Activities and Properties of Peptidylarginine Deiminases of Several Vertebrate Brains

Hidenari Takahara, Kuni Sueyoshi and Kiyoshi Sugawara

Laboratory of Biochemistry, Department of Agricultural Chemistry, Ibaraki University, Ami-machi, Inashiki-gun, Ibaraki 300-03, Japan

Received January 13, 1986

Extracts from brains of mouse, rat, rabbit, pig, chicken, and duck were assayed for peptidylarginine deiminase, which catalyzes the deimination of arginy1 residues. There was peptidylarginine deiminase in the extracts from all the brains that we tested, suggesting the widespread occurrence of this enzyme in vertebrate brains. The levels of the activity in the brains of 8 different inbred strains of mice could be divided into two groups. There is a difference of over 300% between the high and low groups. Furthermore peptidylarginine deiminase purified from the brains of mouse, pig, and chicken showed that there is no great difference in their MW and the optimal conditions for the activity. However, the substrate specificity of the enzyme from chicken brain was somewhat different from those of the enzymes from mammalian brains.

Peptidylarginine deiminase (protein-arginine deiminase, protein L-arginine iminohydrolase, EC 3.5.3.15) catalyzes the formation of citrullyl residues form arginy1 residues. This enzyme requires Ca2+ as an essential factor, and reducing reagents such as dithiothreitol (DTT) stimulate the activity.1,2) Peptidylarginine deiminase activity was first found in an extract from hair follicles by Rogers and co-workers.3) Although peptidylarginine deiminases have been detected in many mammalian tissues4-7) and purified from bovine brain8) and rabbit skeletal muscle,9-11) little is known about the functions of the enzyme in the cell. When the universal roles of peptidylarginine deiminase in the cell are considered, universal properties of the enzyme through species are expected. From this point of view, we measured the activities of peptidylarginine deiminases from the same tissues but of different animals, and found that the enzyme was widespread in mammals and birds in the brain. Moreover, we have purified peptidylarginine deiminase from the brain of mouse, pig, and chicken, and compared their properties.

MATERIALS AND METHODS


Extraction and purification of enzyme. To measure the activity of peptidylarginine deiminase in brain, the tissue quickly removed from each animal was homogenized in 5 volumes of 20 mM Tris–HCl, pH 7.6, containing 10 mM 2-mercaptoethanol, 1 mM EDTA, and 0.43 mM phenylmethylsulfonyl fluoride with a Ultra Turrax at 0°C. The homogenate was filtered through nylon gauze and the filtrate centrifuged at 10,000 × g for 30 min at 4°C. The supernatant was dialyzed against the homogenizing buffer and assayed the activity.

For comparison of the properties of peptidylarginine

Abbreviations: DTT, dithiothreitol; Bz, benzoyl; O-Et, O-ethylester; O-Me, O-methylester; -NH2, -amide; Tos, tosyl; Ac, acetyl.
deiminase from brains of mouse (strain NC), pig, and chicken, the enzyme was purified from the extract of each animal brain by ammonium sulfate fractionation, DEAE-Sepharose ion-exchange, and soybean trypsin inhibitor (Kunitz)-Sepharose affinity chromatography as described previously.\(^1\)

**Assay of enzyme activity.** Unless otherwise specified, peptidylarginine deiminase activity was measured by the formation of citrulline in proline using a colorimetric assay described previously.\(^9\) One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 \(\mu\)mol of citrulline in proline per hour at 55°C.

**Measurement of protein.** Protein was measured by the method of Read and Northcote\(^12\) using bovine serum albumin as a standard.

**RESULTS AND DISCUSSION**

**Activity of peptidylarginine deiminase in several vertebrate brains**

The activity from the brains of several mammals and birds are shown in Table I. The enzymes were apparent in all of the mammals and birds that we tested. The level of the activity in mouse brain varied considerably among eight inbred strains; these mice can be divided into two groups. There is a difference of more than 300% between the high and low groups. Also it is clear from these data that the nervous tissues of mice from genetically closely related strains\(^13\) appear to have similar levels of peptidylarginine deiminase activity. These results suggest that the basal level of the enzymatic activity is set by genetic factors. Recently, Kubilus and Baden\(^7\) also inquired whether the enzyme occurred in the corresponding tissues of other vertebrates. As shown in Table I, together with their comparative values, significant amounts of the activity were present in brains of tortoise, frog, and carp. Thus, the representatives of our and their studies demonstrated that peptidylarginine deiminase is widespread in the central nervous system throughout the vertebrates.

**General properties of the enzymes purified from brains of mouse, pig, and chicken**

All the vertebrate brains tested contain

<table>
<thead>
<tr>
<th>Animal</th>
<th>Units/g of fresh brain</th>
<th>Units/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (KK)</td>
<td>2.27</td>
<td>0.121</td>
</tr>
<tr>
<td>(NC)</td>
<td>1.69</td>
<td>0.198</td>
</tr>
<tr>
<td>(NC/brp)</td>
<td>1.95</td>
<td>0.149</td>
</tr>
<tr>
<td>(AA)</td>
<td>1.46</td>
<td>0.140</td>
</tr>
<tr>
<td>(C 57/BL)</td>
<td>0.56</td>
<td>0.046</td>
</tr>
<tr>
<td>(DDK)</td>
<td>0.46</td>
<td>0.039</td>
</tr>
<tr>
<td>(RR)</td>
<td>0.44</td>
<td>0.041</td>
</tr>
<tr>
<td>(Balb/C)</td>
<td>0.42</td>
<td>0.021</td>
</tr>
<tr>
<td>Rat (Balb/C)</td>
<td>0.62</td>
<td>0.040</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.86</td>
<td>0.043</td>
</tr>
<tr>
<td>Pig</td>
<td>1.58</td>
<td>0.153</td>
</tr>
<tr>
<td>Cow(^*)</td>
<td>3.30</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>0.98</td>
<td>0.056</td>
</tr>
<tr>
<td>Duck</td>
<td>0.79</td>
<td>0.051</td>
</tr>
<tr>
<td>Tortoise(^**)</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Frog(^**)</td>
<td>7.60</td>
<td></td>
</tr>
<tr>
<td>Carp(^**)</td>
<td>4.07</td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) From ref. 8.  
\(^**\) From ref. 7.

**Fig. 1.** Effects of Temperature (A) and pH (B) on the Activities of the Purified Peptidylarginine Deiminases from Mouse (○), Pig (●), and Chicken Brain (△).

The enzyme activity was assayed at different temperatures or pHs under the conditions described in MATERIALS AND METHODS. Buffers used were MES-NaOH buffer (pH 5.6 ~ 6.8) and Tris–HCl buffer (pH 6.8 ~ 8.8). The activity was expressed as a percentage of the maximal enzyme activity.
Peptidylarginine Deiminases of Vertebrate Brains

The enzyme activity was assayed at different concentrations of Ca²⁺ or DTT under the conditions described in Materials and Methods. The activity was expressed as a percentage of the maximal enzyme activity.

Peptidylarginine deiminases that are active under identical conditions of assay. Peptidylarginine deiminases purified to homogeneity from brains of mouse, pig, and chicken had maximal activity at 55~60°C, and the optimum pHs for these enzymes were 6.8 to 7.6 (Figs. 1A and 1B). All of these enzymes were inactive in the absence of Ca²⁺. As shown in Fig. 2A, the profiles of the Ca²⁺ sensitivities of these three enzymes were indistinguishable from each other. Half maximal activation of each enzyme occurred at approximately 0.5 mM and the activity reached a plateau at 1 to 2 mM Ca²⁺. Furthermore, the presence of reducing reagents such as DTT was not essential for the activity but a similar stimulation of the activity of the enzymes from these three animal brains were observed with a maximal activation in 6~10 mM (Fig. 2B). In addition, purified enzymes from brains of mouse, pig, and chicken had similar molecular weights at about 81,000, 83,000, and 79,000, respectively, by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (data not shown). These physicochemical and enzymatic properties of peptidylarginine deiminases from mouse, pig, and chicken brain resemble those of the enzymes of bovine brain and rabbit skeletal muscle. It is of interest that peptidylarginine deiminases of some vertebrates had similar properties irrespective of their phylogenetic relations, suggesting the conservation of this enzyme in evolution.

Substrate specificity

As shown in Table II, the enzymes from mammalian brains preferentially catalyzed the formation of citrulline derivatives from arginine derivatives of which both the amino and the carboxyl groups are substituted. Of the five synthetic arginine derivatives of which ε- amino and ε-carboxyl groups are substituted, Bz-L-Arg-OEt is an excellent substrate for these mammalian enzymes. On the other hand, either ε-amino-free or ε-carboxyl-free arginyl derivatives, except for Bz-L-Arg, were poorer substrates compared to above both side-substituted derivatives and protamine. These substrate specificities of the enzymes from mouse and pig brain resemble those of the enzymes from rabbit and bovine brain. The lack of species-specificity of substrate specificity of peptidylarginine deiminase from mammalian brain, therefore, suggests that these enzymes catalyze the deamination of an arginyl residue of peptides or proteins having a
common structure in brain.

In contrast, the substrate specificity of the enzyme from chicken brain was somewhat different from those of the mammalian tissues (Table II). The chicken enzyme greatly favored the deimination of protamine compared to synthetic arginine derivatives. Of the arginyl derivatives tested, the chicken enzyme showed a noticeable activity towards the \(\alpha\)-carboxyl-free derivatives as well as towards the derivatives which were substituted on both sides. However, lower activity was observed towards the \(\alpha\)-amino-free arginine derivatives as compared with the \(\alpha\)-carboxyl-free or both side-substituted derivatives. Furthermore, the activity towards the arginine derivatives the \(\alpha\)-amino groups of which were substituted with a tosyl group was lower than that towards the derivatives substituted with benzoyl or acetyl groups. These results suggest that the enzymatic activity of peptidylarginine deiminase from chicken brain is greatly affected by the character of the \(N\)-substituted neighboring groups of the arginine residue. The difference in the substrate specificity between the mammalian and bird enzyme suggest a different activity in the deimination of arginyl residues in natural proteins. The specificities of these peptidylarginine deiminases with proteins as substrates are currently being studied in this laboratory.

Acknowledgment. We are grateful to Dr. N. Goto, First Research Division, National Institute of Animal Health, for supplying inbred mice.

REFERENCES