The Amino Acid Sequence of the Procoagulant- and Prothrombin-binding Domain Isolated from Staphylocoagulase*

(Received for publication, August 6, 1985)

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The primary structure of the procoagulant- and prothrombin-binding domains, the 43- and 30-kDa fragments previously isolated from staphylocoagulase, has been determined by sequencing peptides derived from various chemical (CNBr and 2-(2-nitrophenylsulfonyl)-3-methyl-3-bromoindolenine) and enzymatic (trypsin and α-chymotrypsin) cleavages. Carboxypeptidase Y was also used to deduce the COOH-terminal sequence. The 43-kDa fragment contained 324 amino acids and had a calculated molecular weight of 38,098. It included the entire structure of the 30-kDa fragment located in the COOH-terminal portion (positions 126–324). The 43-kDa fragment had an unusual amino acid composition based on the sequence, in which the sum of Asp (28 residues), Asn (35), Glu (9), and Lys (52) residues accounted for more than 45% of the total. In addition, the frequent occurrence of repetitions of various kinds of dipeptides was found along the whole sequence.

Structural comparison of the NH2-terminal portion of the 43-kDa fragment of staphylocoagulase with that of streptokinase did not reveal any obvious sequence homologies. There was also no sequence homology with that of trypsin, α-chymotrypsin, and elastase.

Staphylocoagulase (M, 64,000) is an extracellular protein produced by staphylococci (1) that specifically forms a complex with human prothrombin in a molar ratio of 1:1 (2). The staphylocoagulase-zymogen complex displays the ability to clot fibrinogen and to hydrolyze the chromogenic and fluorogenic tripeptide substrates for α-thrombin (3–7). Staphylocoagulase also interacts with human α-thrombin through the COOH-terminal region of α-thrombin B chain (8) and alters the microenvironment of the active site of α-thrombin by this complex formation (9). Our previous studies have demonstrated that limited α-chymotryptic cleavage of staphylocoagulase yields two large fragments, fragments of 43 and 30 kDa, and these fragments retain the ability to bind to prothrombin (10). We have also showed that the 43-kDa fragment, which is located in the NH2-terminal portion of intact staphylocoagulase, has prothrombin activator activity equivalent to that of the parent molecule.

The present study was undertaken to determine the complete amino acid sequence of the functionally active 43-kDa fragment as an approach to understand the structure-function relationship of staphylocoagulase.

EXPERIMENTAL PROCEDURES AND RESULTS

RESULTS AND DISCUSSION

The amino acid sequence of the procoagulant- and prothrombin-binding domains, 30- and 43-kDa fragments, isolated from staphylocoagulase has been determined by automated Edman degradation of these fragments and of their component peptides (Fig. 1). The primary peptides used in the sequencing were produced by cyanogen bromide and chymotrypsin cleavages. The secondary peptides required for overlaps were produced by trypsin cleavage at the 6 arginine residues after citraconylation and by chemical cleavages at the 1 tryptophan residue (30-kDa fragment) with 2-(2-nitrophenylsulfonyl)-3-methyl-3-bromoindolenine and at the two Asp-Pro bonds with 70% formic acid. As a result of these studies, the positions of all 324 amino acid residues of the 43-kDa fragment, which includes the 30-kDa fragment in its COOH-terminal portion, have been unambiguously determined, as shown in Fig. 2. As staphylocoagulase lacks cystine (Table 1), this represents the complete covalent structure of the 43-kDa fragment.

* This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed.
The most notable aspect of this sequence is the high content of Asp (28 residues), Asn (22), Glu (35), Gln (9), and Lys (52) residues. The sum of these residues accounts for more than 45% of the total residues. Moreover, there are some unusual features in the amino acid sequence of the 43-kDa fragment. One is the uneven distribution of certain amino acid residues: most of the tyrosine and leucine residues are found in the COOH-terminal half, while the proline residues are mainly distributed in the NH2-terminal half of the molecule (Fig. 2). Another unique feature is the frequent occurrence of repetitions of dipeptides: Lys-Lys is repeated seven times and Glu-Glu five times. In addition to these dipeptides, repetitions of tripeptides: Lys-Lys-Lys is repeated five times, and Asp-Lys-Glu (35), Gln (9), and Lys (52) residues. The sum of these residues accounts for more than 45% of the total residues. Moreover, there are some unusual features in the amino acid sequence of the 43-kDa fragment. One is the uneven distribution of certain amino acid residues: most of the tyrosine and leucine residues are found in the COOH-terminal half, while the proline residues are mainly distributed in the COOH-terminal half of the molecule (Fig. 2). Another unique feature is the frequent occurrence of repetitions of dipeptides: Lys-Lys is repeated seven times and Glu-Glu five times. In addition to these dipeptides, repetitions of Lys-Glu (eight times), Thr-Lys (six times), Val-Lys (five times), and Asp-Lys (four times) are found along the whole sequence.

The calculated molecular weight based on the sequence is 23,086 for the 30-kDa fragment and 40,500 for the 43-kDa fragment (data not shown), which are in good agreement with those calculated from the sequence data.

The secondary structure of the 43-kDa fragment predicted by Chou-Fasman analysis (17) contains 32.4% \( \alpha \)-helix, 18.2% \( \beta \)-sheet, and 36.1% \( \beta \)-turn. In spite of the unusual structure described above, these values are consistent with the average frequency for \( \alpha \)-helices (0.38), \( \beta \)-sheets (0.20), and \( \beta \)-turns (0.32) found in ordinary proteins (18).

It is of interest to compare the proposed structure of the 43-kDa fragment to that of streptokinase, which activates human plasminogen by forming a complex in a molar ratio of 1:1 (19), like staphylocoagulase. Jackson and Tang (20) have recently reported that the NH2-terminal 245 residues of streptokinase reveal homology to the sequences of several serine proteases and also contain internal sequence homology. However, structural comparison of the 43-kDa fragment derived from staphylocoagulase with that of streptokinase does not reveal any obvious sequence homologies. There is also no internal homology in the sequence of the 43-kDa fragment.
protein complex. On the other hand, the complex of the 30-kDa fragment located in the COOH-terminal portion, which includes the prothrombin-binding site in the 43-kDa fragment is specific for the binding of prothrombin toward fibrinogen and the fluorogenic tripeptide substrate for trypsin, and elastase. Moreover, there is no sequence homology with those of serine proteases including trypsin, chymotrypsin, and elastase.

Amino acid compositions of the 30- and 43-kDa fragments

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>30-kDa fragment</th>
<th>43-kDa fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>29.0</td>
<td>17</td>
</tr>
<tr>
<td>Asn</td>
<td>13</td>
<td>50.0</td>
</tr>
<tr>
<td>Thr</td>
<td>16</td>
<td>21.3</td>
</tr>
<tr>
<td>Ser</td>
<td>6.1</td>
<td>11.1</td>
</tr>
<tr>
<td>Glu</td>
<td>33.0</td>
<td>26</td>
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<tr>
<td>Gln</td>
<td>7</td>
<td>46.9</td>
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<td>Pro</td>
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<tr>
<td>Gly</td>
<td>5.4</td>
<td>10.1</td>
</tr>
<tr>
<td>Ala</td>
<td>9.0</td>
<td>14.1</td>
</tr>
<tr>
<td>(\frac{1}{2})Cys (^a)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Val (^a)</td>
<td>15.2</td>
<td>20.8</td>
</tr>
<tr>
<td>Met</td>
<td>1.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Ile</td>
<td>8.5</td>
<td>15.2</td>
</tr>
<tr>
<td>Leu</td>
<td>10.9</td>
<td>22.6</td>
</tr>
<tr>
<td>Tyr</td>
<td>5.9</td>
<td>19.9</td>
</tr>
<tr>
<td>Phe</td>
<td>5.8</td>
<td>9.3</td>
</tr>
<tr>
<td>Lys</td>
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<td>53.7</td>
</tr>
<tr>
<td>His</td>
<td>3.2</td>
<td>5.7</td>
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<tr>
<td>Arg</td>
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</tr>
<tr>
<td>Trp (^d)</td>
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<td>2.2</td>
</tr>
</tbody>
</table>

Total 199 324

\(^a\)Calculated from extrapolated or average values estimated from 24-48, and 72-h hydrolysates.

\(^b\)Calculated from the 72-h hydrolysate.

\(^c\)Determined by the \(4\)M methanesulfonic acid method.

The bank containing approximately 2700 sequences has been done using the program described by Toh et al. (22). A number of proteins were observed to display a low degree of similarity to limited sequences (less than 20 residues in all cases) of the 43-kDa fragment. Moreover, there is no sequence homology with those of serine proteases including trypsin, \(\alpha\)-chymotrypsin, and elastase.

As reported previously (10), the 43-kDa fragment derived from staphylococcalase binds strongly with human prothrombin \(\left(K_T = 1.7 \text{nM} \right)\), comparable to intact staphylococcalase \(\left(K_T = 0.49 \text{nM} \right)\), and the enzyme activity of the complex with prothrombin toward fibrinogen and the fluorogenic tripeptide substrate \((\text{t-butoxycarbonyl-Val-Pro-Arg-4-methylcoumaryl-7-amide})\) does not differ appreciably from that of the intact protein complex. On the other hand, the complex of the 30-kDa fragment with prothrombin shows little clotting activity, although it still exhibits binding with prothrombin \(\left(K_T = 120 \text{nM} \right)\). These results, in addition to the present data, suggest that the prothrombin-binding site in the 43-kDa fragment is located in the COOH-terminal portion, which includes the entire structure of the 30-kDa fragment, and that the functional region of staphylococcalase for activating prothrombin is localized in the NH₂-terminal half of the molecule. Further studies on the identification of functional residues from these regions in staphylococcalase activity will be required.

Acknowledgments—We wish to express our thanks to Kazuko Usui-Kawaguchi for amino acid analysis and to Chizuko Takabayshi for high performance liquid chromatography analysis. We thank Dr. Walter Kochel (Department of pathology, University of New Mexico, Albuquerque) for his help in preparing the English manuscript. We also thank Mizumo Akiyoshi for her expert secretarial assistance.

REFERENCES


Continued on next page.
Amino Acid Sequence of Staphylococcal Fragments

**MATERIALS AND METHODS**

To the Amino Acid Sequence of Preproenol and Proenol-Binding Protein

**EXPERIMENTAL PROCEDURES**

Materials — The sources of materials used in this work are: Golldin — Cytochrome changes and 30,000-Dalton bands from Poultry Chemical Co., Inc. (Olmstead, Georgia). Staphylococcus aureus was obtained from the American Type Culture Collection. All other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, Missouri).

Amino acid sequence was determined by an Applied Bio-Systems 4020A automated sequencer. The instrument was operated in accordance with the manufacturer’s instructions. Amino acids were degraded by dithiothreitol and the resulting amino acids were analyzed by a Beckman 119 amino acid analyzer. All samples were stored at -20°C until analyzed.

**RESULTS**

The amino acid sequence of the 30-ko fragment and the 41-ko fragment were determined by sequencing techniques. The results are shown in Table 1. The amino acid compositions of the two fragments are shown in Table 2. The sequence of the 30-ko fragment is also shown in Figure 1. The sequence of the 41-ko fragment is shown in Figure 2. The sequence of the 30-ko fragment was determined by sequencing the purified 30-ko fragment after the cleavage reaction. The sequence of the 41-ko fragment was determined by sequencing the purified 41-ko fragment after the cleavage reaction. The sequence of the 30-ko fragment was determined by sequencing the purified 30-ko fragment after the cleavage reaction. The sequence of the 41-ko fragment was determined by sequencing the purified 41-ko fragment after the cleavage reaction.

**DISCUSSION**

The results presented in this paper demonstrate the usefulness of the conformational changes and 30,000-Dalton bands from Poultry Chemical Co. Inc. as probes for the study of the conformational changes and 30,000-Dalton bands from Poultry Chemical Co. Inc. The results also demonstrate the usefulness of the conformational changes and 30,000-Dalton bands from Poultry Chemical Co. Inc. as probes for the study of the conformational changes and 30,000-Dalton bands from Poultry Chemical Co. Inc. The results further demonstrate the usefulness of the conformational changes and 30,000-Dalton bands from Poultry Chemical Co. Inc. as probes for the study of the conformational changes and 30,000-Dalton bands from Poultry Chemical Co. Inc. The results thus demonstrate the usefulness of the conformational changes and 30,000-Dalton bands from Poultry Chemical Co. Inc. as probes for the study of the conformational changes and 30,000-Dalton bands from Poultry Chemical Co. Inc.
### Table M1

**Amino Acid Sequence of Staphylococcal Fragments**

<table>
<thead>
<tr>
<th>Residue/Molecule</th>
<th>Amino Acid</th>
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<th>30kDa-CHO</th>
<th>30kDa-CHO</th>
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<tbody>
<tr>
<td>Asp</td>
<td>7.1(2)</td>
<td>6.9(5)</td>
<td>5.6(6)</td>
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</tr>
<tr>
<td>Thr</td>
<td>1.1(2)</td>
<td>0.8(1)</td>
<td>0.7(1)</td>
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<tr>
<td>Gly</td>
<td>2.0(2)</td>
<td>2.0(2)</td>
<td>5.5(7)</td>
<td>5.0(2)</td>
</tr>
<tr>
<td>Pro</td>
<td>0.1(1)</td>
<td>0.1(1)</td>
<td>0.1(1)</td>
<td>0.1(1)</td>
</tr>
<tr>
<td>Leu</td>
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<td>1.2(2)</td>
<td>1.4(2)</td>
<td>1.4(2)</td>
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<tr>
<td>Ala</td>
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<td>0.9(1)</td>
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<tr>
<td>Val</td>
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<td>Ile</td>
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*Calculated from values of non-nonspecific and homoserine lactone.

### Table M14

**N-terminal sequence of the 43-kDa fragment**

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<th>PPN Yield</th>
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### Table M15

**Amino acid compositions of sphytopeptides from the 43-kDa fragment**

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<td>8.0(15)</td>
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<td>Thr</td>
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<tr>
<td>Glu</td>
<td>5.8(8)</td>
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<td>5.8(8)</td>
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<tr>
<td>Pro</td>
<td>2.1(1)</td>
<td>2.1(1)</td>
<td>2.1(1)</td>
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<tr>
<td>Ile</td>
<td>6.9(7)</td>
<td>6.9(7)</td>
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<tr>
<td>Met</td>
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<td>0.0(1)</td>
<td>0.0(1)</td>
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<tr>
<td>Ser</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
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<td>48</td>
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</table>

*Calculated from values of non-specific and homoserine lactone.