Hawke, D., Yuan, P.-M., & Shively, J. E. (1982) *Anal. Biochem. 120*, 302–311.
Heinemann, F. S., & Ozols, J. (1982) *J. Biol. Chem. 257*, 14988–14999.
Heinemann, F. S., & Ozols, J. (1983) *J. Biol. Chem. 257*, 4195–4201.
Jones, B. N., Paabo, S., & Stein, S. (1981) *J. Liq. Chromatogr. 4*, 565–586.
Levin, W., Wood, A., Chang, R., Ryan, D., Thomas, P., Yagi, H., Thakker, D., Vyas, K., Boyd, C., Chu, S.-Y., Conney, A. H., & Jerina, D. M. (1982) *Drug Metab. Rev. 13*, 555–580.
Poulos, T. L., Alden, R. A., Wagner, G. C., & Gunsalus, I. C. (1983) *J. Mol. Biol.* (in press).
Reik, L. M., Levin, W., Ryan, D. E., & Thomas, P. E. (1982) *J. Biol. Chem. 257*, 3950–3957.
Ryan, D. E., Thomas, P. E., & Levin, W. (1980) *J. Biol. Chem. 255*, 7941–7955.
Ryan, D. E., Thomas, P. E., Reik, L. M., & Levin, W. (1982a) *Xenobiotica 11*, 727–744.
Ryan, D. E., Thomas, P. E., & Levin, W. (1982b) *Arch. Biochem. Biophys. 216*, 272–288.
Shively, J. E. (1981) *Methods Enzymol. 79*, 31–48.
Tarr, G. E., Black, S. D., Fujita, V. S., & Coon, M. J. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6552–6556.
Thakker, D. R., Levin, W., Yagi, H., Conney, A. H., & Jerina, D. M. (1982) in *Biological Reactive Intermediates II* (Snyder, R., Parke, D., Kocsis, J. J., Jollow, D. J., Gibson, G., & Witmer, C., Eds.) pp 501–523, Plenum Press, New York.
Thomas, P. E., Korzeniowski, D., Ryan, D. E., & Levin, W. (1979) *Arch. Biochem. Biophys. 192*, 524–532.
Thomas, P. E., Reik, L. M., Ryan, D. E., & Levin, W. (1983) *J. Biol. Chem. 258*, 4590–4598.
White, R. E., & Coon, M. J. (1980) *Annu. Rev. Biochem. 49*, 315–356.
Yuan, P.-M., Nakajin, S., Haniu, M., Shinoda, M., Hall, P. F., & Shively, J. E. (1983a) *Biochemistry 22*, 143–149.
Yuan, P.-M., Ryan, D. E., Levin, W., & Shively, J. E. (1983b) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1169–1173.

Amino Acid Sequence of Human D of the Alternative Complement Pathway†

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ABSTRACT: The primary structure of human D, the serine protease activating the C3 convertase of the alternative complement pathway, has been deduced by sequencing peptides derived from various chemical (CNBr and *o*-iodosobenzoic acid) and enzymatic (trypsin, lysine protease, *Staphylococcus aureus* V8 protease, and chymotrypsin) cleavages. Carboxypeptidase A was also used to confirm the COOH-terminal sequence. The peptides were purified by high-pressure liquid chromatography. The proposed sequence of human D contains 222 amino acids and has a calculated molecular weight of 23 748. It exhibits a high degree of homology with other serine proteases, especially around the NH_2-terminus as well as the three residues corresponding to the active-site His-57, Asp-102, and Ser-195 (chymotrypsinogen numbering). This sequence homology is highest (40%) with plasmin, intermediate (35%) with pancreatic serine proteases, such as elastase, trypsin, chymotrypsin, and kallikrein, and least (30%) with the serum enzymes thrombin and factor X. D, however, exhibits only minimal amino acid homology with the other sequenced complement serine proteases, C1r (25%) and Bb (20%). The substitution of a basic lysine for a neutral amino acid three residues NH_2-terminal to the active-site serine as well as a small serine residue for a bulky aromatic amino acid at position 215 (chymotrypsinogen numbering) in the binding pocket may be important in determining the exquisite substrate specificity of D. The presence of His-40 which interacts with Asp-194 (chymotrypsinogen numbering) to stabilize other serine protease zymogens [Freer, S. T., Kraut, J., Robertus, J. D., Wright, H. T., & Xuong, N. H. (1970) *Biochemistry 9*, 1997] argues in favor of such a D precursor molecule.

D, the protease responsible for activating the C3 convertase of the alternative pathway, is one of the least abundant of the complement components in human serum. It consists of a single polypeptide chain with a molecular weight of approximately 23 000 (Volanakis et al., 1977) and is irreversibly inhibited by diisopropyl fluorophosphate (Fearon et al., 1974) which led to its characterization as a serine protease. Its proteolytic attack is directed exclusively against a single arginyl–lysyl peptide bond of B (Lesavre et al., 1979) which becomes susceptible to D only when B is complexed with C3b. The resulting C3bBb complex is the C3 convertase of the alternative complement pathway.

The study of D has been hampered by its low serum concentration and by the difficulty in removing contaminants of similar apparent molecular weight and behavior on ion-exchange chromatography (Johnson et al., 1980). Recently, however, several reports have appeared in the literature detailing the isolation of purified D in sufficient quantities to carry out partial amino acid sequence analysis (Davis et al., 1979; Volanakis et al., 1980; Davis, 1980; Johnson et al., 1980; Reid et al., 1981). We now report the determination of the essentially complete amino acid sequence of this key complement component.

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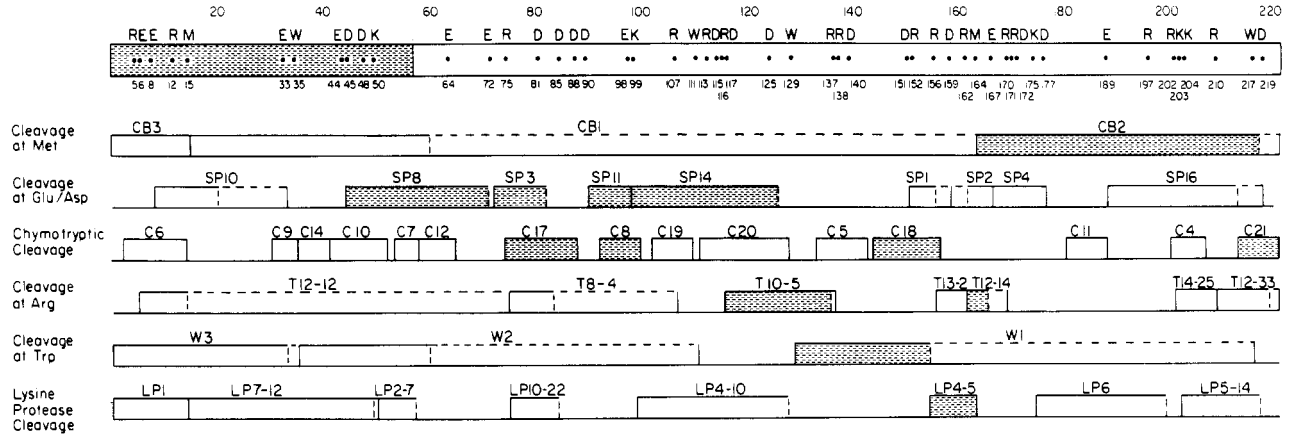

FIGURE 1: Summary of sequencing strategy. Standard one-letter abbreviations are used to designate residues defining the different fragments obtained from D. CB = cyanogen bromide peptides; W = tryptophan peptides; T = tryptic peptides; LP = lysine protease peptides; SP = *Staphylococcus aureus* V8 protease peptides; C = chymotryptic peptides. Fragments are numbered consecutively in order of their elution off HPLC. Numbers separated by hyphens indicate that these peptides were recycled before being sequenced. The peptides isolated from each of the above cleavages are outlined in rectangular boxes. The solid-line part of each box represents the sequenced region of each peptide, while the dashed-line part indicates the putative remaining unsequenced portion. Since it was not clear where two *Staphylococcus aureus* V8 protease peptides terminated and three lysine protease peptides were derived from cleavages at residues other than lysine, only the sequenced region of these peptides is shown. Shading denotes those portions of the peptides that were used to deduce the overall sequence of D presented in Figure 2.

Materials and Methods

Protein Purification. Human D was purified by a combination of ion-exchange chromatography on Bio-Rex 70 and gel filtration on Bio-Gel P-60 (Bio-Rad Laboratories) followed by hydroxylapatite and reverse-phase high-pressure liquid chromatography (HPLC)[1] (Niemann et al., 1984).

Chemical and Enzymatic Cleavages. Prior to cleavage, D was reduced with DTT and carboxymethylated with iodo-[^{14}C]acetic acid by a modification of the method of Crestfield et al. (1963).

Cleavage by CNBr was performed in 70% formic acid at room temperature for 24 h according to Gross (1967). Cleavage at tryptophanyl–X peptide bonds was carried out by using the method of Mahoney & Hermodson (1979) as modified by Mahoney et al. (1981).

Digestion by *Staphylococcus aureus* V8 protease was performed under conditions reported to favor cleavage at glutamyl–X over aspartyl–X peptide bonds (Houmard & Drapeau, 1972). D was cleaved by endoproteinase Lys-C (Boehringer Mannhein) and by TosPheCH_2Cl–trypsin (Worthington) at substrate:enzyme ratios of 50:1 and by TosLysCH_2Cl–chymotrypsin (Sigma) at a substrate:enzyme ratio of 100:1. The latter two reactions were carried at 37 °C for 4 h, while the former one was carried out at the same temperature for 27 h.

The amino acid sequence at the COOH-terminus was determined enzymatically with CPA (Millipore) essentially as described by Bhown et al. (1980b). The released amino acids were identified by reverse-phase HPLC using a precolumn derivatization and fluorescence detection system.

High-Pressure Liquid Chromatography. HPLC was performed at room temperature by using a Waters Associates chromatographic system. Molecular-exclusion separations were performed by using four I-125 0.78 i.d. × 30 cm protein analysis columns (Waters Associates) in series. The developing solvent was 20% glacial acetic acid–15% 1-propanol. For reverse-phase separations, a 0.39 i.d. × 30 cm C_{18} μBondapak column (Waters Associates) was used. Chromatograms were first developed for 10 min in the A solvent (0.1% TFA) and then eluted with a linear gradient to the indicated acetonitrile concentration.

Protein Sequencing. Edman degradations were performed in a Beckman Model 890C sequencer by employing the slightly modified (Bhown et al., 1980a) dilute Quadrol program of Brauer et al. (1975). Less than 10 nmol of each peptide was used for sequencing. Repetitive yields were generally between 96 and 99%. Most residues were determined at least twice on different preparations to substantiate each assigned amino acid residue. PthCS amino acids were identified by at least two independent methods, usually HPLC (Bhown et al., 1981) and TLC (Summers et al., 1973).

Results

Sequencing Strategy. NH_2-terminal sequence analysis coupled with chemical cleavage of methionyl–X peptide bonds as well as enzymatic digestion with *Staphylococcus aureus* V8 protease provided almost 80% of the primary amino acid sequence of D. The remaining 20% of the sequence except for three residues (66–68) plus the overlapping peptides necessary for the alignment of all but one of these initial fragments was provided by an additional chemical cleavage at tryptophanyl–X peptide bonds as well as enzymatic cleavages at arginyl–X peptide bonds and less specific cleavages using chymotrypsin and lysine protease. This sequencing strategy is summarized in Figure 1, and the proposed overall primary amino acid structure of D is presented in Figure 2.

CNBr. As expected on the basis of amino acid analysis, cleavage at methionyl–X peptide bonds produced three major cleavage peptides which were fractionated by molecular exclusion HPLC as illustrated in Figure 3A. As illustrated in Figure 1, the CB3 peptide was completely sequenced (15 amino acids terminating with homoserine), while the CB1 and CB2 peptides were sequenced through 45 and 54 amino acids, respectively. This included the previously undetermined sequence from residues 196–201 (Figure 2). CB2 was thus critical in deducing the overall sequence of D.

Staphylococcus aureus V8 Protease. D was subjected to digestion by *Staphylococcus aureus* V8 protease under conditions reported to favor glutamyl–X peptide bond cleavage. According to the amino acid composition data, anywhere from

[1] Abbreviations: CPA, carboxypeptidase A; DTT, DL-dithiothreitol; HPLC, high-pressure liquid chromatography; PthCS, phenylthiohydantoin; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; TosLysCH_2Cl, L-1-(tosylamido)-2-lysyl chloromethyl ketone; TosPheCH_2Cl, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone.

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1               10                  20                  30                  40
I L G G R E A E A H A R P Y M A S V Q L N G A H L C G G V L V A E Q W V L S A A

41              50                  60                  70                  80
H C L E D A A D G K V Q V L L G A T H L P Q P E P X X X I T I(E)V L R A V P H P

81              90                 100                 110                 120
D S Q P D T I D(H D)L L L L Q L S E K A T L G P A V R P L P W Q R V D R D V A P

121             130                140                 150                 160
G T L C D V A G W G I V N H A G R(R)P D S L Q H V L L P V L D R A T C R L Y D V

161             170                180                 190                 200
L R L M C A E S N R R D S C K G D S G G P L V C G G V L E G V V T S G S R V C G

201             210                220
N R K K P G I Y T R V A T Y A A W I D H V L

NUMBER OF RESIDUES = 222

MOLECULAR WEIGHT OF THE POLYPEPTIDE CHAIN = 23,748
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Composition

22 ALA (A)
15 ARG (R)
4 ASN (N)
16 ASP (D)
8 CYS (C)
8 GLN (Q)
9 GLU (E)
21 GLY (G)
9 HIS (H)
7 ILE (I)
28 LEU (L)
5 LYS (K)
2 MET (M)
0 PHE (F)
15 PRO (P)
10 SER (S)
9 THR (T)
4 TRP (W)
4 TYR (Y)
23 VAL (V)
3 UNK (X)

FIGURE 2: Amino acid sequence and composition of human D. The above sequence was deduced by aligning and overlapping the indicated peptides from Figure 1. Amino acid residues in parentheses were obtained from the previously published sequence of Reid et al. (1981). Residues number 97, 141, 151, and 220 were not confirmed. X denotes unidentified amino acids. Nonoverlapping peptides have been placed on the basis of sequence homology with other serine proteases as well as according to the sequence of D proposed by Reid et al. (1981).

20 to 40 peptides could have been generated. The resultant peptides were fractionated by reverse-phase HPLC (Figure 3B). Nine of these peptides were isolated and sequenced. Six were derived from glutamyl–X peptide bond cleavages, and three were derived from aspartyl–X peptide bond cleavages. Four of these peptides, SP8, SP3, SP11, and SP14, were utilized in deducing the overall sequence of D (Figure 1). The most informative of these peptides was SP8. It extended the known NH_2-terminal sequence of D and elucidated the previously undetermined region from residues 59–71 (Figure 2).

Chymotrypsin. Fifteen chymotryptic peptides were isolated and sequenced. Four, C17, C8, C18, and C21, were utilized in deducing the overall sequence of D (Figure 1). The most informative of these peptides was C21. It suggested the existence of a previously undetected amino acid at residue 220. In addition, peptide C17 extended the known NH_2-terminal sequence of D, while C8 provided the overlap between peptide SP11 and SP14 and C18 provided the overlap between peptide W1 and LP4-5. Finally, peptide C12 extended the known sequence into the previously undetermined region between residues 59 and 71 (Figure 2).

Trypsin. Cleavage of acetylated D at arginyl–X peptide bonds by digestion with trypsin was expected to generate between 16 and 19 peptides. The resultant peptides were thus initially fractionated by molecular-exclusion HPLC followed by reverse-phase HPLC. Seven of these peptides were isolated and sequenced. Two peptides, T10-5 and T12-14, were essential in deducing the overall sequence of D (Figure 1). T10-5 overlapped peptide SP14 with W1, while T12-14 overlapped the LP4-5 and CB2 fragments. Peptide T12-33 also proved to be useful. It identified a previously undetected histidine at residue 220 (Figure 2).

o-Iodosobenzoic Acid. Following cleavage at tryptophanyl–X peptide bonds by treatment with *o*-iodosobenzoic acid, three major cleavage fragments of D were separated by molecular-exclusion HPLC. Almost the complete amino acid sequence (33 out of 34 residues) of the smallest NH_2-terminal peptide, W3, and also the 25 NH_2-terminal residues of the peptide derived from cleavage at tryptophan-35, W2, have been identified (Figure 1). The remaining large peptide, W1, was thus deduced to be derived from the COOH-terminus of D. It proved to be critical in deducing the overall sequence of D. It established the alignment of peptide T10-5 and C18 as well as confirmed the identity of arginine-137 and histidine-144 (Figure 2).

Lysine Protease. Cleavage at lysyl–X peptide bonds by digestion with endoproteinase Lys-C commercially available from Boehringer Mannhein was expected to generate between eight and nine peptides. The digest was fractionated by using a combination of molecular-exclusion and reverse-phase HPLC. Seven of these peptides were isolated and sequenced. Peptide LP2-7 beginning with valine-51 suggested the presence of a lysine at position 50 (Figure 2), not a tyrosine as had previously been reported (Reid et al., 1981). This amino acid identification was confirmed by peptides C10 and W2. Three of these lysine protease peptides, LP7-12, LP10-22, and LP4-5, however, did appear to be derived from the non-lysine-specific cleavage of D. Such nonspecific cleavages using this enzyme have been previously reported (Jörnvall, 1977). One of these peptides, LP4-5, was essential in deducing the overall sequence

of D. It provided the overlap between peptides W1 and T12-14. Another of these peptides, LP7-12, also proved to be useful. It clearly identified methionine, not valine as had previously been reported (Volanakis et al., 1980), at residue 15 (Figure 2).

Carboxypeptidase A. Digestion of D with CPA indicated a COOH-terminal sequence of Val-Leu-COOH which differs significantly from that of other previously described serine proteases which usually terminate in an asparaginyl residue. This dipeptide corresponded to the COOH-terminal sequence of our chymotryptic peptide C21, suggesting that this peptide was the COOH-terminal one.

Discussion

A sequence of 222 amino acid residues with a calculated molecular weight of 23 748 has been determined for human D of the alternative complement pathway. The most notable aspects of this overall amino acid composition on the basis of the sequence of D are the relatively high proline content and the absence of phenylalanine. Proline is usually associated with "bends" in the tertiary structure of a protein, while the lack of phenylalanine is unusual in a protein of this size.

Our strategy in deriving the overall sequence of D is outlined as follows: (1) The sequence from residues 1–71 was derived, as is illustrated in Figure 1, from the indicated overlapping peptides. (2) The COOH-terminal dipeptide of the molecule was determined to be Val-Leu-COOH. This corresponded to the COOH-terminal sequence of our chymotryptic peptide C21. Working backward from the COOH-terminal peptide, the sequence from residues 91–222 was also derived, as is illustrated in Figure 1, from the indicated overlapping peptides. (3) This placed two remaining overlapping peptides, SP3 and C17, between these extended NH_2- and COOH-terminal sequences, although no overlapping peptides formally substantiating the insertion of this sequence into this region of the molecule have been isolated. Reid et al. (1981), however, had previously published an extensive amino acid sequence of D identifying the amino acid residues at the NH_2- and COOH-terminus of these overlapping fragments. Thus, on the basis of the extensive amino acid homology between D and other serine proteases and the sequence published by Reid et al. (1981) and assuming no insertions in this region of the molecule, the overall amino acid sequence of human D was deduced. In addition, although our proposed sequence still contains seven unidentified amino acid residues, several of these have already been identified. Reid et al. (1981), for example, have identified the amino acids at position 72 as a glutamyl residue and at position 90 as an aspartyl residue. This is consistent with the results of our *Staphylococcus aureus* V8 protease cleavage. The unidentified amino acids at positions 89 and 138 have similarly been identified by Reid et al. (1981) as histidyl and arginyl residues, respectively. This then leaves only residues 66–68 remaining unidentified.

Several discrepancies between the present sequence and previously published ones exist. Proline, for example, had previously been identified at position 10 and valine at position 15 (Volanakis et al., 1980). These two residues have now been established to be histidine and methionine, respectively. Residue 24 was identified as histidine, 48 as aspartic acid, and 50 as lysine. This is in agreement with the work of Davis et al. (1979, 1980) but differs from the glutamic acid, glycine, and tyrosine determinations, respectively, published by Reid et al. (1981) at these positions. In addition, we have identified residue 38 as serine, residue 58 as threonine, residue 112 as glutamine, residue 154 as threonine, and residue 213 as threonine, whereas Reid et al. (1981) have identified threonine, isoleucine, glycine, lysine, and serine, respectively, at these positions. We were also able to identify three other previously unknown amino acids at positions 41, 52, and 107 as well as residues 59–65, 69–71, and 196–201. The histidine identification at position 41 was especially critical as this particular amino acid residue is involved in the active site (His-57 of chymotrypsinogen) in all other serine proteases. Finally, we have identified a previously overlooked amino acid at position 220 between the COOH-terminal aspartyl and valyl residues; it is probably histidine.

The three residues corresponding to the active-site His-57, Asp-102, and Ser-195 (chymotrypsinogen numbering) are found at positions 41, 88, and 178 of the D sequence, respectively. The primary binding site at residues 176–181 (Gly-Asp-Ser-Gly-Gly-Pro) is absolutely conserved. This is to be expected as even minor substitutions would alter the conformation of the binding pocket (Stroud et al., 1971). The "binding site" amino acid at position 189 (chymotrypsinogen numbering) found at position 172 of the D sequence is also identical with the aspartyl residue in trypsin that binds the basic residue side chain of the substrate in keeping with the trypsin-like specificity of D. The secondary binding site corresponding to residues 193–195 (Thr-Ser-Gly), however, is not conserved. This sequence is thought to form hydrogen bonds with P2 and P3 residues of the substrate (Segal et al., 1971). The substitution of a threonine for the invariant serine initiating this tripeptide sequence as well as the presence of a small seryl residue instead of a bulky aromatic amino acid at position 215 (chymotrypsinogen numbering) in the binding pocket may be important in determining the exquisite substrate specificity of D. In addition, the substitution of a basic lysine for a neutral amino acid three residues NH_2-terminal to the active-site serine may also be critical in this regard.

Serine proteases generally contain three conserved disulfide bonds forming the histidine loop and the methionine loop and linking the primary and secondary binding sites. Although the disulfide bonds of D have not yet been determined, it is likely that these three commonly conserved disulfide bonds are represented by Cys-26–42, Cys-155–165, and Cys-174–199, respectively. In addition, D appears to contain a fourth conserved disulfide bond linking Cys-124–184 (the B–C chain bridge) of the pancreatic serine proteases found in plasmin, trypsin, kallikrein, chymotrypsin, and elastase, but not thrombin and factor X (Hartley, 1970).

D has maintained a remarkable degree of amino acid sequence homology with other serine proteases. Only two of the 29 "invariant" amino acid residues of the serine proteases (Young et al., 1978) have been altered. Serine-214 (chymotrypsinogen numbering) at the secondary binding site has been conservatively replaced by a threonine (193), and a small, neutral glycyl residue at position 184 (chymotrypsinogen numbering) has been replaced by a large, charged glutamyl acid residue (167). This latter substitution may be important in determining the three-dimensional structure of D, since glycine residues are often found at "corners" of peptide chains. Most of the "conserved" amino acid residues of the serine proteases (Young et al., 1978) have also been retained. Of these residues, 48 remain identical while 19 have been "conservatively" substituted and only 17 have been "radically" substituted.

Overall, D exhibits about 40% residue identity with the B chain of human plasmin and an average of 35% with the pancreatic enzymes porcine kallikrein, bovine trypsin, bovine chymotrypsin, and porcine elastase, but only approximately 30% with the serum enzymes bovine thrombin and bovine

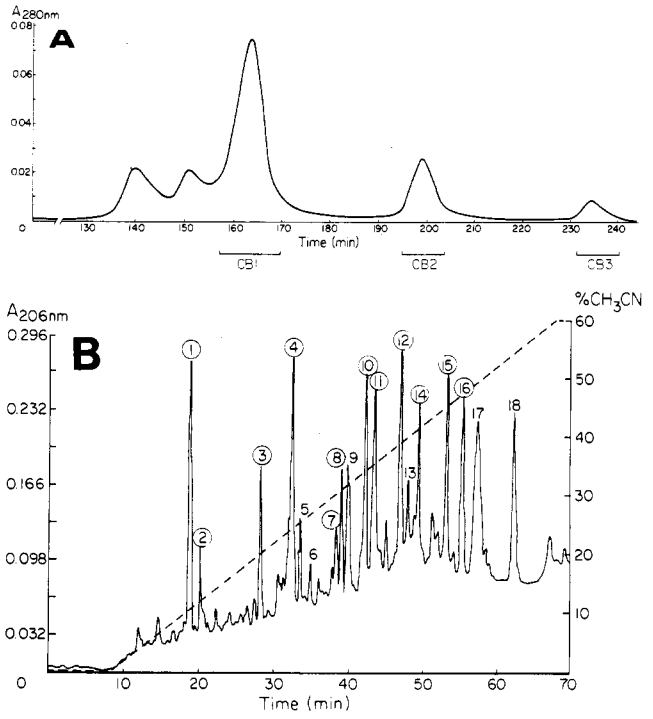

FIGURE 3: (A) Separation of the three major CNBr cleavage peptides of D by molecular-exclusion HPLC. The A_{280}-nm peak eluting between 158 and 170 min contains the large middle CNBr cleavage peptide CB1 (M_r 15 500). The A_{280}-nm peak eluting between 195 and 205 min contains the intermediate-sized COOH-terminal CNBr cleavage peptide CB2 (M_r 7000). The A_{280}-nm peak eluting between 233 and 241 min contains the small NH_2-terminal CNBr cleavage peptide CB3 (M_r 1700). The A_{280}-nm peaks eluting between 138–144 and 149–154 min contain uncleaved D. The flow rate was 0.2 mL/min. Additional experimental details are given in the text. (B) Separation of the *Staphylococcus aureus* V8 cleavage peptides of D by reverse-phase HPLC. The circled peptide peaks have been sequenced. The dashed line indicates acetonitrile (CH_3CN) concentration. The flow rate was 1.5 mL/min. Additional experimental details are given in the text.

factor X. Interestingly, however, D exhibits only minimal homology with other sequenced complement serine proteases. It has 25% of its amino acid residues in common with the b chain of human C1r (Arlaud & Gagnon, 1983), including a unique four-residue sequence from position 101 to 104 (Thr-Leu-Gly-Pro) in one of the structurally conserved regions (Greer, 1981; Furie et al., 1982) of this class of enzymes. It also has only 20% of its amino acid residues in common with the COOH-terminal sequence of human Bb (Christie & Gagnon, 1983). This suggests that D has evolved differently from C1r and Bb.

Finally, the activity of most other similar molecular weight serine protases is regulated by their synthesis as relatively inactive zymogens followed by their subsequent activation, usually involving the cleavage of a small (6–14 amino acid residues) NH_2-terminal peptide, allowing these previously inactive proteins to assume their enzymatically active conformations. The existence of such a putative D precursor molecule has been suggested on the basis of functional analysis (Fearon et al., 1974). The presence of His-40 which interacts with Asp-194 (chymotrypsinogen numbering) to stabilize other serine protease zymogens (Freer et al., 1970) argues in favor of such a D precursor molecule. In addition, similar to other active serine proteases, the NH_2-terminal sequence of D is folded inside the molecule and thus unavailable for reaction with monoclonal antibodies of predetermined specificity for that region of the enzyme (Niemann et al., 1984). Taken together, these structural features suggest that, like other serine proteases, D is probably synthesized as an inactive zymogen.

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References

Arlaud, G. J., & Gagnon, J. (1983) *Biochemistry 22*, 1758.
Bhown, A. S., Cornelius, T. W., Mole, J. E., Lynn, J. D., Tidwell, W. A., & Bennett, J. C. (1980a) *Anal. Biochem. 102*, 35.
Bhown, A. S., Bennett, J. C., & Hunter, E. (1980b) *J. Biol. Chem. 255*, 6962.
Bhown, A. S., Mole, J. E., & Bennett, J. C. (1981) *Anal. Biochem. 110*, 355.
Brauer, A. W., Margolies, M. N., & Harber, E. (1975) *Biochemistry 14*, 3029.
Christie, D. L., & Gagnon, J. (1983) *Biochem. J. 209*, 61.
Crestfield, A. M., Moore, S., & Stein, W. H. (1963) *J. Biol. Chem. 238*, 622.
Davis, A. E., III (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4938.
Davis, A. E., III, Zalut, C., Rosen, F. S., & Alper, C. A. (1979) *Biochemistry 18*, 5082.
Fearon, D. T., Austen, K. F., & Ruddy, S. (1974) *J. Exp. Med. 139*, 355.
Freer, S. T., Kraut, J., Robertus, J. D., Wright, H. T., & Xuong, N. H. (1970) *Biochemistry 9*, 1997.
Furie, B., Bing, D. H., Feldmann, R. J., Robison, D. J., Burnier, J. P., & Furie, B. C. (1982) *J. Biol. Chem. 257*, 3875.
Greer, J. (1981) *J. Mol. Biol. 153*, 1027.
Gross, E. (1967) *Methods Enzymol. 11*, 238.
Hartley, B. S. (1970) *Philos. Trans. R. Soc. London, Ser. B 257*, 77.
Houmard, J., & Drapeau, G. R. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 3506.
Johnson, D. M. A., Gagnon, J., & Reid, K. B. M. (1980) *Biochem. J. 187*, 863.
Jörnvall, H. (1977) *Eur. J. Biochem. 72*, 425.
Lesavre, P. H., Hugli, T. E., Esser, A. F., & Muller-Eberhard, H. J. (1979) *J. Immunol. 123*, 529.
Mahoney, W. C., & Hermodson, M. A. (1979) *Biochemistry 18*, 3810.
Mahoney, W. C., Smith, P. K., & Hermodson, M. A. (1981) *Biochemistry 20*, 443.
Niemann, M. A., Kearney, J. F., & Volanakis, J. E. (1984) *J. Immunol. 132*, 809.
Reid, K. B. M., Johnson, D. M. A., Gagnon, J., & Prohaska, R. (1981) *Methods Enzymol. 80*, 134.
Segal, D. M., Powers, J. C., Cohen, G. H., Davies, D. R., & Wilcox, P. E. (1971) *Biochemistry 10*, 3728.
Stroud, R. M., Kay, L. M., & Dickerson, R. E. (1971) *Cold Spring Harbor Symp. Quant. Biol. 36*, 125.
Summers, M. R., Smythers, G. W., & Oroszlan, S. (1973) *Anal. Biochem. 53*, 624.
Volanakis, J. E., Schrohenloher, R. E., & Stroud, R. M. (1977) *J. Immunol. 119*, 337.
Volanakis, J. E., Bhown, A. S., Bennett, J. C., & Mole, J. E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1116.
Young, C. L., Barker, W. C., Tomaselli, C. M., & Dayhoff, M. O. (1978) in *Atlas of Protein Sequence and Structure* (Dayhoff, M. O., Ed.) Vol. 5, Suppl. 3, p 73, National Biomedical Research Foundation, Washington, DC.