
The polypeptide consists of 262 amino acid residues. One of the two disulfide loops links Cys-101 and Cys-104 and the other Cys-173 and Cys-209. Two carbohydrates side chains are attached at Asn-18 and Asn-160.

A variety of DNases have been found in different tissues and species. DNase I, the first-discovered DNase, has a pH optimum near 7, an obligatory requirement for divalent cations, and produces 5'-phosphoryl nucleotides on hydrolysis of DNA (1). Bovine pancreatic DNase is the most studied of the DNases and its three-dimensional structure has recently been revealed (5) at 2.5 Å resolution. For a better understanding of the structure-function relationship, as well as evolution of an enzyme, a comparison of the structures of the enzyme from various sources is useful. Thus, this paper and the accompanying communication (6) describe the investigation and comparison of the primary structures of bovine, ovine, and porcine pancreatic DNases. Among the three DNases, porcine is the most difficult to purify. Herein, we also describe a procedure that permits us to obtain a homogeneous form of porcine DNase for sequence analysis.

EXPERIMENTAL PROCEDURES AND RESULTS

The procedure for purification of ovine pancreatic DNase (25) was not entirely applicable to purification of porcine DNase because a drastic loss of activity resulted in the first several steps and the enzyme remained nonhomogeneous throughout the procedure. We therefore modified the procedure to include a BaSO4 precipitation step and a Blue Dextran-Sepharose step and to exclude the gel filtration step. A summary of the purification steps is shown in Table I. Blue Dextran-Sepharose has been used as a purification medium for many nucleotide binding enzymes (27, 28), including porcine spleen acid DNase (29). As indicated in Table I, porcine pancreatic DNase with a specificity for nucleotides also binds Blue Dextran-Sepharose and this Blue Dextran-Sepharose step results in 10-fold purification.

There were no significant differences between bovine and porcine DNases when the effects of pH and divalent cations on activity were tested. The specific activity was increased by approximately 10-fold when the pH of the assay solution was raised from 5.0 to 7.0. Both enzymes were most active in the presence of Mn2+ as a single cation and were stimulated by Ca2+. However, the specific activity of porcine DNase is only 650 units/A280 (Table I) as compared with 1000 units/A280 of bovine (9) or ovine (25) DNase. This difference is not due to the higher molar absorption coefficient of porcine DNase at 280 nm. In fact, as compared with bovine (4) or ovine (6) DNase, porcine DNase has the same number of tryptophans and three less tyrosines. Thus, on a mole basis, the specific activity of porcine DNase is 60% of that of bovine or ovine DNase.

The proposed amino acid sequence of porcine DNase and the logic of derivation of the sequence are shown in Fig. 6.
The derivation of the sequence is based on sequence data from manual Edman degradation of short peptides and the results of automated Edman degradation of the intact protein and of the two largest CNBr fragments. The sequence was determined using approximately 500 nmol of the protein as compared with over 50 pmol used in the sequencing of bovine DNase (3, 4).

The amino acid compositions in Table II show that Peptides Cb2 and Cb4 each contain two Cys/mol. Since the four CNBr peptides were obtained from the protein with the disulfide bridges intact, there must be one disulfide loop each in Peptides Cb2 and Cb4. Therefore, the first disulfide loop connects Cys-101 and Cys-104, and the second Cys-173 and Cys-209 (Fig. 6). The two Cys(Cm)-containing peptides (Tr2, Tr3, see Table III), derived from the tryptic digest of the reduced and S-carboxymethylated DNase, provide the positioning of the 4 half-cystine residues.

The presence of two different glycopeptides (Tr1 and Tr2, see Table III) in the tryptic digest of the reduced and S-carboxymethylated porcine DNase indicates that there are two different carbohydrate attachment sites per molecule of DNase. Although, among the four CNBr fragments, only Peptide Cb2 is a glycopeptide (Table I), there are actually two tryptic glycopeptides (Peptides Cb2-Tr1 and Cb2-Tr8, see Table III) and two chymotryptic glycopeptides (Peptides Cb2-Ch1 and Cb2-Ch8, see Table IV) within Peptide Cb2. Thus, in contrast to one carbohydrate attachment site each in bovine (4) and ovine (6) DNases, porcine DNase has two. The first carbohydrate attachment site is established using the glycopeptide (Cb2-Tr1-Thr) containing 4 amino acid residues (Ser, Asx, Ala, Thr), isolated from the thermolytic digest of Peptide Cb2-Tr1 (Table III). In the first and third cycles of manual Edman degradation, the identified PTH-derivatives were PTH-Ser and PTH-Ala, respectively; free Thr was detected in the aqueous phase after the third cycle (Table III). The unidentified PTH in the second cycle must be from the Asx residue with attached carbohydrate side chain, which was not extracted by the organic solvent used in the Edman degradation procedure. Thus, the 2nd residue of this glycotetrapeptide is an Asn with a carbohydrate side chain. This, in

The abbreviations used are: PTH-, phenylthiohydantoin; HPLC, high performance liquid chromatography; TFA, trifluoroacetic acid.
Amino Acid Sequence of Porcine DNase

...turn, based on the position of Peptide Cb2-Tr1-Th1 in the intact polypeptide of porcine DNase (Fig. 6), places the first carbohydrate side chain at Asn-18, the same position as those of bovine (4) and ovine (6) DNases.

The second carbohydrate attachment site is found in Peptide Tr2-Pr2, another glycotetrapeptide (Gly, Asx2, Thr), isolated from a Pronase digest of Peptide Tr-2. As shown in Table III, manual Edman degradation of Peptide Tr2-Pr2 indicates that the 1st residue is Gly and the 3rd Asp, with the 2nd and 4th unidentified. Since mild acid hydrolysis of Peptide Tr2 has a cleavage site between Asp and Thr (Table III) and Thr is the NH₂ terminus of the next nonglycosylated peptide (Ac5), the 4th residue in Peptide Tr2-Pr2 is assigned to Thr. By difference, the unidentified 2nd residue in Peptide Tr2-Pr2 must be the Ness-carrying carbohydrate. From the position of Peptide Tr2-Pr2 in the intact polypeptide (Fig. 6), the second carbohydrate attachment site in porcine DNase is placed at Asn-106.

REFERENCES


Amino Acid Sequence of Porcine DNase

Table II

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<tr>
<th>Infant Theme</th>
<th>OCH Peptide</th>
<th>OCH Peptide</th>
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The compositions from amino acid analyses are expressed as residues/mole. Values of less than 0.5 are omitted. The numbers in parentheses are the compositions based on the determination of the peptides. The sequence of the infant peptide is also determined (Table I) so that the sequence is consistent with 10 residues.

Fig. 4. HPLC profiles of the tryptic and chymotryptic fragments of Peptide OCH1 and the tryptic digest of Peptide OCH1. One fourth of Peptide I or Peptide II (Fig. 2) was isolated by Affigel 15 and digested with chymotrypsin (5 μg) or trypsin (5 μg). The digested peptides were analyzed by reverse-phase HPLC. Peptide I and II were identified by Edman degradation.

Fig. 5. Profiles illustrating isolation of the trypsin peptides derived from the tryptic digest and purification of the major trypsin peptide. Amino acid compositions and chromatographic properties of the peptides are shown in Table I. Peptide OCH1 was collected, analyzed, and purified by reverse-phase HPLC on a C18 column. Peptides were identified by Edman degradation.

4) The data are the average of four analyses.
5) Determined by the amino acid analyses and other factors and isolation data.
6) These values were obtained from analysis of the non-hydrolyzed

7) Determined by the method of Wurtz et al. (19).
Table III
Amino Acid Sequence of Porcine DNase

<table>
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<tr>
<th>Peptide</th>
<th>Amino Acid Sequence</th>
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<td>PII-Trb</td>
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The amino acid sequence of PII-Trb is shown in Table III.

sequence data of Peptide PII-Trb are shown in Table III.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino Acid Sequence</th>
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The amino acid sequence of PII-Trb is shown in Table III.

sequence data of Peptide PII-Trb are shown in Table III.
Amino Acid Sequence of Porcine DNase

### Table IV

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<td>Cycle 1, 2, 0.9, 1.0</td>
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<tr>
<td>C46-098</td>
<td>Asp-Thr-Val-Leu-Ile-Pro</td>
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### Table V

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Table IV

Sequence determination of the synthetic peptides

For comparison, refer to the peptide under Table III for isolation of peptides, refer to Fig. 3 and 4.

* Refer to Table III for the residues Ile at the N-terminus of C46-097.

### Table VI

<table>
<thead>
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Table V

Some properties of the synthetic peptides

The sequences are expressed as single-letter codes. For comparison of peptides, refer to the Table III for isolation of peptides, refer to Fig. 3 and 4.