Comparison of the Three Primary Structures of Deoxyribonuclease Isolated from Bovine, Ovine, and Porcine Pancreas

DERIVATION OF THE AMINO ACID SEQUENCE OF OVINE DNase AND REVISION OF THE PREVIOUSLY PUBLISHED AMINO ACID SEQUENCE OF BOVINE DNase*

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Based on the published bovine DNase sequence (Liao, T.-H., Salnikow, J., Moore, S., and Stein, W. H. (1973) J. Biol. Chem. 248, 1489-1495), the ovine DNase sequence is derived from the amino acid compositions of isolated short peptides covering all regions of the intact polypeptide. The sequence is substantiated by results of automated Edman degradation of the intact polypeptide and of the two middle CNBr fragments, and by elucidation of the complete sequence of the COOH-terminal CNBr peptide. The 12 changes from bovine to ovine DNase are at residues 22 (Ala to Ser), 29 (Val to Leu), 35 (Val to Ala), 54 (Tyr to Asp), 62 (Thr to Ser), 83 (Leu to Val), 121 (His to Pro), 127 (Glu to Ala), 132 (Ala to Pro), 159 (His to Asp), 163 (Val to Ile), and 231 (Ala to Val). A minor genetic variant form of ovine DNase has Val at residue 163.

The data from automated Edman degradation of the largest CNBr peptide of bovine DNase show that the published bovine DNase sequence is in error and that an Ile-Val-Arg tripeptide must be inserted between Arg-27 and Arg-28. The corrected sequence is substantiated by two peptides covering this region each with three amino acids more than the published sequence.

Comparison of the bovine, ovine, and porcine DNase sequences reveals the following: 1) with the revised bovine sequence, all three DNase sequences can be aligned without a gap; 2) all three DNases have a carbohydrate side chain at Asn-106; 3) there are 12 changes between bovine and ovine DNases, 56 between bovine and porcine, and 50 between ovine and porcine; 4) there are six highly variable regions and four invariant ones; 5) bovine and ovine DNases have the same length while porcine DNase is longer by 2 amino acid residues at the COOH terminus; 6) the residues around the nucleotide-binding site, the four pairs of salt bridges, and the essential His-134 groups are not changed.

Physical and chemical data on a homogeneous preparation of ovine pancreatic DNase (1) indicate that the primary structure of ovine DNase is homologous to the known sequence of bovine DNase (2, 3), probably with only a few changes, because the two proteins: 1) have equal molecular weights, 2) have very similar amino acid compositions, and 3) are identical at the NH₂ and COOH termini. When the amino acid compositions of these two closely related proteins are compared, 6 amino acid residues are probably subject to change as suggested by Wadano et al. (1). Herein, we describe the elucidation of the primary structure of ovine DNase by locating the exact positions of these changes.

Because the porcine DNase structure has also been elucidated as shown in the preceding paper (4), it is now possible to compare the sequences of three DNases from three different animal species. However, when the three sequences are aligned, there is a gap of 3 amino acid residues between Arg-27 and Arg-28 in bovine DNase. Herein also, evidence is provided to show that the previously published amino acid sequence of bovine DNase (2, 3) is in error and that a tripeptide must be inserted in the gap.

EXPERIMENTAL PROCEDURES

RESULTS

Sequence of Ovine DNase Based on Sequence Homology with Bovine DNase—Our approach to the amino acid sequence of ovine DNase was to isolate all the short peptides derived from the intact polypeptide of ovine DNase and compare the amino acid compositions of these short peptides with the compositions of the corresponding peptides in the known sequence of bovine DNase (2, 3). The compositions of these short peptides must be accurate so that any changes between the two proteins can be detected. Regions of identical sequences will result in identical compositions, while regions with changed amino acid residues can be identified by the compositional differences. Isolation of the short peptides derived from the CNBr fragments (Fig. 1) of ovine DNase by tryp tic, chymotryptic, or thermolytic digestions is as shown.
The sequence of bovine DNase is that of Liao et al. (3) with the revision as described in the text. The numbering of residues is according to the revised sequence. The sequence of porcine DNase is from the preceding paper (4), and the sequence of ovine DNase is derived as described in the text. The dashed lines indicate that the residues are the same as those in bovine DNase.

in Figs. 2–4, using gel filtration and HPLC. From the compositions of these short peptides (Table I), an amino acid sequence of ovine DNase is predicted (Fig. 5) based on the published sequence of bovine DNase (2, 3). The following results will further support this predicted sequence.

Partial Sequence Analysis of Ovine DNase—The NH2-terminal sequences of 17 residues of the intact polypeptide, 42 residues of CNBr Peptide Cb2, and 31 residues of CNBr Peptide Cb4 are deduced from the results of automated Edman degradation (Table II). The complete sequence of CNBr Peptide Cb5 (15 residues) is obtained from the results of manual Edman degradation of its four thermolytic peptides (Table III). In no case are these sequences contradictory to those predicted. Thus, except for a few special residues discussed below, it is evident that the amino acid compositions of short peptides should suffice for sequence deductions in regions lacking sequencing data. Furthermore, comparison of the peptide profiles of ovine DNase (Figs. 2–4) with those of bovine DNase (data not shown) shows that for each pair of corresponding peptides, if their amino acid compositions are the same, their relative elution positions are also the same, suggesting that their sequences are identical. On the other hand, if a pair of peptides differ in one or two amino acids, their elution positions are quite different.

CNBr Fragments of Ovine and Bovine DNases—When ovine DNase is compared with bovine, the amino acid compositions of their CNBr Peptides Cb1, Cb3, or Cb5 are identical, whereas those of the CNBr Peptides Cb2 or Cb4 differ slightly (Table IV). Because of this extremely high degree of similarity in composition between the five ovine and five bovine CNBr peptides, it is concluded that the CNBr cleavage sites, and hence the positions of the 4 methionine residues in the two proteins, are the same. The slight compositional differences between Peptides Cb2 and Peptides Cb4, shown in Table IV, can account for the differences between the ovine and bovine DNase sequences (Fig. 6).

Assignment of the Changed Residues in Ovine DNase—The assignments of Ser-22, Leu-29, and Ala-35 are based on the amino acid compositions of Peptides Cb2-Cb2 and Cb2-Tr1 for Ser-22, Peptides Cb2-Tr2 for Leu-29, and Peptides Cb2-Ch4 and Cb2-Tr3 for Ala-35 (Table I). These three changed positions are confirmed by automated Edman degradation of Peptide Cb2 (Table II). The amino acid composition of Peptide Cb2-Ch5a indicates that Tyr and Thr in bovine DNase are changed to Asx and Ser in ovine DNase. The Asp substituting for the Tyr at position 54 is identified by results of automated Edman degradation of Peptide Cb2-Tr2 (Table II) and manual Edman degradation of Peptide Cb2-Tr5 (Table III). By inference, Ser is placed at position 23, substituted for Thr. The following assignments are based on the amino acid compositions of the peptides shown in Table I: Val-83 of Peptides Cb2-Ch11 and Cb2-Tr7, Pro-121 of Peptides Cb2-Ch17, Cb2-Ch17a, and Cb2-Tr10; Ala-127 of Peptide Cb2-Tr11a-Th1; Pro-132 of Peptide Cb2-Tr11a-Th2. Within Peptide Cb2-Ch21 or Cb2-Tr12 (Table I), His and Val in bovine DNase are replaced by Asx and Ile in ovine DNase. The Asp is assigned at position 159, replacing His, based on the result of manual Edman degradation of Peptide Cb2-Tr12 (Table III). By inference, Ile is placed at position 163, substituting for Val. However, there is a genetic variant with retention of Val at this position as a peptide containing Val rather than Ile has been isolated (Table I, Peptide Cb2-Tr12a). The assignment of Val at position 231, replacing Ala, is based on the amino acid compositions of Peptides Cb4-Ch4-Th3, Cb4-Th4, and Cb4-Th4a (Table I). However, because there are two alanines in this region of the bovine sequence (Ala-230 and Ala-231), it is necessary to establish which one is replaced by Val. Since after 2 h at 37 °C, only t.0 residue of Ala was liberated from Peptide Cb4-Ch4-Th3 by aminopeptidase M (data not shown), Val should be placed at position 231.

The Carbohydrate Attachment Site—Because Peptides Cb2-Ch1 and Cb2-Tr1 contain glucosamine (Table I), it is likely that Asx-18 may have a carbohydrate side chain, as in bovine DNase. This attachment site is confirmed by the fact that the attachment site is confirmed by the fact that the carbohydrate attachment site.

Revision of the Sequence of Bovine DNase—The results of
automated Edman degradation of bovine Peptide Cb2 (Table II) indicate that a tripeptide, Ile-Val-Arg, is inserted between Arg-27 and Arg-28 of the published sequence of bovine DNase (3). Although a trypptic tripeptide, Ile-Val-Arg, was isolated and sequenced previously (2), it was mistakenly positioned for residues 25-27, assuming a chymotryptic-like cleavage at Tyr-24 by trypsin. This mistake is understandable because, in the revised sequence (Fig. 6), this tripeptide is repeated between residues 25 and 30, and the chymotryptic peptide (residues 25-32) covering this region has never been isolated (2). To substantiate this revised sequence, bovine DNase was cleaved at the only Asp-Pro (residues 59-60) bond of the molecule with 88% formic acid at 37 °C (6), and the peptide containing residues 1-59 (Peptide Ac1) was isolated using HPLC (Fig. 7a). Peptide Ac1 was further hydrolyzed at Asp-33 in 0.003 N HCl at 110 °C (7), and the peptide containing residues 1-33 (Peptide Ac2) was isolated using HPLC (Fig. 7b). The amino acid compositions of Peptides Ac1 and Ac2 (Table V) clearly show that 3 extra residues (Ile, Val, and Arg as underlined) are indeed present in this region of the sequence.

Comparison of the Three Primary Structures of Pancreatic DNase—As shown in Fig. 6, ovine DNase is structurally very close to bovine DNase. There are only 12 changes between the two amino acid sequences. All changes occur due to single base changes in codons. However, if the comparison is made between the genetic variant forms of bovine DNase (i.e. DNases C and D, see Ref. 5) and that of ovine DNase, there are only 10 changes; bovine DNases C and D have Pro instead of His at residue 121 (5), and the variant form of ovine DNase has Val instead of Ile at residue 163 (Table I). Porcine DNase (4) is structurally less closely related to bovine than is ovine DNase. It differs from bovine DNase by as many as 56 amino acid residues. With five exceptions, all changes occur due to single base changes. It should be noted that the 12 changes from bovine to ovine are also changed in porcine, with two exceptions (residues 127 and 231). On the other hand, 8 residues (residues 22, 29, 35, 83, 121, 132, 159, and 163), in which ovine and porcine DNases are identical, are changed in bovine DNase. Therefore, there are only 50 changes between ovine and porcine DNases. Thus, in an evolutionary sense, ovine DNase is more closely related to porcine DNase than is bovine DNase. There are two highly variable residues (residues 54-59 from bovine to ovine are also changed in porcine, with two which ovine and porcine DNases are identical, are changed in porcine DNase). Nevertheless, bovine parotid DNase, which has the same primary structure as that of bovine pancreatic DNase, is glycosylated at both Asn-18 and Asn-106 (17). This seems to contradict the observations made above in the pancreatic DNases. However, because the carbohydrate side chains in bovine parotid DNase contain both glucosamine and galactosamine, differing from only glucosamine in many of the Asn-linked oligosaccharides (17), the polypeptide processing system in parotid may be operating differently. Therefore, in bovine parotid, glycosylation of Asn-106 could precede disulfide locking or occur in the presence of disulfides.

The pig differs from cows and sheep in its digestive system. Glycosylation of RNase has been found to be related to the type of digestive system in mammals (18). Non-ruminants with cecal digestions, like the pig, horse, and guinea pig, produce RNase with a greater number of carbohydrate attachment sites than ruminants do (18). DNase follows a similar pattern; porcine DNase is more glycosylated (4) than bovine and ovine DNases. Thus, RNase and DNase are consistently glycosylated, and the amount of glycosylation depends upon the digestive system of the animal in which the enzymes have to function. However, in RNase and DNase, sugar side chains are not involved in enzymatic activity as removal of the carbohydrate side chains from the two enzymes does not change enzymatic activity (19).

Although the three-dimensional structure of only bovine DNase is known (13), it is probable that these three proteins may have very similar structures. Natural selection pressure favors conservation of the three-dimensional structure and permits only changes that do not disrupt protein function (20). When the sequences of all three DNases are compared, there are six regions (residues 35-39, 121-127, 159-163, 188-191, 204-208, and 223-231) that are highly variable; every region contains more than three changes and more than 55% of the amino acid residues are variable. All six regions either contain a loop or are associated with a loop region. Conservative changes may not alter the structure of the protein, but changes in the three regions between residues 121 and 127, between 159 and 163, and between 188 and 191 are not conservative in the sense of French and Robson (21). These nonconservative areas must be situated either outside or away around Asn-106 is an exposed but undefined loop. Based on the calculation of Chou and Fasman (10), the changes near Asn-106 (Ser-105 to Pro-103, Ser-108 to Thr-108, and Ser-110 to Asn-110) from bovine to porcine DNase (4) should not affect the turn structure, and thus porcine DNase should also have an exposed loop in this region. Yet, Asn-106 is glycosylated in porcine DNase, but not in bovine. Therefore, it is not the loop structure alone that determines whether or not a carbohydrate side chain will be attached. The nonglycosylation of bovine Asn-106 could be due to disulfide locking preceding glycosyltransferase action during polypeptide processing, since glycosylation of proteins occurs in the endoplasmic reticulum (14) where the enzyme catalyzing disulfide cross-linking also is situated (15). This hypothesis is substantiated by the fact that bovine Asn-106 cannot be glycosylated by the oviduct membrane system in the native form of the enzyme but can be after the opening of the disulfide by reduction and S-alkylation (16). Assuming that the polypeptide processing system is the same for oviduct and pancreas, the glycosylation of porcine Asn-106 (4) could then be the result of the change of Ser-103 to Pro-103 in the primary structure. Because the pyrrolidine ring of proline can forbid the rotation of the N,C,C bond, disulfide locking of Cys-101 and Cys-104, with Pro-103 in between, may be interrupted to allow glycosylation of Asn-106 during the processing of porcine DNase. However, bovine parotid DNase, which has the same primary structure as that of bovine pancreatic DNase, is glycosylated at both Asn-18 and Asn-106 (17). This permits only changes that do not disrupt protein function (8). Bovine (2)
from the regions involving the conformation and function of the enzyme (13). On the other hand, four regions are invariable; each contains more than 10 amino acid residues. All four regions are involved in \(\beta\)-structure or \(\alpha\)-helix; two of the four are located at the NH\(_2\) or COOH termini. These observations suggest that in certain regions of the molecule changes are not allowed. Furthermore, certain residues of functional importance are not changed at all; for example, the residues around the nucleotide-binding site (Asn-7, Arg-9, Asp-42, Glu-78, Arg-111, Asp-168, Asp-251, and His-252) (13), the four pairs of salt bridges (Arg-73 and Asp-139; Asp-93 and Lys-157; Asp-149 and 185; Arg-187 and Asp-198) (13), and the active site His-134 (22).

Porcine DNase, as compared with ovine and bovine DNases, is longer by 2 amino acid residues (Arg-Ala) at the COOH terminus of bovine DNase is essential for the retention of the active conformation of the enzyme and the three-dimensional structure (13) even at 2.5 \(\AA\) resolution. The presence of the positively charged Lys and Arg, along with two extra amino acids at the COOH terminus in porcine DNase, may distort a portion of the three-dimensional structure and thus lower the enzymatic activity. Functionally related proteins can show marked similarity in their active site sequences (20). The substrates for pancreatic DNase and RNase are very similar, and the two enzymes catalyze essentially the same reaction, hydrolysis of a phosphodiester bond. When the active site sequences of RNase at His-119 from three species are compared with those of DNase at His-134 from bovine, ovine, and porcine, there is great sequence homology (Table VI). There is consistently a Val residue at the third position from the NH\(_2\) side of His. The second position from the NH\(_2\) side and the third from the COOH side of His are either Pro or Ala. A Leu or a Val occupies the 1st residue on the NH\(_2\) side. This sequence homology would have been difficult to recognize if only the sequence of bovine DNase were available. From this homology of active site sequence, it is reasonable to conclude that DNases and RNases belong to the same enzyme family (histidine nucleases) and are as closely related as trypsin and chymotrypsin (serine proteases).

The elucidation and comparison of the primary structures of a functional protein isolated from various sources can provide information as to the correctness of the sequences. In the present study of pancreatic DNase, the mistake of a missing peptide in bovine DNase probably would have gone unnoticed if the sequences of ovine and porcine DNases were not determined. This error is not observed in the crystal structure (13) even at 2.5 \(\AA\) resolution.

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**TABLE VI**

Comparison of the active site sequences of pancreatic ribonuclease and deoxyribonuclease

| Enzyme   | Active site sequence                                      |
|----------|----------------------------------------------------------|
| Bovine RNase  | Pro-Tyr-Val-Pro-Val-His-Phe-Asp-Ala                     |
| Equine RNase  | Pro-Pro-Val-Pro-Val-His-Phe-Asp-Ala                     |
| Bovine DNase  | Val-Pro-Val-Pro-Val-His-Phe-Asp-Ala                     |
| Ovine DNase   | Ala-Ile-Val-Ala-Leu-His-Ser-Ala-Pro                      |
| Porcine DNase | Ala-Ile-Val-Pro-Leu-His-Ser-Ala-Pro                      |

* From Smyth et al. (24) and Heinrikson et al. (25).
* From Jackson and Hirs (26).
* From Scheffer and Beintema (27).
* From Liao et al. (3).
* From the preceding paper (4).
Primary Structures of DNase

**EXPERIMENTAL PROCEDURE**

**Materials and equipment**
- DNase I was purified to apparent homogeneity from bovine pancreatic DNase by the method of Sambrook et al. (1989).
- The enzyme was subjected to acid hydrolysis and then reconstituted with 50 mM Tris-HCl, pH 8.0, and 1 mM EDTA.
- The resulting solution was used for the preparation of primary structures.

**Methods**
- The primary structures were determined by amino acid analysis, mass spectrometry, and circular dichroism measurements.

**Results**
- The primary structures were determined for the DNase I enzyme.
- The results were compared with those obtained by other methods.

**Discussion**
- The primary structures of DNase I were consistent with previous reports.
- The differences in the primary structures were likely due to variations in the enzyme preparation and the methods used.

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**Table 1**

| Peptide | Composition |
|---------|-------------|
| P1      | Ala-Thr-Glu | 0.4 0.5 0.6 |
| P2      | Gln-Asp-Glu | 0.5 0.6 0.7 |
| P3      | Ser-Asp-Glu | 0.6 0.7 0.8 |
| P4      | Glu-Asp-Glu | 0.7 0.8 0.9 |

**Figures**

- Figure 1: Peptide profiles of the primary structures of DNase I and DNase II.
- Figure 2: Peptide profiles of the primary structures of DNase III.
- Figure 3: Peptide profiles of the primary structures of DNase IV.

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**Supplementary Material**

**Comparison of the Three Primary Structures of DNase I**

**Isolated from Bovine Pancreatic DNase**

**Building the Primary Structures**
- The primary structures were derived from homogenates of bovine pancreases.
- The homogenates were then digested with trypsin and the resultant peptides were analyzed by high-performance liquid chromatography (HPLC).
- The peptides were further purified by HPLC and characterized by amino acid analysis.

**Experimental Procedures**
- The HPLC profiles were compared with those obtained by other methods.
- The results were consistent with previous reports.

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Primary Structures of DNase

Table III

| Peptide  | Peptide  | Peptide  | Peptide  | Peptide  | Peptide  |
|----------|----------|----------|----------|----------|----------|
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |

Table IV

| Peptide  | Peptide  | Peptide  | Peptide  | Peptide  | Peptide  |
|----------|----------|----------|----------|----------|----------|
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |

Table V

| Peptide  | Peptide  | Peptide  | Peptide  | Peptide  | Peptide  |
|----------|----------|----------|----------|----------|----------|
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |
| A       | B        | C        | D        | E        | F        |

Fig. 1. The amino acid sequence of ovine DNase. The sequence is derived based on the partial sequence of the ovine DNase and the CD-DNA and C2-DNA of ovine DNase (Table I). The sequence of a, b, c, d, e, and f are derived from Table II and Table III. The sequence of g, h, i, and j are derived from the sequence of ovine DNase and the CD-DNA of ovine DNase. The sequence of k, l, m, n, and o are derived from the sequence of ovine DNase and the CD-DNA of ovine DNase. The sequence of p, q, r, s, and t are derived from the sequence of ovine DNase and the CD-DNA of ovine DNase.