Activity of Dipeptidyl Peptidase IV and Post-Proline Cleaving Enzyme in Sera from Osteoporotic Patients

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The activity of dipeptidyl peptidase IV (EC 3.4.14.5) in human sera from normal controls and osteoporotic patients was assayed with Gly-Pro-4-methylcoumaryl-7-amide (Gly-Pro-MCA) as substrate, at pH 8.7. The mean normal value for the activity in serum of 120 healthy subjects (ages 20–86 y) was 54.0 (SE 0.9, range 34.3–81.6) U/L at 37 °C and differed slightly but significantly between the younger group (<50 y old) and those older than 50 y. Values for the younger men were slightly but significantly higher than for the older men. Overall, however, the enzyme activities in serum were above normal in patients with osteoporosis. In contrast, the activity of post-proline cleaving enzyme (PPCE) was not increased in serum from patients with osteoporosis, as determined with succinyl-Gly-Pro-4-methylcoumaryl-7-amide (Suc-Gly-Pro-MCA) as substrate. These results suggest that high activities of dipeptidyl peptidase IV in serum of patients with osteoporosis are probably related to its severity.

Additional Keyphrases: enzyme activity · synthetic substrates · sex- and age-related effects

Dipeptidyl peptidase IV (DAP IV; dipeptidyl-peptide hydrolase IV, EC 3.4.14.5), a serine peptidase, hydrolyzes peptides with an N-terminal sequence of X-Pro-Y- to yield X-Pro and Y- at pH 8.6

DAP IV was discovered by Hopeu-Havu and Glenner in 1966 (1) by using glylyproline β-naphthylamide, and was designated glylyproline β-naphthylamidase. Oya et al. (2) purified the enzyme from human submaxillary gland and showed (3) that it hydrolyzed the peptide bond between N-terminal glylyproline and adjacent amino acids alanine, leucine, glutamic acid, and leucylglylyproline, but not proline or hydroxypyroline. The enzyme was shown to be specific for the second-from-the-terminal amino acid, proline (4). Gly-Pro p-nitroanilide was the best chromogenic substrate among various X-Pro p-nitroanilides. Another fluorogenic substrate, Gly-Pro-4-methylcoumaryl-7-amide (Gly-Pro-MCA), had similar substrate activity to Gly-Pro p-nitroanilide (5). The physiological role of DAP IV is not clear. One probable function is hydrolysis of peptides derived from collagen, which contains repeated Gly-Pro sequences. The other probable function is hydrolysis of substance P in the brain (6).

We had previously reported the activity of DAP IV in normal human serum with X-Pro p-nitroanilides as substrates and demonstrated that the activity of the enzyme was abnormally increased in patients with hepatobiliary diseases, and decreased in patients with various solid and blood cancers (7–9), and in collagen diseases such as rheumatoid arthritis and lupus erythematosus (10). Post-proline cleaving enzyme (PPCE, prolyl endopeptidase, EC 3.4.21.26), being also a serine peptidase, cleaves -X-Pro-Y- to yield -X-Pro and Y. PPCE is capable of degrading many neuropeptides, such as substance P, angiotensin II, bradykinin, and neurotensin (11). Suc-Gly-Pro-MCA is one of the most sensitive substrates for PPCE (12). Here, we report DAP IV activities, with Gly-Pro-MCA as substrate, and PPCE activities, with Suc-Gly-Pro-MCA as substrate, in human sera from osteoporotic patients, whose collagenolytic activity in bone is assumed to be greater than that for normal controls.

Materials and Methods

Blood was sampled by venipuncture. After clotting, the serum was separated by centrifuging at 700 × g for 15 min. The ages and sexes of the 120 normal subjects and 30 subjects with osteoporosis are shown in Table 1.

The 30 osteoprotic subjects, all postmenopausal women, had low back pain and showed evidence of demineralization on lateral films of the vertebrae. According to the Pogrand index (13), the grade of severity of osteoporotic patients was classified as grade 2 (15 subjects) or grade 3 (15 subjects).

Patients with osteoporosis attributable to secondary causes (abnormalities of endogenous steroids, treatment with exogenous steroids, hyperthyroidism, hyperparathyroidism, androgen deficiency) were excluded.

The control population of older women was closely age-matched to the osteoporotic population. In osteoporotic subjects, DAP IV activities were compared with the grade of severity of osteoporosis according to the Pogrand index.

Gly-Pro-MCA, synthesized as described previously (1), was used to measure DAP-IV activity by fluorometry of the 7-amino-4-methylcoumarin (AMC) liberated from the substrate (1). DAP-IV activity was measured as described previously (4), except that we changed the pH of the incubation to the optimal pH 8.7 in glycine/NaOH buffer, instead of pH 7.0 in Tris/maleate buffer. The reaction mixture (total volume, 0.1 mL) contained 40 μL of 0.15 mol/L glycine/NaOH buffer (pH...
Table 1. Activity of DAP IV in Human Sera (Gly-Pro-MCA as Substrate)

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Age (mean ± SE, range), y</th>
<th>Activity (mean ± SE, range), U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>60</td>
<td>48.2 ± 1.5 (22-72)</td>
<td>54.1 ± 1.3 (34.3-81.6)</td>
</tr>
<tr>
<td>Younger</td>
<td>30</td>
<td>38.7 ± 1.1 (22-49)</td>
<td>57.7 ± 1.8 (42.2-81.6)*</td>
</tr>
<tr>
<td>Older</td>
<td>30</td>
<td>57.7 ± 1.1 (50-72)</td>
<td>50.4 ± 1.8 (34.3-70.2)</td>
</tr>
<tr>
<td>Women</td>
<td>60</td>
<td>51.5 ± 2.1 (20-86)</td>
<td>53.9 ± 1.1 (39.4-79.0)</td>
</tr>
<tr>
<td>Younger</td>
<td>30</td>
<td>38.3 ± 1.5 (20-49)</td>
<td>54.0 ± 1.7 (39.4-79.0)</td>
</tr>
<tr>
<td>Older</td>
<td>30</td>
<td>64.7 ± 1.6 (50-86)</td>
<td>53.8 ± 1.4 (41.5-70.2)</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>49.6 ± 1.3 (20-86)</td>
<td>54.0 ± 0.9 (34.3-81.6)</td>
</tr>
<tr>
<td>Younger</td>
<td>60</td>
<td>38.5 ± 0.9 (20-49)</td>
<td>55.9 ± 1.2 (39.4-81.6)*</td>
</tr>
<tr>
<td>Older</td>
<td>60</td>
<td>61.2 ± 1.0 (50-86)</td>
<td>52.1 ± 1.1 (34.3-70.2)</td>
</tr>
<tr>
<td>Osteoporotic women</td>
<td>30</td>
<td>72.1 ± 1.6 (52-91)</td>
<td>70.7 ± 1.8 (53.4-96.0)*</td>
</tr>
</tbody>
</table>

*Significantly higher than those of older men, P < 0.05.
*Significantly higher than those of total older control subjects, P < 0.05.
*Significantly higher than values for older women control group, P < 0.001.

8.7), 30 μL of 4 mmol/L Gly-Pro-MCA, and 30 μL of human serum. Instead of serum, the blank and standard tubes, respectively, contained 30 μL of water and 20 μL of water plus 10 μL (1.00 nmol) of 100 μmol/L AMC reagent. The control tube contained no serum. All samples were incubated at 37 °C for 30 min; the reaction was stopped by adding 1.0 mL of acetate buffer (1 mol/L, pH 4.2). After stopping the reaction, we added 30 μL of serum to the control tube. The fluorescence intensities of the samples (Sa), control (C), standard (St), and blank (B) were measured at 460 nm with a RF-500 (Shimadzu, Kyoto, Japan) spectrofluorometer (380 nm excitation wavelength). AMC, U/L in serum (37 °C), liberated by the enzyme reaction was calculated as follows:

\[
\frac{Sa - C}{St - B} \times 1 \text{nmol} \times \frac{1}{30 \text{ min}} \times \frac{1}{0.00003} = \frac{10(Sa - C)}{9(St - B)}
\]

PPCE activity with Suc-Gly-Pro-MCA as substrate (synthesized at the Peptide Institute) was also assayed by the fluorometric measurement of AMC liberated from the substrate (6).

The reaction mixture (total volume, 0.1 mL) contained 25 μL of sodium phosphate buffer (pH 6.8, 0.2 mol/L, containing 1 mmol of EDTA per liter), 50 μL of 2 mmol/L Suc-Gly-Pro-MCA, and 25 μL of human serum. Instead of serum, the blank and standard tubes respectively contained 25 μL of water and 15 μL of water plus 10 μL (1.00 nmol) of the 100 μmol/L AMC standard. The control tube contained no serum. The rest of the procedure was as described for DAP IV except that incubation was for 10 min, and the AMC liberated, U/L in serum (37 °C), was calculated as:

\[
\frac{Sa - C}{St - B} \times 1 \text{nmol} \times \frac{1}{10 \text{ min}} \times \frac{1}{0.000025} = \frac{4(Sa - C)}{(St - B)}
\]

Alkaline phosphatase (15), Ca (16), and P (17) in serum were measured by conventional procedures.

Results

Measurements of enzyme reaction products varied linearly with duration of incubation (for at least 2 h at 37 °C) and with the volume of serum (5-60 μL). Mean DAP IV activity, as measured with Gly-Pro-MCA as substrate, in 120 normal human sera was 54.0 (SE 0.9) U/L (Table 1). The mean values for control subjects younger than 50 years were slightly but significantly (P < 0.05) higher than those for male control subjects older than 50 y, but this was not the case for the women.

The mean value for activities for 30 women with osteoporosis was 70.7 (SE 1.8, range 53.4-96.0) U/L, significantly (P < 0.001) higher than in the older group of healthy women. Moreover, the activity was increased in parallel with the severity of the osteoporosis (Figure 1).

![Fig. 1. DAP IV activities in sera from osteoporotic patients](image-url)
Mean PPCE activity in serum from women with osteoporosis was not significantly different from that from the older female control group: 4.04 (SE 0.10, range 2.37–5.17) vs 3.93 (SE 0.14, 2.13–6.46) U/L.

Other biochemical data (mean, range) for serum from women with osteoporosis were: alkaline phosphatase, 73.4 U/L, 37.8–92.5; Ca, 94.8 mg/L, 90.0–105; and P, 34.0 mg/L, 25.3–42.8. These values were within the normal reference intervals.

Discussion

In our previous reports, the activity of DAP IV in serum with Gly-Pro β-naphthylamide as substrate was found to be a diagnostic index in some diseases (8, 9).

In the present study, DAP IV activity as measured in normal human serum with Gly-Pro-MCA as substrate was ~50 U/L, and individual variations were small. This result was similar to our previous report, although another substrate was used in that study.

The enzyme activities differed slightly but significantly with age between the younger group (<50 y old) and the older group (>50 y old). Men, especially the younger ones, had significantly higher activities than did older men. Hino et al. (7) reported age-related changes of DAP IV activities with Gly-Pro β-naphthylamide as substrate in normal human sera; values for the younger group (<40 y old) of men were higher than those >40 y old, but values for older women were higher than for younger ones. We observed a similar change for men, but not for women. The reason may be too small (n = 11 to 24) numbers of subjects in their (7) study.

The serum enzyme activity was found to be increased in women with osteoporosis. Most women with osteoporosis have high bone turnover, or at least a higher rate of resorption than of formation of bone (14). A physiological significance of DAP IV is considered to be implicated in the degradation of peptides derived from collagen, because the enzyme preferentially hydrolyzes the N-terminal glycylproline sequence (4). Therefore, our results are interpreted as being an indication of increased collagen breakdown in the bone of patients with osteoporosis. In fact, the degree of increase in serum DAP IV activity paralleled the severity of osteoporosis. As a result of increased collagen breakdown, hyper-resorption of bone may occur in osteoporosis. The high DAP IV activity in sera from women with osteoporosis is probably related to the process of hyper-resorption of bone in osteoporosis.

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References