Isolation and Characterization of an Inhibitor of Factor XIIa from Bovine Plasma*

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An inhibitor of factor XIIa has been purified to homogeneity from bovine plasma. The purification steps included precipitation of contaminating proteins with polyethylene glycol and chromatography on DEAE-cellulose, Affi-Gel blue, and immobilized wheat germ lectin. The apparent molecular weight of the XIa inhibitor (called INH1) was 85,000, reduced, and 92,000, nonreduced, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The extinction coefficient (ε280) of INH1 is 1.3, and the protein contains 17.7% carbohydrate. Purified antibody to INH1, raised in either rabbits or chickens formed a precipitin line of identity with purified INH1, and a component of bovine plasma, but there was no reaction with purified human inhibitors or with any component of human plasma. INH1 inhibits bovine and human XIIa, bovine and human C1-esterase, and human kallikrein, but does not inhibit bovine trypsin, bovine trypsin, human plasmin, or human thrombin. This activity is similar to that of C1-inhibitor but different from antithrombin III, α2-antiplasmin, or α1-protease inhibitor. INH1, at a physiological concentration (0.47 μM) causes rapid inactivation of XIIa. The two molecules react in a 1:1 stoichiometry with a second-order rate constant of 1.23 × 10^6 M⁻¹ min⁻¹.

Factor XIIa and kallikrein are serine proteases which participate in the contact phase of blood coagulation. Several investigators have reported that C1-inhibitor (C1INH) is the major physiological inhibitor of human factor XIIa (1, 2). Other proteins which have been found to inhibit human factor XIIa are antithrombin III (ATIII) (1-3), α2-antiplasmin (α2-AP) (4), α2-macroglobulin (α2-M) (5), and the plasminogen-activation inhibitor described by Hedner (6). Pixley et al. (2) have recently shown that C1INH contributes over 90% of the factor XIIa inhibitory activity of human plasma and that α2-AP, α2-M, and ATIII are responsible for the remainder of the inhibition. Schapira et al. (7) showed that human C1NH is responsible for 58% of the inhibition of human kallikrein, that α2-M is responsible for 38%, and that ATIII, α2-AP, and α1-proteinase inhibitor (α1-PI) are together responsible for the remaining 4% inhibition of human kallikrein.

The protease inhibitors of bovine plasma and their roles in the regulation of the bovine blood coagulation system have been less well studied. A protein analogous to human C1INH has not been identified in bovine plasma. α2-Macroglobulin has been purified in several species including the bovine (8-11). Bovine α2-M has been shown to inhibit trypsin and thrombin (9), but does not inhibit kallikrein (11) or bovine factor XIIa (12). Bovine ATIII has been purified (13), but its inhibitory activity toward factor XIIa or kallikrein has not been established in the bovine system.

This study was initiated to clarify the role of bovine inhibitors in regulating the initial phase of blood coagulation in bovine plasma. We report a purification procedure for a major inhibitor of bovine factor XIIa, describe some of its properties, and compare these properties to those of known human inhibitors in an attempt to identify this inhibitor. This inhibitor, which we have called INH1, to indicate that it is the first of the inhibitors of the bovine contact system which we have purified and characterized, is one of at least three inhibitors of XIIa in bovine plasma. INH1 is similar to human C1INH, but there are differences between the two proteins. INH1 is an effective inhibitor of Factor XIIa and forms a 1:1 stoichiometric complex with it. A preliminary report of this study has been presented (14).

EXPERIMENTAL PROCEDURES

Immunological Techniques—New Zealand White rabbits or Rhode Island White pullets were immunized initially with INH1 in an emulsion of Freund’s complete adjuvant. Rabbits and one hen received native INH1, and one hen received INH1 which had been denatured, reduced, and alkylated. Subcutaneous or intramuscular injections were given in Freund’s incomplete adjuvant at 2-week intervals and later in 0.01 M Tris, 0.15 M NaCl, pH 7.4, at 1-month intervals. Blood was obtained from the rabbit’s marginal ear vein and from the vein under the hen’s wing 1 week after each booster injection. All eggs were collected for purification of IgY.

Antiserum was rendered specific for INH1 by adsorption with DEAE-cellulose column fractions which contained contaminants but did not contain INH1. The adsorbed rabbit antiserum was precipitated by the addition of saturated ammonium sulfate to 40%. The precipitate was dissolved in saline according to the method of Miles et al. (15). Antiserum was concentrated and dialyzed against saline.

* Portions of this paper (including part of “Experimental Procedures," Figs. 8 and 9, and Tables III and IV) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 87M-562, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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The abbreviations used are: C1NH, C1-esterase inhibitor or C1-inhibitor; α1-PI, α1-proteinase inhibitor; α2-AP, α2-antiplasmin; α2-M, α2-macroglobulin; ATIII, antithrombin III; BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; IgG, immunoglobulin G; INH1, bovine XIIa inhibitor; PEG, polyethylene glycol; SDS, sodium dodecyl sulfate; STI, soybean trypsin inhibitor.
Bovine Protease Inhibitor of Factor XIIa

tate containing IgG was purified on columns of DEAE-Affi-Gel blue by the prescribed procedure (Bio-Rad). Antiserum titer was determined by the method of Sewell (16). The IgY was purified by the method of Polson et al. (17) as modified by Carroll and Stollar (18). Protein containing IgY was obtained from the yolks by a 3.5-12.0% polyethylene glycol precipitation followed by chromatography on DEAE-Affi-Gel blue with 0.02 M potassium phosphate, pH 8.0. The IgY eluted at 0.15 M potassium phosphate. Double immunodiffusion assays were performed by the method of Ouchterlony (15), using 0.5% agarose in 0.05 M sodium barbital buffer, pH 8.6. Precipitin reactions between IgY and INH were only observed in the presence of high salt (1.5 M sodium chloride).

The competitive enzyme-linked immunoabsorption assay for human C1INH was performed as described by Schmaier et al. (19). Rocket immunoelectrophoresis was performed using the technique of Laurell (20).

\[ E + 1 = \frac{k_1}{k_{-1}} E - I \rightarrow EI \]  

where \( E \) is the free protease, \( I \) is the free inhibitor, \( E - I \) is a reversible complex, and \( EI \) is a stable nondissociable complex of inhibitor and protease. The dissociation constant for the reversible reaction, \( K_d \), is equal to \( k_{-1}/k_1 \).

When experiments are performed under pseudo-first order conditions, the concentration of the inhibitor greatly exceeds the concentration of the protease and the enzyme activity remaining at any time, \( t \), can be expressed as

\[ \ln \frac{E}{E_0} = -k_{app}(t) \]

where \( E \) is the remaining activity and \( E_0 \) is the initial concentration of free protease. \( I_0 \) is the initial concentration of free inhibitor, and

\[ k_{app} = \frac{k_2}{1 + K_d/I_0}. \]

Experimentally, \( k_{app} \) is obtained by measuring residual protease activity as a function of time for several concentrations of INH. A plot of \( \ln E/E_0 \) versus time is used to obtain a value of \( k_{app} \) from the slope of each line. A replot of \( k_{app} \) versus \( I_0 \) yields a slope of \( k_2/K_d \), a y intercept of \( k_1 \), and an x intercept of \(-K_d\).

**RESULTS**

**Characterization of INH**—Purified INH was analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Fig. 1, INH was greater than 95% pure by electrophoresis and was a single-chain molecule both in the presence and absence of reducing agents. Its \( M_r \), under reducing conditions was 85,000 and the \( M_r \) without reduction was 92,000 as compared with standard reduced proteins. We have not been able to demonstrate the presence of a lower \( M_r \) subunit of INH when INH was reduced and run on more highly cross-linked polyacrylamide gels. Periodate-Schiff staining gave a strongly positive reaction indicating the presence of carbohydrates.

Monospecific rabbit antiserum prepared against purified INH formed a single precipitin line with bovine plasma and a line of identity with INH (Fig. 2, top). The concentration of INH in bovine plasma was measured by Laurell rocket immunoelectrophoresis using purified monospecific rabbit IgG and purified INH as a standard. The concentration of INH in plasma was found to be 40 \( \mu \)g/ml, or 0.47 \( \mu \)M.

To determine whether purified INH was the same size as INH in plasma or whether it had been proteolyzed during purification, INH was compared by gel chromatography with the corresponding inhibitor in bovine plasma. As shown in Fig. 3, INH eluted as a single peak slightly later than IgG, the internal standard, whether purified INH or plasma was applied. This suggests that the purification procedure had not significantly altered the size of the protein. When purified INH was subjected to gel chromatography in the presence of either 1.0 M NaCl or 0.1% SDS, the elution pattern was observed.

The amino acid and carbohydrate composition of INH was given in Table III (Miniprint). The extinction coefficient \( (E_{280})^{0.1} \) was found to be 1.3. A sample of INH whose protein concentration was 0.69 mg/ml (as determined by amino acid

**Fig. 1.** SDS-polyacrylamide gel electrophoresis of purified INH. Electrophoresis was performed by the method of Weber and Osborn (54), either with or without 100 mM dithiothreitol (DTT). Gels were stained for protein with Coomassie Blue and for carbohydrate with periodate-Schiff reagent.
and carbohydrate analysis) had an apparent concentration of 0.79 mg/ml when assayed by the Lowry method using BSA as a standard.

Interaction of INH₁ with Proteases—INH₁ was tested for its inhibitory action on several proteases, as shown in Table 1. INH₁ inhibited bovine and human factor XIIa and human plasma kallikrein. INH₁ also inhibited bovine and human C₁-esterase. However, INH₁ did not inhibit bovine kallikrein, human plasmin, or human thrombin, and it inhibited bovine trypsin only to a small degree.

The spectrum of activity for INH₁ was compared with the activities of several known human protease inhibitors (Table 1). INH₁ does not resemble ATIII because INH₁ does not inhibit thrombin. INH₁ does not inhibit plasmin, which suggests that INH₁ is not similar to α₂AP. Bovine INH₁ is most similar to human C₁INH in its specificity. Both INH₁ and C₁INH are able to inhibit bovine and human factor XIIa, bovine and human C₁-esterase, and human kallikrein. Neither inhibits bovine kallikrein. Human C₁INH is a weak inhibitor of plasmin (34).

Immunological Data—Ouchterlony reactions were carried out to detect any cross-reactivity between INH₁ and human C₁INH (Fig. 2). The rabbit antiserum raised against INH₁ reacted with INH₁ and with a single component in bovine plasma (Fig. 2, top). However, rabbit anti-INH₁ did not react with human C₁INH or with any component of human plasma. Conversely, antiserum to human C₁INH did not react with INH₁ or with any component of bovine plasma, although rabbit anti-C₁INH did react with human C₁INH and with a component of human plasma (Fig. 2, bottom). When INH₁ was assayed with a competitive enzyme-linked immunosorbent assay for human C₁INH, no reaction was observed in the range of 5–500 ng of INH₁. Reaction with human C₁INH could be detected at 5 ng of C₁INH.

The results of the Ouchterlony reactions showed no cross-reactivity between bovine and human proteins with rabbit antiserum raised against bovine INH₁ or against human C₁INH. This raised the possibility that the proteins in the three mammalian species were very similar. If this were true, then rabbits might raise antibodies only to the differences in the proteins. Denatured and native INH₁ were injected into chickens to obtain specific antibodies to INH₁. Reactions of

![Fig. 2. Ouchterlony immunodiffusion reactions.](image-url)

**Fig. 2.** Ouchterlony immunodiffusion reactions. Ouchterlony double immunodiffusion assays (15) were performed on glass microscope slides (1 x 3 inches) containing 2 ml of 0.5% agarose in 0.05 M sodium barbital buffer, pH 8.6. Samples of bovine plasma (BP), human plasma (HP), purified INH₁, or human C₁INH were applied to 1-mm diameter wells and allowed to diffuse at room temperature for 24 h. Slides were washed thoroughly in 0.01 M, 0.07 M sodium chloride buffer, pH 8.6, followed by deionized water. The gel was air dried, stained for protein with Coomassie Brilliant Blue, and destained as described (54). A, rabbit antiserum to bovine INH₁; B, rabbit antiserum to human C₁INH.

![Fig. 3. Comparison of gel filtration profiles of purified INH₁ and of INH₁ in plasma.](image-url)

**Fig. 3.** Comparison of gel filtration profiles of purified INH₁ and of INH₁ in plasma. Purified INH₁ or bovine plasma obtained by venipuncture was applied to a column of Bio-Gel A-0.5m (0.9 x 40 cm) in 0.01 M Tris, 0.15 M sodium chloride, 0.01 M EDTA buffer, pH 7.4, with 1 mg/ml BSA. Fluorescein isothiocyanate-IgG (M₀ = 160,000) was added to each sample prior to chromatography. Fractions were assayed for INH₁ antigen by Laurell rocket immunoelectrophoresis. Plasma INH₁ (A); purified INH₁ (O).
chickens immunized with denatured INH. Neither IgG from the antiserum of hens nor IgY from the egg yolks reacted with INH, and a single component from bovine plasma.

Interaction of INH, with Factor XIIa—To demonstrate complex formation between INH, and XIIa, 125I-labeled bovine factor XIIa was run on gel chromatography either alone or after incubation with excess INH, (Fig. 5). Factor XIIa alone on gel filtration had an apparent M, of 110,000, as compared with an internal standard of fluoresceinated IgG of 160,000. When labeled XIIa was preincubated with excess unlabeled INH, two radioactive peaks were seen on gel filtration, one with an M, greater than IgG and the other corresponding to the position of 125I-labeled XIIa alone. The higher M, peak would be expected if factor XIIa were in complex with INH,.

The proposed mechanism (Equation 1) suggests a 1:1 stoichiometry between XIIa and INH,. To quantitatively determine the stoichiometry of the reaction, a fixed amount of XIIa was incubated with different amounts of INH, at 37 °C for 24 h, and the remaining activity of XIIa was measured. The long incubation time was necessary to ensure that the reaction had gone to completion. A graph comparing the amount of INH, added with residual XIIa is shown in Fig. 6. Extrapolation to zero XIIa activity corresponds to 49 pmol of INH,. Since 45 pmol of XIIa was added to each reaction mixture, this evidence supports a 1:1 stoichiometry.

The kinetics of inactivation of bovine factor XIIa by INH, was investigated under pseudo-first order conditions. Fig. 7A is a plot of percent remaining XIIa activity versus time, using a different concentration of INH, for each reaction. The slopes obtained by linear regression of the data obtained for each concentration of INH, were replotted in Fig. 7B as the slope (kobs), versus the INH, concentration (see Equation 3). The dissociation constant K, for the initial reversible reaction (obtained from the intercept at the abscissa) was found to be 0.78 µM. The rate constant for the irreversible reaction obtained from the start of XIIa inactivation (the ordinate intercept) was 0.95/min. The second-order rate constant, k2/K1, is 1.23 × 10−4 M−1 min−1. When compared with other plasma protease inhibitors (Table II), INH, was found to be comparable to ClINH in its effectiveness.

**DISCUSSION**

An inhibitor of bovine factor XIIa has been purified to homogeneity from bovine plasma. The inhibitor, which we have called INH,, is a single-chain glycoprotein with an apparent M, of 85,000, reduced, and 92,000, nonreduced, by SDS-polyacrylamide gel electrophoresis. INH, is present in bovine plasma at a concentration of 0.47 µM.

Because the major inhibitor of human factor XIIa (2) and of human kallikrein (7) is ClINH, the first two steps in the isolation of INH, (PEG fractionation and chromatography on DEAE-cellulose) were based on procedures for the purification of human ClINH (40, 41). However, at all stages of the purification, INH, was identified and pooled on the basis of its ability to inhibit bovine factor XIIa. The Affi-Gel blue chromatography step, not used in ClINH purifications, was successful in separating INH, from contaminants other than albumin. Albumin was eliminated by the wheat germ lectin-
**Table II**

**Kinetic data on selected inhibitors**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Pro tease</th>
<th>$k_0/K_i$</th>
<th>Inhibitor plasma concentration</th>
<th>$k_{\text{rm}}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_1$PI</td>
<td>Human neutrophil elastase</td>
<td>3,900</td>
<td>3.7</td>
<td>14,430</td>
<td>(50)</td>
</tr>
<tr>
<td>$a_1$PI</td>
<td>Porcine trypsin</td>
<td>150</td>
<td>3.7</td>
<td>555</td>
<td>(50)</td>
</tr>
<tr>
<td>ATIII</td>
<td>Human thrombin</td>
<td>0.50</td>
<td>3.4</td>
<td>1.7</td>
<td>(51)</td>
</tr>
<tr>
<td>ATIII</td>
<td>Human thrombin + heparin</td>
<td>450</td>
<td>3.4</td>
<td>1,530</td>
<td>(51)</td>
</tr>
<tr>
<td>$a_2$AP</td>
<td>Human plasmin</td>
<td>1,400</td>
<td>1.0</td>
<td>1,400</td>
<td>(52)</td>
</tr>
<tr>
<td>ClINH</td>
<td>Human Clr</td>
<td>2.6</td>
<td>1.7</td>
<td>4.4</td>
<td>(53)</td>
</tr>
<tr>
<td>Human Cls</td>
<td>2.8</td>
<td>1.7</td>
<td>4.8</td>
<td>(53)</td>
<td></td>
</tr>
<tr>
<td>Human kallikrein</td>
<td>1.0</td>
<td>1.7</td>
<td>1.7</td>
<td>(7, 49)</td>
<td></td>
</tr>
<tr>
<td>Human XIIa</td>
<td>0.22</td>
<td>1.7</td>
<td>0.37</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>INH$_1$</td>
<td>Bovine XIIa</td>
<td>1.23</td>
<td>0.47</td>
<td>0.68</td>
<td>This paper</td>
</tr>
<tr>
<td>INH$_1$</td>
<td>Human kallikrein</td>
<td>0.29</td>
<td>0.47</td>
<td>0.14</td>
<td>This paper</td>
</tr>
</tbody>
</table>

**Fig. 7. Kinetics of inhibition of bovine factor XIIa by INH$_1$.**

All reactions were performed at 37°C. A, bovine factor XIIa (80 nM) was added to tubes containing INH$_1$ at the following concentrations: 167 nM (A), 222 nM (O), 333 nM (△), 500 nM (□), or 1120 nM (☆) in 0.1 M Tris buffer, pH 8.0, containing 1 mg/ml BSA and 1 mg/ml aprotinin. At the indicated times, aliquots of 0.1 ml of reaction mixture were removed and assayed for residual factor XIIa activity by the addition of 0.4 ml of S2302 (0.5 mM). B, replotted data in A to determine $k_0$ and $K_i$.  

Sepharose step, similar to the ClINH purification of Sim and Reboul (40) which used the high carbohydrate content of ClINH to bind it to concanavalin A-Sepharose.

A number of criteria were used to compare the characteristics of INH$_1$ with those of known inhibitors in order to identify IT-TI$_1$. The majority of inhibitors which have been thoroughly characterized are of human origin, and species difference could mask similarities. One of the major guides to identification of INH$_1$ is the spectrum of protease inhibition (Table I). Only human inhibitors were compared by activity since inhibitors from bovine and other species have not been thoroughly characterized in this manner. The inhibitor which most closely resembles INH$_1$ by activity is ClINH. Both INH$_1$ and ClINH inhibit C1-esterase, the activated first component of the classical complement system. Human ClINH was first described as an inhibitor of human C1-esterase (42), and it is the only human plasma inhibitor of C1-esterase. In addition, both INH$_1$ and human ClINH inhibit bovine and human XIIa and human kallikrein, and neither inhibits bovine kallikrein. Because INH$_1$ does not inhibit either plasmin or thrombin, it is unlikely that INH$_1$ is analogous to either $a_2$AP or ATIII.

The apparent $M_r$ of INH$_1$ on SDS-polyacrylamide gel electrophoresis is higher than that of human $a_2$AP (67,000), $a_1$PI (54,000), ATIII (58,000), or bovine ATIII (56,000). INH$_1$ is closer in size to the $M_r$, 75,000 plasminogen activation inhibitor (6), but INH$_1$ is retained on DEAE-cellulose while Hedner's inhibitor is not (44). Since the activity of the plasminogen activation inhibitor on the proteases in Table I has not been reported, a comparison by activity was not possible. The plasma concentration of INH$_1$ is lower than the concentrations of known human inhibitors (43) with the exception of the plasminogen activation inhibitor.

A comparison of physical characteristics of INH$_1$ with those of known inhibitors confirms that INH$_1$ is most similar to ClINH, although the two molecules are not identical. The $M_r$ of INH$_1$ (92,000 nonreduced and 85,000 reduced, by SDS-polyacrylamide gel electrophoresis) is 20,000 less than that of ClINH (112,000 nonreduced and 105,000 reduced). This difference is not a result of INH$_1$ being proteolytically cleaved during purification, as purified INH$_1$ and ClINH in plasma elute at the same position on gel filtration (Fig. 3). The slight decrease in the apparent $M_r$ of ClINH measured by SDS-polyacrylamide gel electrophoresis upon reduction, compared with its $M_r$ in the absence of reducing agents (45), has been explained by the single-chain ClINH molecule being held in a very rigid asymmetric conformation by disulfide bonds.
Electron micrographs of ClINH indicate that it is a very elongated molecule (46), and ClINH has a high relative Stokes radius on gel filtration (46).

The apparent $M_c$ of INH is also slightly higher nonreduced than reduced on SDS-polyacrylamide gel electrophoresis. This could be due to the loss of a small peptide when the disulfide bonds are broken upon reduction, or INH could be very asymmetric as is ClINH. Even with very large quantities of protein stained with Coomassie Blue or labeled with 125I, no smaller $M_c$ band of INH has been seen upon reduction. The apparent $M_c$ of INH, by gel filtration (150,000 in the presence of buffer, SDS, or high salt) suggests that INH is a very asymmetric molecule. Both INH and ClINH have a high concentration of carbohydrate (17.7 and 34.7%, respectively). Their amino acid compositions are fairly similar (Table III), although the two molecules cannot be compared directly (in terms of number of amino acid residues/molecule) since the $M_c$ of the two proteins are different.

Rabbit antiserum to INH did not react with human ClINH or any component of human plasma. Rabbit antiserum to human ClINH also did not react with INH, or bovine plasma. Lack of immunological reaction could mean that the two proteins share no antigenic determinants (13) or that the analogous proteins are so similar to those of the animal producing the antibodies that antibodies were only raised to small differences in the molecule (48). To test this alternative, antibodies were raised to denatured INH, in chickens. Although chickens as well as lower vertebrates have a complement system (48) it is probable that any ClINH-like molecules in chickens are not as similar to the bovine as are the rabbit’s ClINH-like molecules. Chicken anti-INH does not react with human plasma or ClINH, indicating that there is no recognition of a molecule in human plasma. Lack of cross-reactivity does not rule out protein similarities, and the immunological data could not be used to confirm identification of INH with a human protein.

The proposed mechanism of inhibition, in which a complex forms between an inhibitor and a protease (Equation 1), is a common mechanism among plasma protease inhibitors. The higher molecular weight peak of radioactivity seen on gel chromatography when $^{125}$I-labeled XIIa was incubated with excess inhibitor (Fig. 5) suggests that a stable complex also forms between INH and bovine factor XIIa. We have confirmed by end-point activity measurements (Fig. 6) that INH and XIIa interact with a 1:1 stoichiometry.

Results of kinetic studies on the inhibition of bovine factor XIIa by INH show a second-order rate constant ($k_r/K_i$) of $1.23 \times 10^4$ M$^{-1}$ min$^{-1}$ (Table II). This is faster than the rate reported by Pikey et al. (2) for the inhibition of human factor XIIa by human ClINH (2.2 $\times 10^4$ M$^{-1}$ min$^{-1}$). The physiological effectiveness of different inhibitors can be compared directly by comparing their values of $k_{on}$, the product of the second-order rate constant, and the plasma concentration of the inhibitor (2). Table II lists kinetic data and plasma concentrations of several human inhibitors and of INH, for direct comparison. Although ClINH is a relatively inefficient inhibitor, compared with $a_1PI$, ATIII, and $a_2AP$, it is the most effective inhibitor of XIIa (2). INH is also a relatively inefficient inhibitor but is twice as effective as ClINH in inhibiting XIIa. The relative effectiveness of INH in bovine plasma can only be definitively determined by comparison with other as yet uncharacterized bovine inhibitors of XIIa. Based on comparison with human plasma inhibitors INH appears to be an effective inhibitor of XIIa.

Acknowledgments—We thank Patrick McDevitt, Dr. Jerry Mendowski, and Daniel Roberts for providing bovine factor XIIa and kallikrein, and Barbara Keller for performing the amino acid analysis. We also acknowledge Drs. Robert Colman, Alvin Schmaier, Robin Pfixey, and Daniel Kari for their critical evaluation of this manuscript. The faculty and students from W. B. Saul Agricultural High School, particularly Barbara Mitchell, Gerald Brown, Stephen Feick, Maryann Dobbs, Lacinene Howard, and Tyrone Winkle, have been helpful in the preparation of chicken immunoglobulins and the obtaining of bovine venipuncture blood.

REFERENCES

Bovine Protease Inhibitor of Factor XIIa

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Additional references and materials are provided at the end of the text.
Bovine Protease Inhibitor of Factor XIIa

Figure 8. Elution profile on DEAE cellulose.

Bovine plasma (see Table I) was fractionated by the addition of solid PEG to ES. The supernatant (1800 ml) was dialyzed and applied to a DEAE cellulose column (4.5 x 3.5 cm) at a flow rate of 7.5 ml/hr, and fractions of 4 ml were collected. The column was equilibrated with 0.9 M KCl, 0.01 M potassium phosphate, 0.1 M potassium chloride, and 0.1 M potassium chloride, pH 7.5, and absorbed proteins were eluted with a linear gradient of 0-0.9 M KCl in 0.01 M potassium phosphate, pH 7.5. Fractions were assayed for protein by absorption at 280 nm (D280), for inhibition of bovine factor XIIa (D388), and for inhibitory activity by Laurell rocket immunoelectrophoresis (D07).

Figure 9. Elution profile on Affi-gel Blue.

The pooled fractions from the DEAE cellulose column (Figure 8) were applied to an Affi-gel blue column (1.6 x 28.5 cm). The flow rate was 6.1 ml/hr, and fractions of 8 ml were collected. The area indicates the start of a gradient of 150 ml each of 0.5 M sodium phosphate, pH 7.8, and 0.5 M sodium phosphate, pH 7.8, containing 0.5 M potassium chloride, 0.1 M potassium chloride, and 0.1 M potassium chloride, pH 7.5, and for inhibitory activity by Laurell rocket immunoelectrophoresis (D07).

Table III

Comparison of amino acid and carbohydrate composition of IMH with reported composition for C12H5

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Residues per 85,000</th>
<th>% Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>value</td>
<td>IMH</td>
<td>IMH</td>
</tr>
<tr>
<td>Asp</td>
<td>11.7</td>
<td>7.6</td>
</tr>
<tr>
<td>Thr</td>
<td>4.04</td>
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</tr>
<tr>
<td>Ser</td>
<td>62.0</td>
<td>18.3</td>
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<tr>
<td>Gly</td>
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<td>16.7</td>
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<td>Ala</td>
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<td>Asn</td>
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<td>Lys</td>
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<td>3.6</td>
</tr>
<tr>
<td>Arg</td>
<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Cys</td>
<td>4.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Val</td>
<td>8.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Pro</td>
<td>9.4</td>
<td>2.9</td>
</tr>
<tr>
<td>His</td>
<td>9.6</td>
<td>3.1</td>
</tr>
<tr>
<td>Tyr</td>
<td>35.4</td>
<td>10.9</td>
</tr>
<tr>
<td>Met</td>
<td>11.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Arg</td>
<td>17.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Ser</td>
<td>20.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Cys</td>
<td>3.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Carbohydrate (%)

- D.A.: 21.7
- N.A.: 39.4
- Glc: 41.0
- Gal: 16.1
- Total: 17.7

Table IV

Purification of IMH

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Protein (mg)</th>
<th>IMH (mg)</th>
<th>Purification</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine plasma</td>
<td>1000</td>
<td>48,088</td>
<td>40,200</td>
<td>1.0</td>
<td>100.0</td>
</tr>
<tr>
<td>ES PEG</td>
<td>3755</td>
<td>64,039</td>
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</tr>
<tr>
<td>DEAE pool</td>
<td>346</td>
<td>496</td>
<td>32,807</td>
<td>96</td>
<td>56.7</td>
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<tr>
<td>Affi-gel Blue pool</td>
<td>117</td>
<td>86</td>
<td>11,088</td>
<td>96</td>
<td>27.6</td>
</tr>
<tr>
<td>Wheat Germ</td>
<td>6.3</td>
<td>3.7</td>
<td>3,700</td>
<td>2080</td>
<td>9.1</td>
</tr>
</tbody>
</table>

(a) IMH, this paper
(b) S.A., specific activity; n.n., neutral sugar; g.l., glucuronic; g.l., galactosamine.

(c) not including 2.5E imported for adverse.

Table V

(a) IMH, concentration determined by Laurell rocket immunoelectrophoresis (D07)

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