Activities of Dipeptidyl Peptidase II and Dipeptidyl Peptidase IV in Mice with Lupus Erythematosus-like Syndrome and in Patients with Lupus Erythematosus and Rheumatoid Arthritis

Masako Hagihara, Masaru Ohhashi, and Toshiharu Nagatsu

We examined the activities of peptidases in plasma and tissues of the New Zealand Black (NZB) mouse as an animal model of human systemic lupus erythematosus, and also in serum from patients with rheumatoid arthritis and systemic lupus erythematosus. Activities of dipeptidyl peptidase II (DAP II) and post-proline cleaving enzyme (PPCE) were increased, and dipeptidyl peptidase IV (DAP IV) activity was decreased in plasma and spleen of NZB mice, as compared with the control BALB/c mice. Likewise, the activity of DAP II was increased and that of DAP IV was decreased in serum of patients with rheumatoid arthritis and systemic lupus erythematosus. These results indicate the importance of hydrolytic enzymes in the pathogenesis of autoimmune diseases.

Additional Keyphrases: prolyl endopeptidase · autoantibodies · autoimmune disease

Dipeptidyl peptidase II (EC 3.4.14.2; DAP II) and dipeptidyl peptidase IV (EC 3.4.14.5; DAP IV), both dipeptidyl-peptide hydrolases, cleave dipeptide from unsubstituted NH₂ termini of dipeptide derivatives.¹ DAP II differs from DAP IV in its substrate specificity, subcellular localization, and pH optimum. DAP II, a serine peptidase with a restricted substrate specificity, hydrolyzes preferentially Lys-Ala-2-naphthylamide at pH 5.5, but also cleaves the N-terminal dipeptide from 2-naphthylamides and tripeptides with a penultimate alanine or prolyl residue (1). DAP IV, also a serine peptidase, hydrolyzes peptides having an N-terminal sequence of X-Pro-Y to yield X-Pro and Y at pH 8.0 (2).

Using a new and specific assay for DAP II activity with 7-Lys-Ala-4-methylcoumarinamide (Lys-Ala-MCA) as a fluorogenic substrate, we recently found that DAP II activity was increased in serum from cancer patients, whereas DAP IV activity was decreased (3, 4). We also found that serum DAP II activity was increased in tumor-bearing mice having increased ratios of DAP II/DAP IV activities (4).

In a recent study, Aoyagi et al. (5) noted changes in the activities of post-proline cleaving enzyme (PPCE; prolyl endopeptidase, EC 3.4.21.26) in the spleen of an animal model of lupus erythematosus [hybrids of New Zealand Black (NZB) and New Zealand White mice]. The activity of PPCE progressively increased with age in the hybrid mice with lupus, in contrast to the opposite tendency in control mice. Thus this peptidase may have some biochemically measurable relation to the development of immunological abnormalities.

We have examined DAP II and DAP IV activities, and their ratios, in plasma and tissues of NZB mice with lupus erythematosus-like syndrome and in human serum from patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). We also examined the activities of PPCE and collagenase-like peptidase (CL-peptidase) in plasma and tissues of NZB mice, and compared these with the DAP II and DAP IV activities.

Materials and Methods

Samples. NZB mice and BALB/c mice were obtained from Charles River Laboratories, Japan Inc., Atsugi, Kanagawa 243-02, Japan. BALB/c mice were used as controls. We used only female mice in these experiments, killing them by decapitation at six, 12, 15, 20, or 25 weeks of age. We also assayed serum samples from patients with RA or SLE. Mouse tissues and human sera were stored at −80°C until assay. We homogenized the mouse tissues in 0.25 mol/L sucrose solution, centrifuged the homogenate at 30,000 × g for 20 min, and measured enzymatic activities in the supernate.

Substrates for enzyme assay. The following substrates were obtained from the Peptide Institute, Protein Research Foundation (Minoh, Osaka 562, Japan): 7-amino-4-methylcoumarin, Lys-Ala-MCA, Gly-Pro-MCA, (succinyl-Gly-Pro)-MCA, and (succinyl-Gly-Pro-Leu-Gly-Pro)-MCA.

Buffers. We used the following buffers: "universal" buffer (0.2 mol/L of sodium borate and 0.05 mol/L of citrate per liter, adjusted to pH 5.3 with 0.1 mol/L sodium phosphate buffer) for the DAP II assay, glycine–NaOH buffer (0.15 mol/L each, pH 8.7) for DAP IV, 0.2 mol/L sodium phosphate buffer (pH 6.8) containing 1 mmol of EDTA per liter for PPCE, and Tris–maleate buffer (0.2 mol/L each, pH 8.0) containing 20 mmol of CaCl₂ per liter for CL-peptidase.

Enzyme activity. All enzyme activities were assayed fluorometrically by measuring the enzymatic formation of 7-amino-4-methylcoumarin. DAP II activity was measured by "high-performance" liquid chromatography with fluorometric detection, with Lys-Ala-MCA as substrate (2). DAP IV activity was measured fluorometrically, with Gly-Pro-MCA as substrate, as described previously (6). PPCE activity was determined by fluorometry, with (succinyl-Gly-Pro)-MCA as substrate (7). CL-peptidase activity, with (succinyl-Gly-Pro-Leu-Gly-Pro)-MCA as substrate, was assayed by fluorometry of the 7-amino-4-methylcoumarin liberated from the reaction product Gly-Pro-MCA by the second enzyme reaction with DAP IV (8). Protein was assayed by the method of Lowry et al. (9). IgG, IgA, and complement components C₃ and C₄ were determined by single radial immunodiffusion (10, 11).
Results

Table 1 compares the plasma enzyme activities of NZB mice and the control mice. At three weeks of age, the NZB mice had activities of DAP II, DAP IV, PPCE, and CL-peptidase that were significantly lower than those of the controls. Activities of three of the enzymes stayed below that of the controls, but between 15 and 20 weeks of age the activity of DAP II activity began to increase, leading to a significantly increased ratio DAP II/DAP IV in NZB mice (P <0.005). By 25 weeks of age, the significance of the increased DAP II/DAP IV ratio was P <0.001.

Table 2 similarly compares the changes in various enzyme activities in the spleen and kidney of NZB and control mice.

The activities of DAP II and DAP IV in human serum from control patients, patients with RA, and patients with SLE are shown in Table 3. Data on IgG, IgA, CH50, C9, and C3 are also included. DAP II activity significantly exceeded control values in patients with RA (P <0.01) and in patients with SLE (P <0.001), whereas DAP IV activity was significantly lower in patients with RA (P <0.01) and in patients with SLE (P <0.001). Therefore, the DAP II/DAP IV ratio was also significantly increased in patients with RA (P <0.01) and in patients with SLE (P <0.001).

Discussion

Some studies indicate that hydrolytic enzymes participate in the pathogenesis of immunoallergic disturbances (12–14), but the specific roles of these enzymes have not yet been elucidated.
Table 3. DAP II and DAP IV Activities (Mean ± SD) in Serum from Patients with RA or SLE

<table>
<thead>
<tr>
<th>Serum</th>
<th>n</th>
<th>DAP II</th>
<th>DAP IV</th>
<th>(DAP II/DAP IV) × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>25</td>
<td>1.23 ± 0.24</td>
<td>41.30 ± 4.78</td>
<td>2.93 ± 0.47</td>
</tr>
<tr>
<td>(lg G, 1120 ± 230 mg/dL; Ig A, 264 ± 85 mg/dL; CH₅₀, 90 ± 20 units/mL; C₉, 90 ± 20 mg/dL; C₆, 36 ± 23 mg/dL)</td>
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<tr>
<td>RA</td>
<td>21</td>
<td>1.75 ± 0.25*</td>
<td>34.54 ± 3.15*</td>
<td>5.13 ± 0.93***</td>
</tr>
<tr>
<td>(lg G, 1198 ± 226 mg/dL; Ig A, 309 ± 103 mg/dL)</td>
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<tr>
<td>SLE</td>
<td>21</td>
<td>2.22 ± 0.64***</td>
<td>29.94 ± 64.6***</td>
<td>7.23 ± 0.39***</td>
</tr>
<tr>
<td>(CH₅₀, 33 ± 12 units/mL; C₉, 85 ± 20 mg/dL; C₆, 19 ± 10 mg/dL)</td>
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Significance of results indicated as in Table 1.

To our knowledge, this is the first report of DAP II activity in mice with SLE-like syndrome and in patients with RA or SLE. An interesting finding was the opposite changes in the activities of DAP II and DAP IV, which is similar to our previous findings concerning tumor-bearing animals and cancer patients (4).

We found in the present study that plasma DAP II activity increased with age in NZB mice, whereas plasma DAP IV activity decreased. Plasma PPCE and CL-peptidase activities were lower in NZB mice than in the control mice. During maturational development, DAP II activity in the spleens of NZB mice increased and DAP IV activity decreased; PPCE activity increased gradually with age and became significantly high at 25 weeks of age. The increase in PPCE activity in the spleen with age agrees with the previous report by Aoyagi et al. (5). Kidney DAP II activity also increased in NZB mice with age. The origin of the DAP II and DAP IV in plasma is not yet clear, but the activities of the two enzymes may reflect changes of the peptidases in various tissues.

The results in NZB mice prompted us to examine activities of DAP II and DAP IV—and the ratios of these two activities—in the serum of patients with RA or SLE. The results tended to agree with the changes seen in NZB mice: DAP II activity in serum was increased and DAP IV activity in serum was decreased, confirming our previous report (15), and the ratio of DAP II/DAP IV activities in serum was significantly increased. The increase in this ratio was more pronounced in patients with SLE than in patients with RA.

The mechanism and the clinical significance of the increase in this ratio in patients with RA or SLE remain to be further examined. Perhaps the ratio can be developed to be a biochemical index of some autoimmune disease.

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References