A Method for the Estimation of Human Plasma Kininogens I and II

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A practical method for the estimation of plasma kininogens I and II was described. The level of kininogen I was calculated as difference between total kininogen and kininogen II. One-tenth milliliter of heparinized plasma was incubated with 1.2 Frey units of human plasma kallikrein in 0.05 M phosphate buffer, pH 7.4, at 37°C for 45 minutes to deplete kininogen I completely. It was then heated at 62°C for 15 minutes and incubated with 100 μg of trypsin at 37°C for 45 minutes to release bradykinin from kininogen II. To measure the total kininogen level (kininogens I and II), 0.1 ml of plasma was first heated at 62°C for 15 minutes in 0.05 M phosphate buffer, pH 7.4. It was then incubated with 100 μg of trypsin at 37°C for 45 minutes.

Released plasma kinin was assayed on the isolated rat uterus against synthetic bradykinin as a standard. Total kininogen and kininogen II levels were expressed as the amount of bradykinin released by these procedures.

In 10 healthy subjects, the estimated levels for plasma kininogens I and II were 2.48 ± 0.28 and 3.15 ± 0.55 μg bradykinin equivalent per milliliter, respectively. It seems essential to estimate the changes of kininogens I and II instead of total kininogen for the study of plasma kallikrein-kinin system in pathological and physiological conditions.

Since Diniz and his collaborators (1961) first described a quantitative determination of kininogen levels in blood plasma,1 most of the studies on kininogen levels in the physiological and pathological conditions have been done by estimating total kininogen in human plasma.2–10

Margolis (1963) was the first who reported that kininogen was not a single entity but included at least two substrates which possessed widely differing affinities for the releasing enzymes. He activated plasma kallikreinogen (Component A) to plasma kallikrein by contact with glass surface which in turn released plasma kinin from kininogen I (Component B). Total kininogen was measured by the kinin released with trypsin or urinary kallikrein.11

There have since been other studies supporting the presence of two kininogens in the human blood plasma.12 According to Suzuki and collaborators, plasma kallikrein hydrolyzes only kininogen I. In this, it differs from glandular kallikrein and snake venom that digest both kininogens I and II.13

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MATERIALS AND METHODS

Materials

Human plasma kallikrein was prepared by casein activation according to the method of Habermann and Klett and standardized on dog blood pressure with swine pancreatic kallikrein (Padutin, Bayer A.G.) as a standard. Trypsin, 2×cryst., was a product of Worthington Biochem. Corp. Soybean trypsin inhibitor, 5×cryst., was a product of Nutritional Biochem. Corp. Synthetic bradykinin was supplied by Sandoz A.G., Basel, Switzerland. Glasswares which came into contact with plasma were all silicone coated. Plastic syringes or containers were preferred if available. Heparinized blood was drawn from antecubital vein, chilled in iced water bath immediately and centrifuged at 2,500×g at 4°C for 20 minutes to separate plasma.

Methods

1) Estimation of kininogen II

One-tenth milliliter of plasma was incubated with 1.2 Frey units of plasma kallikrein in 0.4 ml of 0.05 M phosphate buffer, pH 7.4, at 37°C for 45 minutes. During this procedure, kininogen I was hydrolyzed to release plasma kinin which was soon inactivated by plasma kininase. The kininogen I-free incubation mixture was then heated at 62°C for 15 minutes to inactivate kininase and then incubated with 100 μg of trypsin at 37°C for 45 minutes to release bradykinin from kininogen II. After incubation, 100 μg of soybean trypsin inhibitor (SBTI) were added to inactivate trypsin.

2) Estimation of total kininogen

The procedures were the same as above, except that incubation with plasma kallikrein was omitted. Bradykinin was released from heat-treated plasma.

Bioassay of released bradykinin was performed on the isolated rat uterus with synthetic bradykinin as a standard. The kininogen level in plasma was expressed as the amount of bradykinin released during incubation with trypsin. The difference between total kininogen and kininogen II levels represented kininogen I (Fig. 1).

3) Bioassay

Virgin female rats of Wistar strain, 150–200 g of body weight, were pretreated with hypodermic injection of 0.1 mg of estradiol dipropionate 16 to 24 hours before use. The animals were sacrificed by a blow on the head and were bled from the carotid artery. One of the uterine horns was cut out and hung up in the muscle bath filled with De Jalon solution and aerated with oxygen. Contraction of the muscle was recorded on a smoked kymograph with a lever. Temperature of the organ bath was kept at 30°C in a thermo unit and a tension of 0.6 g was given to the muscle. Two micrograms per milliliter solution of synthetic brady-
Estimation of Human Plasma Kinogens I and II

Fig. 1. An assay method of human plasma kinogens I and II.

Fig. 2. The effect of incubation time on liberation of bradykinin from 0.1ml of heated plasma (62°C, 15 min) by trypsin at 37°C, at pH 7.4.

Comment on the methods

Fig. 2 showed bradykinin released during incubation of 0.1 ml of heated (62°C, 15 minutes) plasma with 100 μg of trypsin at 37°C. After 30 minutes, all kininogen was hydrolyzed and bradykinin was liberated.

Various doses (5–500 μg) of trypsin were incubated with 0.1 ml of heated plasma in the same conditions as above. As shown in Fig. 3, 50 μg or more trypsin hydrolyzed kininogen completely.

Various doses of plasma kallikrein (0.2–1.6 F.U.) were used to determine the amount of plasma kallikrein enough to deplete kininogen I from the plasma for the estimation of kininogen II. As shown in Fig. 4, bradykinin released by trypsin was minimal when 0.8 F.U. or more plasma kallikrein was used.
Fig. 3. The effect of trypsin concentration on liberation of bradykinin from 0.1 ml of heated plasma (62°C, 15 minutes) at 37°C, at pH 7.4 and 45 minutes' incubation.

Fig. 4. The effect of concentration of human plasma kallikrein on decay of kininogen I in 0.1 ml of plasma. See text.

Fig. 5. The effect of incubation time on decay of kininogen I in 0.1 ml of plasma by human plasma kallikrein. See text.
Fig. 5 also showed bradykinin released by the procedure 1 in which various incubation times for plasma kallikrein were used. Thirty minutes were enough to deplete kininogen I from the plasma under the above-mentioned condition.

RESULTS

Plasma kininogen levels were estimated in 10 healthy human adults consisting of 5 male and 5 female subjects. As shown in Table 1, total kininogen ranged 4.1–6.9 µg bradykinin equivalent per milliliter plasma (mean 5.63±0.76). Kininogens I and II were 2.0–2.9 (mean 2.48±0.28) and 2.0–4.0 (mean 3.15±0.55) µg/ml plasma, respectively.

Table 1. Plasma kininogen levels in healthy subjects

<table>
<thead>
<tr>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>Kininogen µg BK/ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>1. S.E.</td>
<td>F</td>
<td>54</td>
<td>6.9</td>
</tr>
<tr>
<td>2. N.T.</td>
<td>F</td>
<td>20</td>
<td>6.2</td>
</tr>
<tr>
<td>3. T.M.</td>
<td>M</td>
<td>30</td>
<td>6.1</td>
</tr>
<tr>
<td>4. M.S.</td>
<td>F</td>
<td>21</td>
<td>6.0</td>
</tr>
<tr>
<td>5. M.F.</td>
<td>F</td>
<td>45</td>
<td>5.8</td>
</tr>
<tr>
<td>6. I.M.</td>
<td>M</td>
<td>34</td>
<td>5.7</td>
</tr>
<tr>
<td>7. N.I.</td>
<td>M</td>
<td>26</td>
<td>5.5</td>
</tr>
<tr>
<td>8. K.A.</td>
<td>M</td>
<td>35</td>
<td>5.4</td>
</tr>
<tr>
<td>9. N.K.</td>
<td>F</td>
<td>20</td>
<td>4.6</td>
</tr>
<tr>
<td>10. Y.M.</td>
<td>M</td>
<td>28'</td>
<td>4.1</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>5.63±0.76</td>
</tr>
</tbody>
</table>

DISCUSSION

Different names have been coined for the two kininogen components by authors who studied kininogen in blood plasma of mammals. Margolis gave the name Componet B to the substrate in plasma which released kinin by plasma kallikrein (activated Component A). Habermann et al. purified two kininogens of different molecular weight from bovine plasma and named them kininogens I and II, although they did not describe which one of them was susceptible to plasma kallikrein. Jacobsen, and Jacobsen and Kriz also purified two different kininogens from human, dog and rabbit plasma and named them substrates 1 and 2. Substrate 1 released the same amount of kinin when incubated with either plasma kallikrein or glandular kallikrein, while substrate 2 did not produce kinin through the action of plasma kallikrein. Suzuki and collaborators described separation of two different kininogens, kininogens I and II, from bovine plasma. They found that plasma kallikrein which was activated either by glass contact or by casein precipitation hydrolyzed only kininogen I which had a larger molecular weight than kininogen II. On the other hand, snake venom and glandular kallikrein released kinin from both of them.

The studies of Jacobsen and Suzuki are based on enzymological treatment in contrast to other investigations. In addition, both authors obtained so similar
results that it seems reasonable to assume that substrates 1 and 2 of the former author are equal to kininogens I and II of the latter. The nomenclature of Suzuki is used in the present paper.

Table 2 shows human plasma kininogen levels reported by various authors. In the papers of Margolis and Jacobsen, kininogen levels for plasma kallikrein were reported. Blood plasma contains kallikrein inhibitor which can inactivate plasma kallikrein before the substrate for the latter is used up. According to Werle, blood plasma of mammals contains about 1 to 5 K.I.U./ml of kallikrein inhibitor. For this reason, addition of exogenous plasma kallikrein is necessary to ensure hydrolysis of all kininogen I in the plasma. In the present study, 12 Frey units of human plasma kallikrein were used for one milliliter of plasma to overcome kallikrein inhibitor of plasma.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Total kininogen μg BK/ml plasma</th>
<th>Kininogen I μg BK/ml plasma</th>
<th>Kininogen II μg BK/ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diniz</td>
<td>7.4–14.3</td>
<td>1–1.5</td>
<td></td>
</tr>
<tr>
<td>Margolis</td>
<td>3–4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasciolo</td>
<td>6–10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sicuteri</td>
<td>2–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rinvik</td>
<td>4.2–6.7(5.47±0.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trautschold</td>
<td>15–20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jacobsen</td>
<td>2.8–4.5</td>
<td>0.5–1.0</td>
<td></td>
</tr>
<tr>
<td>Brocklehurst</td>
<td>6.1±1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouri</td>
<td>4.1–6.9(5.63±0.76)</td>
<td>2.0–2.9(2.48±0.28)</td>
<td>2.0–4.0(3.15±0.55)</td>
</tr>
</tbody>
</table>

The total kininogen level in the present method was in good accordance with that reported by Rinvik and by Brocklehurst. However, the kininogen I level was higher than that reported by Margolis and by Jacobsen. In the present study, all the kininogen levels are expressed in terms of bradykinin, because trypsin is used to release kinin from total kininogen and from kininogen II (kininogen I depleted substrate).

For the study of plasma kallikrein-kinin system, it seems essential to estimate the changes of kininogens I and II instead of total kininogen. The present method is sensitive enough for practical use in the simultaneous estimation of human plasma kininogens I and II.

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References


