys-Ala-Asp-
          30                                  38
Thr-Val-Ser-Val-Ser-Val-Ala-Tyr-Gln-Asp-Gly-Ala-
```

The first 15 in sequence from the N-terminus of S3 could be overlapped with the 14–28 sequence of tetra-S-carboxymethylated Paim I, so the numbering was assigned as shown above for the S3 fragment. It is revealed that the order of three fragments of *S. aureus* V8 protease digests (S1, S2, and S3) is S2–S3–S1. Since the calculated monoisotopic molecular weight of the peptide corresponding to Ser-14–Asp-36 is 2579.1, it is expected that the peptide (Gly-37 to the S3 C-terminus) whose monoisotopic molecular weight is 1756.0 (observed monoisotopic molecular weight of S3, 4317.1, minus 2579.1 plus molecular weight of H_2O) would be produced by the further digestion of S3 by *S. aureus* V8 protease. After digestion by *S. aureus* V8 protease at 37 °C for 24 h (pH 8.8), the COOH-terminal peptide S3-2 from S3 was purified by HPLC. It contained 19 amino acids (Table II) and showed glycine at the NH_2 terminus. A FAB mass spectrum of S3-2

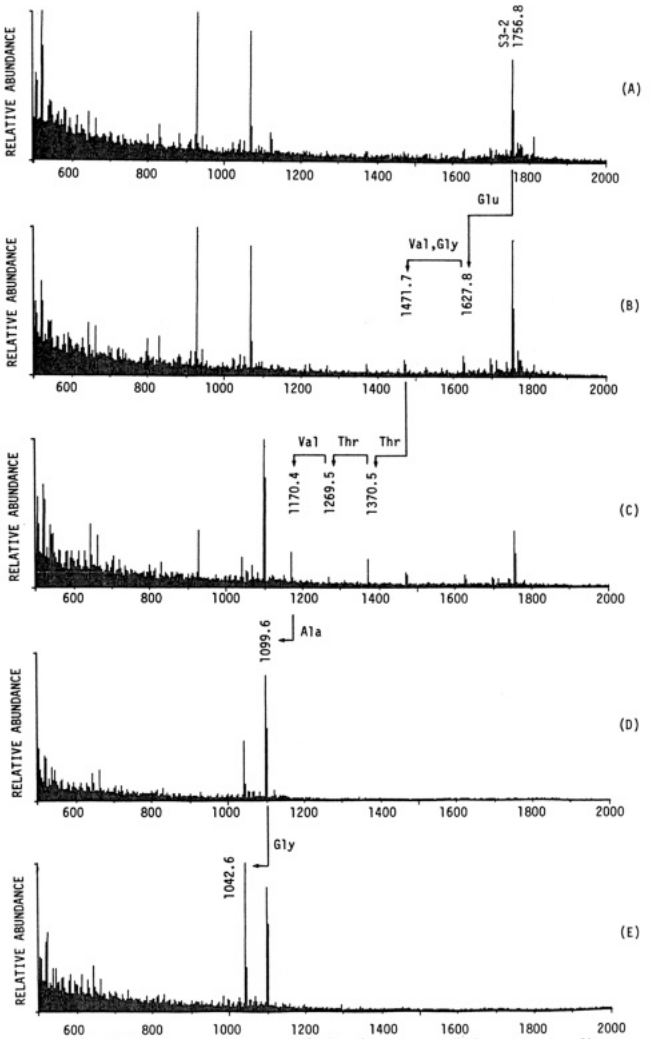

FIGURE 2: FAB mass spectra of the *S. aureus* V8 protease digests of S3 (A) and of the digests with carboxypeptidase Y [(B) 1 min, (C) 10 min, (D) 1 h, and (E) 2 h].

gave a monoisotopic molecular weight of 1755.8. Thus, it is concluded that the peptide S3-2 should be assigned to the Gly-37 to C-terminus fragment of peptide S3. Automated Edman degradation of this peptide revealed the sequence up to the 18th cycle, ending at Gly-54. The sequence of the peptide is

```
37          40
Gly-Ala-Thr-Gly-Pro-CmCys-Ala-Thr-Leu-Pro-Pro-Gly-
     50          55
Ala-Val-X-Y-Val-Gly-Z
```

Amino acid composition of S3-2 showed four threonine residues and one glutamic acid residue. On the basis of *S. aureus* V8 protease specificity, the C-terminus of this peptide must be glutamic acid. Accordingly, unassigned positions 51 (X) and 52 (Y) must be occupied by two threonine residues.

In order to confirm the amino acid sequence of this peptide, a peptide mixture of *S. aureus* V8 protease digests of S3 was subjected to carboxypeptidase Y digestion (Hirayama et al., 1986). After treatment with carboxypeptidase Y, peptide S3-2 gave mass peaks at m/z 1627.8, 1471.7, 1370.5, 1269.5, 1170.4, 1099.6, and 1042.6 on the FAB mass spectra shown in Figure 2. The results indicated that the C-terminal sequence of S3-2 was -Gly-Ala-Val-Thr-Thr-(Val,Gly)-Glu.

The largest of the *S. aureus* V8 protease fragments S3-(14–55) is shown in Figure 3.

The sequence of S1 (COOH-terminal *S. aureus* V8 protease fragment of tetra-S-carboxymethylated Paim I) was deter-

```
 14                    20                                  30
Ser-Trp-Arg-Tyr-Thr-Thr-Ala-Ala-Asn-Asn-Cys-Ala-Asp-Thr-Val-Ser-Val-Ser-Val-Ala-

                      40                                  50
Tyr-Gln-Asp-Gly-Ala-Thr-Gly-Pro-Cys-Ala-Thr-Leu-Pro-Pro-Gly-Ala-Val-Thr-Thr-Val-

     55
Gly-Glu
```

FIGURE 3: Amino acid sequence of the *S. aureus* V8 protease fragment S3.

Table III: Peptides Generated by Enzymatic Cleavage of the Tetra-S-carboxymethylated Paim I[a]

peptide	obsd mass value	theor mass value	matching section (based on enzyme specificity) of
T1	1853.8	1853.8	Ala-1–Arg-16
T2	5820.5[b]	5820.3[b]	Tyr-17–Ser-73
C1	1295.7	1295.6	Ala-1–Tyr-12
C2	1744.8	1744.8	Thr-18–Tyr-34
C3	3929.2	3928.8	Gln-35–Ser-73
C4	1111.7	1111.5	Leu-58–Leu-67
C5	1727.9	1727.8	Leu-58–Ser-73
C6	635.3	635.3	Ala-68–Ser-73
S1	1947.8	1947.9	Gly-56–Ser-73
S2	1424.7	1424.6	Ala-1–Glu-13
S3	4318.1	4317.9	Ser-14–Glu-55
S3-2	1756.8	1756.8	Gly-37–Glu-55
S4	1428.7	1428.6	His-61–Ser-73
S5	2580.2	2580.1	Ser-14–Asp-36
S6	2806.1	2806.3	Thr-27–Glu-55
S7	1068.5	1068.5	Thr-27–Asp-36

[a] A computer program was used to generate all possible peptides and their corresponding molecular weights from a given amino acid sequence. In some cases, more than one possibility existed for a particular value and enzyme specificity was used to assign the peptide. [b] See footnote 2.

mined with Edman degradation and FABMS. Automated Edman degradation of the fragment established the sequence of all 18 amino acids shown below. A FAB mass spectrum of S1 gave a monoisotopic molecular weight of 1946.8, in agreement with the calculated value (1946.9) expected from the sequence.

```
 56              60
Gly-Tyr-Leu-Gly-Glu-His-Gly-His-Pro-Asp-His-Leu-
                70      73
Ala-Leu-CmCys-Pro-Ser-Ser
```

In order to confirm the C-terminal amino acid of the protein, tetra-S-carboxymethylated Paim I was subjected to carboxypeptidase Y digestion. After treatment with carboxypeptidase Y, tetra-S-carboxymethylated Paim I gave an average mass peak at m/z 7570.2[2] on the FAB mass spectrum. It is indicated that the C-terminal amino acid of Paim I is Ser; therefore, S1 must be the C-terminal peptide of Paim I.

Confirming the Primary Structure of the Protein by FABMS.[3] The FAB mass spectrum of the *S. aureus* V8 protease digest indicates the presence of at least eight peptides, four of which are apparent in the partial spectrum shown in Figure 4. The eight *S. aureus* V8 protease peptides could be matched with the sequence shown in Figure 5 on the basis of their molecular weights and enzyme specificity (Table III). The positive-ion FAB mass spectrum of the chymotryptic digests of tetra-S-carboxymethylated Paim I gave at least six peptides. The six chymotryptic peptides (C1 to C6) fit the

[3] A computer program was used to generate all possible peptides and their corresponding molecular weights from a given amino acid sequence. In some cases, more than one possibility existed for a particular value and enzyme specificity was used to assign the peptide.

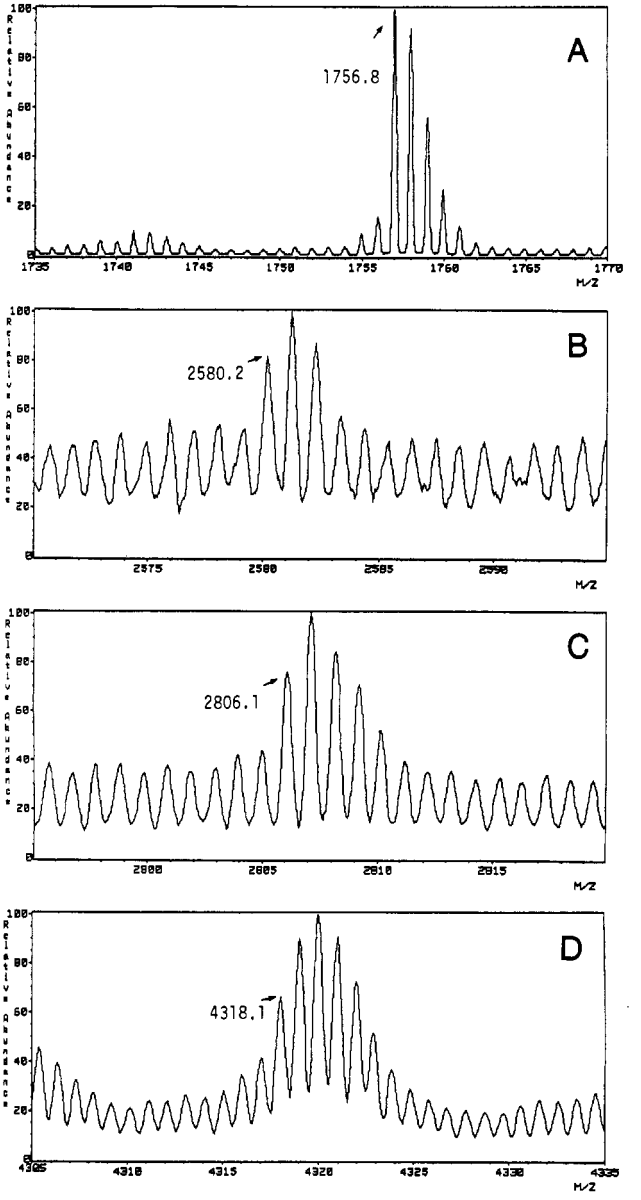

FIGURE 4: FAB mass spectra of the *S. aureus* V8 protease digests of tetra-S-carboxymethylated Paim I: (A) S3-2; (B) S5; (C) S6; (D) S3.

sequence shown in Figure 5 on the basis of their molecular weights and enzyme specificity (Table III).

Furthermore, the two tryptic peptides T1 and T2 were matched with the sequence shown in Figure 5 on the basis of their molecular weights and trypsin specificity (Table III).

The amino acid composition is shown in Table IV, and it is in agreement with that expected from the amino acid sequence.

Discussion

The mold *Streptomyces* has been the source of several low molecular weight proteins that exhibit α-amylase inhibitor activity in Mammalia. They include Haim II, Hoe-467A

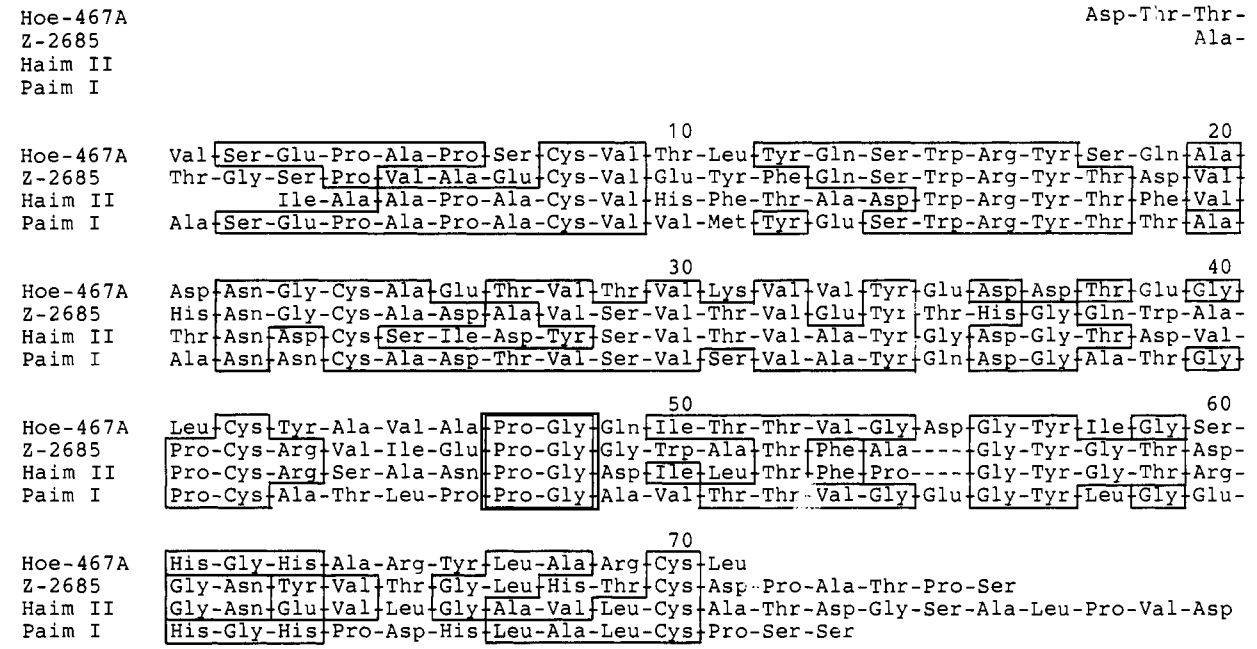

FIGURE 5: Amino acid sequences of Z-2685, Hoe-467A, Haim II, and Paim I. Boxed regions represent sequence homologies.

Table IV: Amino Acid Composition of Tetra-S-carboxymethylated Paim I

amino acid	amino acid analysis[a]	sequence
Asp	5.4	3
Asn		2
Thr	6.8	7
Ser	5.5	6
Glu	5.0	4
Gln		1
Pro	6.9	7
Gly	7.1	7
Ala	11.3	11
Val	6.8	7
Met	0.8	1
Leu	4.1	4
Tyr	3.5	4
His	3.0	3
Arg	0.8	1
Trp[b]	0.8	1
CmCys	3.9	4
total	71.7	73

[a] This amino acid composition was calculated on the basis of 73 residues per mole of tetra-S-carboxymethylated Paim I. [b] Trp was determined after hydrolysis with methanesulfonic acid.

(Aschauer et al., 1981, 1983; Vertesy et al., 1984), Z-2685 (Hofmann et al., 1985), AI-3688 (Vertesy & Tripier, 1985), and Paim I. Since these five proteins inhibit various mammalian α-amylases, it is expected that they have the same amino acid sequence in the active site for mammalian α-amylases. A comparison of the sequence of amino acids in Paim I, Haim II, Hoe-67A, and Z-2685, shown in Figure 5, suggests that a considerable homology exists among these proteins, in spite of the difference in their amino acid composition. Particularly noteworthy are the locations and spacings of the four half-cystine residues and complete agreement of -Trp-Arg-Tyr-17. The similarity of four half-cystine residues would mean the resemblance of their tertiary structure.

The complete backbone of Hoe-467A was drawn for the peptide segments involved in two β-strands and hairpin tight turns by using ^{1}H NMR techniques of Kline (Kline & Wüthrich, 1985; Kline et al., 1986) and X-ray diffraction methods of Pflugrath (Pflugrath et al., 1986). The fact that at least one each of the tyrosine, arginine, and tryptophan residues is essential for α-amylase inhibition activity of Haim II (Arai et al., 1985b) and that one hairpin tight turn contains -Trp-Arg-Tyr-17 strongly suggests that the region is an active site of these proteins.

Animal α-amylases from pig, dog, cow, and horse are inhibited not only by Haim II but also by Paim I. On the other hand, human salivary and pancreatic α-amylases are inhibited by Haim II but are not inhibited by Paim I. Accordingly, Haim II and Paim I should have different amino acid sequences in the binding sites for α-amylases. In Figure 5 Paim I and Haim II have quite different amino acid sequences at Gly-58–Val-68 (Paim I) and Leu-58–Ala–68 (Haim II). It is suggested that these regions might include the binding sites for α-amylases.

The primary structure of Paim I was determined by FABMS in conjunction with the conventional Edman degradation method. In particular, FABMS was used in the early stage of this work to determine the molecular weight of the protein. The combination of techniques employed in our investigation is able to determine the primary structure of proteins more rapidly and reliably than any single method.

ACKNOWLEDGMENTS

We thank Professors H. Matsuda and T. Matsuo (Institute of Physics, College of General Education, Osaka University) for much helpful discussion throughout this work, T. Seino, S. Ozawa, and H. Morimoto for operating the amino acid analyzer, and D. Ejima for operating the automated Edman degradation system.

REFERENCES

Arai, M., Oouchi, N., & Murao, S. (1985a) *Agric. Biol. Chem. 49*, 987–991.

Arai, M., Oouchi, N., Goto, A., Ogura, S., & Murao, S. (1985b) *Agric. Biol. Chem. 49*, 1523–1524.

Aschauer, H., Vĕrtesy, L., & Braunitzer, G. (1981) *Hoppe-Seyler's Z. Physiol. Chem. 362*, 465–467.

Aschauer, H., Vĕrtesy, L., Nesemann, G., & Braunitzer, G. (1983) *Hoppe-Seyler's Z. Physiol. Chem. 364*, 1347–1356.

Buko, A. M., & Fraser, B. A. (1985) *Biomed. Mass Spectrom. 12*, 577–585.

Buonocore, V., Poerio, E., Gramenzi, F., & Silano, V. (1975) *J. Chromatogr. 114*, 109-114.
Burrill, P. H., Brannon, P. M., & Kretchmer, N. (1981) *Anal. Biochem. 117*, 402-405.
Goto, A., Matsui, Y., Ohyama, K., Arai, M., & Murao, S. (1983) *Agric. Biol. Chem. 47*, 83-88.
Goto, A., Matsui, Y., Ohyama, K., Arai, M., & Murao, S. (1985a) *Agric. Biol. Chem. 49*, 435-439.
Goto, A., Arai, M., & Murao, S. (1985b) *Nippon Nogei Kagaku Kaishi 59*, 411-417.
Hartley, B. S. (1970) *Biochem. J. 119*, 805-822.
Hirayama, K., Ando, T., Takahashi, R., & Murai, A. (1986) *Bull. Chem. Soc. Jpn. 59*, 1371-1378.
Hofmann, O., Vértesy, L., & Braunitzer, G. (1985) *Biol. Chem. Hoppe-Seyler 366*, 1161-1168.
Kline, A. D., & Wüthrich, K. (1985) *J. Mol. Biol. 183*, 503-507.
Kline, A. D., Braun, W., & Wüthrich, K. (1986) *J. Mol. Biol. 189*, 377-382.
Kluh, I. (1981) *FEBS Lett. 136*, 231-234.
Maeda, K., Hase, T., & Matsubara, H. (1983) *Biochim. Biophys. Acta 743*, 52-57.
Marshall, J. J., & Lauda, C. M. (1975) *J. Biol. Chem. 250*, 8030-8037.
Murai, H., Hara, S., Ikanaka, T., Goto, A., Arai, M., & Murao, S. (1985) *J. Biochem. (Tokyo) 97*, 1129-1133.
Murao, S., Goto, A., Matsui, Y., & Ohyama, K. (1980a) *Agric. Biol. Chem. 44*, 1679-1681.
Murao, S., Goto, A., & Arai, M. (1980b) *Agric. Biol. Chem. 44*, 1683-1684.
Murao, S., Goto, A., Matsui, Y., Ohyama, K., & Arai, M. (1981) *Agric. Biol. Chem. 45*, 2599-2604.
Murao, S., Oouchi, N., Goto, A., & Arai, M. (1983) *Agric. Biol. Chem. 47*, 453-454.
Murao, S., Oouchi, N., Goto, A., & Arai, M. (1985) *Agric. Biol. Chem. 49*, 107-110.
Nakajima, R., Imanaka, T., & Aiba, S. (1986) *Appl. Microbiol. Biotechnol. 23*, 355-360.
O'Donnell, M. D., FitzGerald, O., & McGeeney, K. F. (1977) *Clin. Chem. (Winston-Salem, N.C.) 23*, 560-566.
Oouchi, N., Arai, M., & Murao, S. (1985) *Agric. Biol. Chem. 49*, 793-797.
Pflugrath, J. W., Wiegand, G., & Huber, R. (1986) *J. Mol. Biol. 189*, 383-386.
Samy, T. S. A., Hahm, K.-S., Modest, E. J., Lampman, G. W., Keutmann, H. T., Umazawa, H., Herlihy, W. C., Gibson, B. W., Carr, S. A., & Biemann, K. (1983) *J. Biol. Chem. 258*, 183-191.
Thoma, J. A., Spradlin, J. E., & Dygert, S. (1971) *Enzymes (3rd Ed.) 5*, 115.
Vértesy, L., & Tripier, D. (1985) *FEBS Lett. 185*, 187-190.
Vértesy, L., Oeding, V., Bender, R., Zepf, K., & Nesemann, G. (1984) *Eur. J. Biochem. 141*, 505-512.
Weiner, A. M., Platt, T., & Weber, K. (1972) *J. Biol. Chem. 247*, 3242-3251.
Yazdanparast, R., Anderews, P., Smith, D. L., & Dixon, J. E. (1986) *Anal. Biochem. 153*, 348-353.
Yergey, J., Heller, D., Hansen, G., Cotter, R. J., & Fenselau, C. (1983) *Anal. Chem. 55*, 353-356.

Amide Proton Exchange in the α-Amylase Polypeptide Inhibitor Tendamistat Studied by Two-Dimensional ^{1}H Nuclear Magnetic Resonance†

Wang Qiwen,‡ Allen D. Kline, and Kurt Wüthrich*
Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule Zürich-Hönggerberg, CH-8093 Zürich, Switzerland
Received March 27, 1987

ABSTRACT: The individual amide proton exchange rates in Tendamistat at pH 3.0 and 50 °C were measured by using two-dimensional ^{1}H nuclear magnetic resonance. Overall, it was found that the distribution of exchange rates along the sequence is dominated by the interstrand hydrogen bonds of the β-sheet structures. The slowly exchanging protons in the core of the two β-sheets were shown to exchange via an EX2 mechanism. Further analysis of the data indicates that different large-scale structure fluctuations are responsible for the exchange from the two β-sheets, even though the three-dimensional structure of Tendamistat appears to consist of a single structural domain.

Tendamistat (HOE-467) is an effective polypeptide inhibitor of mammalian α-amylases (Aschauer et al., 1981; Vértesy et al., 1984). Its binding activity appears to be related to the presence of a specific sequence of Trp-Arg-Tyr (Murai et al., 1985; Vértesy & Tripier, 1985). The conformation of this peptide segment as well as the overall backbone fold of Tendamistat was determined independently by X-ray diffraction in single crystals (Pflugrath et al., 1986) and by nuclear magnetic resonance (NMR)[1] in solution (Kline & Wüthrich, 1985; Kline et al., 1986). These features of the protein coincide closely in the crystal and in solution, with the polypeptide backbone forming an antiparallel β-barrel of six strands. In this paper, the investigations on Tendamistat are extended to

† This work was supported by the Schweizerischer Nationalfonds (Project 3.198-0.85).
‡ Permanent address: Shanghai Institute of Organic Chemistry, Academia Sinica, Shanghai, China.

[1] Abbreviations: NMR, nuclear magnetic resonance; 2D, two dimensional; COSY, 2D correlated spectroscopy; 2QF-COSY, two-quantum-filtered COSY; NOESY, 2D nuclear Overhauser enhancement spectroscopy.

0006-2960/87/0426-6488$01.50/0