Rat Plasma High-molecular-weight Kininogen

A SIMPLE METHOD FOR PURIFICATION AND ITS CHARACTERIZATION*

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Izumi Hayashi, Hisao Kato‡§, Sadaaki Iwanaga§, and Sachiko Oh-ishi

From the Department of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108 and the §Department of Biology, Faculty of Science, Kyushu University 33, Higashi-ku, Fukuoka 812, Japan

High-molecular-weight kininogen has been isolated from rat plasma in three steps in a relatively high yield. The purified preparation gave a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the absence and presence of 2-mercaptoethanol, and the apparent $M$, was estimated as 100,000. On incubation with rat plasma kallikrein, rat high $M$, kininogen yielded a kinin-free protein consisting of a heavy chain ($M_\text{r} = 64,000$) and a light chain ($M_\text{r} = 46,000$), liberating bradykinin. The kinin-free protein was S-alkylated, and its heavy and light chains were separated by a zinc-chelating Sepharose 6B column. The amino acid compositions of rat high $M$, kininogen and its heavy and light chains were very similar to those of bovine high $M$, kininogen and its heavy and fragment 1-2-light chains, respectively. A high histidine content in the light chain of rat high $M$, kininogen indicated the presence of a histidine-rich region in this protein as in bovine high $M$, kininogen, although this region was not cleaved by rat plasma kallikrein.

Rat high $M$, kininogen corrected to normal values the prolonged activated partial thromboplastin time of Brown-Norway Katholie rat plasma known to be deficient in high $M$, kininogen and of Fitzgerald trait plasma. The kinin-free protein had the same correcting activity as intact high $M$, kininogen. Rat high $M$, kininogen also accelerated approximately 10-fold the surface-dependent activation of rat factor XI and prekallikrein, which was mediated with kaolin, amyllose sulfate, and sulfatide. These results indicate that rat high $M$, kininogen is quite similar to human and bovine high $M$, kininogens in terms of biochemical and functional properties.

The rat has been widely used as an experimental model system for studies on the kallikrein-kinin system (1-3), and the physiological role of various tissue kallikreins is being studied in plasma (12, 13), vascular tissue (14), stomach (15), thyroid (16), pituitary gland (17), brain (18, 19), salivary gland (20), urine (21), and kidney (22). Recently, a congenital deficiency of the kallikrein-kinin system in Brown-Norway Katholie rat was reported by Damas and Adam (5, 9). We have studied the same strain of rats and have reported that the impairment is based upon a severe deficiency of high-molecular-weight kininogen and a moderate deficiency of prekallikrein (10, 11). Although the molecular structures and the complementary DNA sequences for bovine high-molecular-weight and low-molecular-weight kininogens have recently been established (23-26, 46), knowledge of the biochemical properties of kininogens in rat plasma is still limited. Thus, it seems important to characterize rat kininogen to obtain basic information that could be used to study its role in the kallikrein-kinin and the intrinsic coagulation systems. Recent findings of the presence of a third kininogen, named T-kininogen, in rat plasma (4-7), also prompted us to attempt to compare rat kininogens with other mammalian kininogens.

In the present paper, a simple method for the purification of rat HMW kininogen is presented, and its biochemical properties are described. A brief account of this work has appeared in an abstract (48).

EXPERIMENTAL PROCEDURES

Purification of High $M$, Kininogen

DEAE-Sephadex A-50 Column Chromatography—One hundred ml of frozen rat plasma was thawed in a water bath at 15°C and dialyzed overnight against 10 liters of 0.02 M Tris-HCl, pH 8.0, containing 0.05 M NaCl and 1 mM EDTA. After centrifugation at 4000 rpm for 15 min, using an HG-4L rotor in a Sorvall RC-3 centrifuge, the clear supernatant was applied to a column (5.5 x 30 cm) of DEAE-Sephadex A-50, equilibrated with 0.02 M Tris-HCl, pH 8.0, containing 0.05 M NaCl, Polybrene (0.5 g/liter), and benzamidine (50 mg/liter). After applying the sample, the column was washed with 2 liters of the equilibration buffer. Protein was eluted with a linear salt gradient formed from 2 liters each of the equilibration buffer and the buffer containing 0.3 M NaCl. As shown in Fig. 2, high $M$, kininogen was eluted with 0.3 M NaCl and separated from prekallikrein and factor XII. Each of the fractions containing high $M$, kininogen, prekallikrein, and factor XII was pooled as indicated by the absorbance at 280 nm.

Zinc-chelating Sepharose 6B Column Chromatography—The pooled high $M$, kininogen fraction from the previous step was applied to a column (1.0 x 16.4 cm) of Sepharose 6B which was equilibrated with 0.02 M Tris-HCl, pH 8.0, containing 0.2 M NaCl and 1 mM EDTA. The column was washed with 1 liter of the equilibration buffer containing 0.2 M NaCl and 1 mM EDTA. The high $M$, kininogen was eluted with a linear salt gradient formed from 2 liters each of the equilibration buffer and the buffer containing 0.5 M NaCl. The pooled high $M$, kininogen fraction was further purified by the zinc-chelating Sepharose 6B column as described above.

1 Portions of this paper (including "Experimental Procedures" and Figs. 10-12) 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. 84M-2814, cite the authors, and include a check or money order for $3.60 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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‡ To whom correspondence should be addressed.
was applied to a column (3.2 x 25 cm) of zinc-chelating Sepharose 6B, equilibrated with 0.02 M Tris-HCl, pH 8.0, containing 0.05 M NaCl. The column was washed with 500 ml of 0.02 M Tris-HCl, pH 8.0, containing 1 M NaCl and subsequently eluted first with a linear gradient formed from 1 liter each of the washing buffer and the same buffer containing 0.05 M EDTA. As shown in Fig. 3, high Mₖ kininogen was found in the fractions eluted with EDTA. The fractions containing high Mₖ kininogen were combined, as indicated by the solid bar, and dialyzed overnight against 10 liters of 0.2 M ammonium bicarbonate. The dialysate was lyophilized and dissolved in 1 ml of 0.02 M Tris-HCl, pH 8.0, containing 0.15 M NaCl.

**Sephadex G-150 Column Chromatography**—The high Mₖ kininogen fraction from the chelating Sepharose step was loaded onto a column of Sephadex G-150, equilibrated with 0.02 M Tris-HCl, pH 8.0, containing 0.15 M NaCl. As shown in Fig. 4, the protein eluted with the equilibration buffer was separated into two peaks, and the high Mₖ kininogen activity was found in a lower molecular weight fraction. The fractions indicated by a solid bar were collected and stored at -80°C. Table I presents a summary of the purification procedure. The overall yield of high Mₖ kininogen from 100 ml of rat plasma was 2.4 absorbance units, and the method was simple and reproducible.

**Purity and Molecular Weight of Isolated Rat High Mₖ Kininogen**—The purified preparation gave a single band on...
TABLE I

<table>
<thead>
<tr>
<th>Purification procedure</th>
<th>Volume (ml)</th>
<th>Total protein (units)</th>
<th>Total units</th>
<th>Specific activity</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>100</td>
<td>4896</td>
<td>123.0</td>
<td>0.025</td>
<td>100</td>
</tr>
<tr>
<td>DEAE-Sephadex A-50</td>
<td>470</td>
<td>412.7</td>
<td>110.0</td>
<td>0.267 (1)</td>
<td>89.4</td>
</tr>
<tr>
<td>Zinc-chelating Sepharose 6B</td>
<td>52</td>
<td>5.3</td>
<td>29.1</td>
<td>5.541 (220.5)</td>
<td>23.7</td>
</tr>
<tr>
<td>Sephadex G-150</td>
<td>55</td>
<td>2.4</td>
<td>28.4</td>
<td>12.0 (477.7)</td>
<td>23.1</td>
</tr>
</tbody>
</table>

*Absorbance unit at 280 nm.

Rat Plasma High-molecular-weight Kininogen

SDS-PAGE in the presence and absence of 2-mercaptoethanol (Fig. 5). By comparison with standard proteins run after reduction, the apparent molecular weight of the kininogen was estimated as 100,000, comparable to that of human high M, kininogen (33).

**Cleavage of Rat High M, Kininogen by Rat Plasma Kallikrein**—Fig. 6a shows an analysis by SDS-PAGE of the degradation of rat high M, kininogen by rat plasma kallikrein. High M, kininogen appeared to have been degraded, and two faint bands at M, 64,000 and 46,000 were detected on the unreduced gel. On the reduced gel, high M, kininogen was clearly shown to be cleaved into two chains, a heavy chain with M, 64,000 and a light chain with M, 46,000. To compare the kallikrein-catalyzed degradation of rat high M, kininogen with that of bovine high M, kininogen, the latter was hydrolyzed with rat plasma kallikrein. Fig. 6b shows that rat plasma kallikrein and bovine plasma kallikrein are capable of liberating the previously known fragment 1-2 (25) and the carbohydrate-free fragment 1-2 (34) very rapidly from bovine high M, kininogen. These results indicate that rat plasma kallikrein cleaves rat high M, kininogen into a protein (kinin-free protein) with two chains bridged by disulfide bonds, liberating kinin, and that it does not liberate any fragments from the kininogen, which is thus different from bovine high M, kininogen.

To characterize the heavy and light chains derived from rat high M, kininogen, the kininogen was incubated with rat plasma kallikrein on a preparative scale as described under "Experimental Procedures." The mixture was applied to a column (1.5 x 62 cm) of Sephadex G-50, equilibrated with 0.2 M ammonium bicarbonate. The result is shown in Fig. 7. A kinin-free protein was eluted as a single peak, which gave a single band without reduction on SDS-PAGE and two bands after reduction, as shown in Fig. 8, lanes 1 and 2. On the other hand, the kinin as assayed by HPLC was found in the fractions between tubes 50 and 60. The kinin-free protein that eluted in the void volume was S-alkylated, and the resulting...
heavy and light chains were separated by using a zinc-chelating Sepharose column, as described under "Experimental Procedures." The heavy and light chains thus isolated gave, respectively, a single band on SDS-PAGE, as shown in Fig. 8, lanes 3 and 4. Their mobilities corresponded to the heavy and light chains detected in the kinin-free protein (lane 2 in Fig. 8), and the molecular weight estimated after reduction on SDS-PAGE was 64,000 for the heavy chain and 46,000 for the light chain.

For identification of the kinin found in the later fractions (Fig. 7), these were collected, lyophilized, and analyzed by reversed-phase HPLC. The HPLC patterns of the kinin fraction are compared with those of synthetic kinins in Fig. 9. The retention time of the sample (Fig. 9 (1)) was equal to that of synthetic bradykinin (Fig. 9 (2)), and it was distinguishable from methionyl/lysyl/bradykinin, lysyl/bradykinin, and isoleucyl/seryl/bradykinin. Moreover, the amino acid composition of the kinin fraction was found to be: serine (1.1), glycine (1.1), phenylalanine (2.0), arginine (2.0), and proline (2.9), the same as the composition of bradykinin. From these results, kinin released from rat high Mₚ kininogen by rat plasma kallikrein was identified as bradykinin.

**Amino Acid Compositions of Rat High Mₚ Kininogen and Its Heavy and Light Chains**—In Table II, amino acid compositions of rat high Mₚ kininogen and its heavy and light chains are shown in comparison with the corresponding proteins.
derived from bovine high \( M \), kininogen. The total number of amino acid residues of rat high \( M \), kininogen was calculated to be 587, quite similar to that of bovine high \( M \), kininogen. The sum of the individual amino acid residues of the two chains and bradykinin was in good agreement with those of bovine high \( M \), kininogen. The amino acid compositions of rat heavy and light chains were also similar to those of the corresponding chains derived from bovine high \( M \), kininogen (23-25), although the integral numbers of several residues in rat light chain, such as Ser, Met, Arg, and Leu, were significantly different from those of bovine fragment 1.2-light chain.

The high content of histidine in rat light chain indicates that the histidine-rich region known in bovine fragment 1.2-light chain is also present in the light chain. Since rat light chain contains only 1 half-cystine residue, the heavy and light chains are believed to be bridged with a single disulfide bond, as in the case of bovine high \( M \), kininogen.

Functional Activity of Rat High \( M \), Kininogen—Fig. 10 shows the correction of the prolonged APTT of Brown-Norway Katholiek (BN-Ka) rat plasma by rat high \( M \), kininogen. The APTT of BN-Ka plasma was about 40 s. By the addition of rat high \( M \), kininogen, the prolonged APTT of BN-Ka plasma was diminished to 20 s. As reported previously (10, 11), the high \( M \), kininogen content in Brown-Norway Kitasato (BN-Ki) rat plasma was normal, as in Sprague-Dawley (BN-Ka) rat plasma.

![Fig. 7. Separation of kinin and kinin-free protein by gel filtration on a column of Sephadex G-50.](image)

![Fig. 8. SDS-PAGE of heavy and light chains derived from rat high \( M \), kininogen.](image)

![Fig. 9. Identification of kinin released from rat high \( M \), kininogen by rat plasma kallikrein.](image)
rat plasma. The APTT of BN-Ki rat plasma was 22 s, as expected. Therefore, this result indicates that rat high M, kininogen corrects the prolonged APTT of BN-Ka rat plasma to normal values. The addition of rat high M, kininogen to BN-Ki rat plasma did not change the APTT. The content of high M, kininogen in normal rat plasma, which was estimated by kinin release after kaolin activation, has been estimated to be about 500 pmol/ml of plasma (35). Since the prolonged APTT of BN-Ka rat plasma was corrected to normal values by the addition of 1 pmol of high M, kininogen to 20 µl of the plasma, normalization required 10% of the total high M, kininogen content of normal plasma.

Rat high M, kininogen, in addition to the kinin-free protein, also corrected the prolonged APTT of high M, kininogen-deficient plasma, Fitzgerald trait plasma, as shown in Fig. 11. The result demonstrates, therefore, that rat high M, kininogen retains its correcting activity after kinin release, as with human high M, kininogen (36, 37, 44).

To ascertain further the functional properties of rat high M, kininogen, its effect on the surface-mediated activation of factor XII and prekallikrein was examined, using preparations of rat factor XII and prekallikrein. As shown in Fig. 12, rat high M, kininogen accelerated the activation of factor XII and prekallikrein in the presence of kaolin, amylose sulfate, and sulfatide used as a surface. The maximal acceleration in the presence of the optimal amounts of high M, kininogen was found to be 5- to 10-fold.

DISCUSSION

Rat plasma is now known to contain at least three kinds of kininogens, each of which shows different susceptibilities to plasma and tissue kallikrein and trypsin (49). Of the three, a kininogen with M, 72,000, which is susceptible only to trypsin and releases T-kinin (Ile-Ser-bradykinin), has been purified recently by Bedi et al. (7) and Sakamoto et al. (6). A kininogen with an apparent M, 110,000, which releases kinin on treatment with trypsin and murine acid protease, is known in rat plasma (38); however, its properties have not been examined in detail. The results described in this paper present definitive functional, physicochemical, and structural criteria that the kininogen isolated is rat high M, kininogen. The purification procedure for rat high M, kininogen is simple and reproducible. It consists of three steps to obtain the homogeneous preparation. A zinc-chelating Sepharose 6B column is very effective for the isolation of high M, kininogen, since it strongly adsorbs the kininogen, which is separated completely from other kininogens (now being purified in our laboratory). Zinc-chelating Sepharose has been shown to be effective for the purification of various kinds of proteins. With regard to blood coagulation factors, zinc-chelating Sepharose has been used for the purification of fibrinogen (39) and factor XII (40). Since high M, kininogen contains a histidine-rich region (24), high M, kininogen was expected to have a high affinity for zinc-chelating Sepharose. In fact, rat high M, kininogen has a higher affinity for zinc-chelating Sepharose than factor XII and prekallikrein. Separation of high M, kininogen from factor XII and prekallikrein is essential for the purification of high M, kininogen, because they degrade high M, kininogen. The present procedure can be applied to high M, kininogens from other experimental animals, because the method does not require large amounts of plasma.

The apparent M, of rat high M, kininogen is about 100,000, as assessed by SDS-PAGE. However, this molecular weight seems not to be correct, as abnormal behavior of bovine high M, kininogen on SDS-PAGE and gel filtration has been described (41). This is probably due to the glycoprotein nature of the material. In fact, rat high M, kininogen on SDS-PAGE

### Table II

**Comparison of amino acid compositions of rat high M, kininogen with bovine high M, kininogen**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Rat*</th>
<th>Bovine*</th>
<th>Rat*</th>
<th>Bovine*</th>
<th>Rat*</th>
<th>Bovine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>68</td>
<td>65</td>
<td>47.0</td>
<td>47</td>
<td>41</td>
<td>25.5</td>
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<tr>
<td>Thr</td>
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<td>43</td>
<td>24.4</td>
<td>24</td>
<td>24</td>
<td>18.8</td>
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<tr>
<td>Ser</td>
<td>42.1</td>
<td>42</td>
<td>22.1</td>
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<tr>
<td>Glu</td>
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<td>60</td>
<td>45.2</td>
<td>45</td>
<td>43</td>
<td>26.6</td>
</tr>
<tr>
<td>Pro</td>
<td>37.5</td>
<td>38</td>
<td>18.1</td>
<td>18</td>
<td>18</td>
<td>17.6</td>
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<tr>
<td>Gly</td>
<td>46.1</td>
<td>46</td>
<td>22.6</td>
<td>22</td>
<td>16</td>
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<tr>
<td>Ala</td>
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<td>24</td>
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<td>Val</td>
<td>31.4</td>
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<td>23.5</td>
<td>24</td>
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<td>Met</td>
<td>4.1</td>
<td>4</td>
<td>4.5</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Ile</td>
<td>24.0</td>
<td>24</td>
<td>16.8</td>
<td>17</td>
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<td>9.2</td>
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<tr>
<td>Leu</td>
<td>36.8</td>
<td>37</td>
<td>24.0</td>
<td>24</td>
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<td>Tyr</td>
<td>14.4</td>
<td>14</td>
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<td>Phe</td>
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<td>Lys</td>
<td>44.9</td>
<td>45</td>
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<td>21</td>
<td>9.1</td>
<td>9</td>
<td>9</td>
<td>10.5</td>
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<tr>
<td>Total</td>
<td>557</td>
<td>603</td>
<td>361</td>
<td>361</td>
<td>361</td>
<td>361</td>
</tr>
</tbody>
</table>

Glucosamine: 17.9 (18)  9.0  11.3
Galactosamine: 4.3 (4)  6.5  8.1

* Hydrolysis with 4 N methanesulfonic acid for 24, 48, and 72 h.
* From Refs. 23-25.
* Determined as cystine.
* Calculated from the carboxymethyl-cysteine content in heavy chain and light chain.
* Determined as carboxymethyl-cysteine.
after reduction shows almost the same mobility as bovine high M, kininogen (data not shown), which has been established to have M, 76,000 based on the chemical structure, including carbohydrate units (24, 25). Moreover, the amino acid composition of isolated rat high M, kininogen is very similar to that of bovine high M, kininogen (Table II). These data indicate that rat high M, kininogen may have a molecular weight of approximately 76,000. Although there are a number of similarities between rat and bovine kininogens, a marked difference is found in the mode of cleavage by plasma kallikrein. As previously reported, plasma kallikrein initially liberates two fragments from bovine high M, kininogen, in addition to bradykinin: fragment 1-2 and carbohydrate-free fragment 1-2, which are extremely rich in histidine and glycine (23, 24, 34). In the case of rat high M, kininogen, only bradykinin is observed on degradation with rat plasma kallikrein. A similar observation with rat high M, kininogen has been reported in human high M, kininogen, which does not liberate the corresponding fragments with plasma kallikrein (42-44). However, the existence of the histidine-rich region corresponding to fragment 1-2 known in bovine high M, kininogen is also suggested in rat high M, kininogen, since the rat light chain is rich in histidine and glycine, and the rat kinin-free protein displays the correcting activity on high M, kininogen-deficient plasma. Therefore, it seems most likely that absence of liberation of the histidine-rich fragment is due to lack of peptide bonds susceptible to plasma kallikrein in rat high M, kininogen and not due to the specificity of rat plasma kallikrein because it can liberate the histidine-rich fragment from bovine high M, kininogen (Fig. 6b).

The degradation sequence of rat high M, kininogen with plasma kallikrein produces bradykinin and the kinin-free protein consisting of the heavy and light chains bridged by a single disulfide bond. SDS-PAGE and amino acid analyses of the resulting derivatives fulfilled all of the requirements for cleavage at two peptide bonds in high M, kininogen, although a possible release of small fragments cannot be excluded. In the degradation sequence of intact high M, kininogen with rat plasma kallikrein, two faint protein bands at M, 64,000 and 46,000 were seen, corresponding to those of the heavy and light chains detected on SDS-PAGE without reduction (Fig. 6a). However, these faint bands were not observed when the samples were treated with SDS at 55 °C instead of 80 °C (data not shown). Although the appearance of these derivatives cannot be explained in the present study, we suggest that the disulfide bond in high M, kininogen is very unstable and is cleaved during SDS-PAGE.

High M, kininogen is now known to have two different physiological functions from studies on high M, kininogens from bovine and human plasma: one is as a natural substrate for plasma kallikrein to generate bradykinin and the other is a role as a cofactor in the surface-mediated activation of factor XII. These functions are also proven for purified rat high M, kininogen as demonstrated in this paper. Rat high M, kininogen corrects not only the prolonged APTT of Fitzgerald trait plasma but also Brown-Norway Katholiek rat plasma, which has recently been identified to be deficient in high M, kininogen (10, 11). Moreover, the purified preparation accelerates all the kaolin-, amylose sulfate-, and sulfatide-mediated activations of factor XII and prekallikrein, which were tested by using the reconstituted system as bovine high M, kininogen (45, 50). Thus, all the results described indicate that rat high M, kininogen is functionally and structurally similar with those of human and bovine high M, kininogens thus far characterized (33).

Acknowledgments—We wish to express our thanks to Dr. T. Nakajima, Faculty of Pharmaceutical Sciences, University of Tokyo, for providing information on the condition for separation of kinins by HPLC. Thanks are given to Kazuko Usui-Kawaguchi for amino acid analyses and to Mizumo Akiyoshi for her expert secretarial assistance.

REFERENCES

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Rat Plasma High-molecular-weight Kinogen

SUPPLEMENTARY MATERIALS

to Rat Plasma High-molecular-weight Kinogenin
A Simple Method for Purification and Its Characterization

By I. Nagashii, H. Kato, G. Inoue and S. Oh-hashi

EXPERIMENTAL PROCEDURES

Syngenta-balinking rat was obtained from Shionogi Laboratory animal farm. Shionogi. Blood was collected and anticoagulated by adding ten-volume of 3.8% sodium citrate as reported previously (10). After the addition of ethanolamine 1.0 ml was added, followed by the addition of 0.1 M sodium to sodium acetate and then 9 ml of 70% ethanol.

RESULTS AND DISCUSSION

1. High-molecular-weight protein was isolated from plasma. Extracts from bovine serum and rat plasma were analyzed by SDS-PAGE. The high-molecular-weight protein had an apparent mol. wt of 150,000 daltons.

2. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

3. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

4. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

5. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

6. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

7. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

8. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

9. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

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11. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

12. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

13. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

14. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

15. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

16. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

17. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

18. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

19. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

20. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

21. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

22. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

23. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

24. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.

25. Kinogenin was isolated from rat plasma by gel filtration. The purified fraction contained the high-molecular-weight protein and was analyzed by SDS-PAGE. The apparent mol. wt of the high-molecular-weight protein was 150,000 daltons.