DEACETYLATION AND DEFORMYLATION OF *N*-ACYL AMINO ACIDS BY KIDNEY ACYLASES

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1. Introduction

Mammalian tissues contain the enzymes deacylating α-*N*-acyl amino acids. Acylase I (EC 3.5.1.14) is of wide substrate specificity and shows a higher activity for the acyl derivatives of hydrophobic amino acids. Acylase II (EC 3.5.1.15) is specific to *N*-acyl L-aspartic acid [1]. Another enzyme which acts preferentially on *N*-acyl L-aromatic amino acids has been separated and designated tentatively as acylase III [2]. The physiological roles of these acylases have not been established.

A very high activity of *N*-formyl-methionine deformylase in animal tissues has been reported [3] and some possible roles of the enzyme, including a role in the protein synthesis as pointed out for the deformylases in micro-organisms, discussed [3]. In this study, acylase I, II, and III of the kidneys of rats and guinea pigs have been separated by DEAE-cellulose column chromatography, and it is shown that the deacetylation and deformylation are common properties of these 3 acylases. The K_m values of formyl-substrates are markedly higher than those of acetyl-substrates. No enzyme that is specific to deacetylation or deformylation alone was observed.

2. Materials and methods

N-Acyl derivatives of amino acids were obtained from Sigma Chemical Co. (St. Louis) or Wako Chemical Industry Ltd. DEAE-cellulose (capacity: 0.92 mequiv./g) was obtained from Brown Co. *N*-Acyl derivatives were dissolved in water and the solution was adjusted to pH 8 with NaOH. The following animals were used: male rats (Wistar, 200–350 g), male guinea pigs (Hartley, 250–400 g).

The enzyme reaction mixture contained 0.3 ml 0.2 M borate buffer (pH 8, prepared from 0.2 M H_3BO_3 and 0.05 M borax), a substrate (4 μmol) and enzyme solution (0.1–0.2 ml) in final vol. 1.0 ml. The assay procedures in [2] were followed. One unit (U) of activity was defined as that amount forming 1 μmol product/min under the conditions above.

3. Results

Acylase I, II, and III activities were represented with the activities towards *N*-acetyl L-methionine, *N*-acetyl L-aspartic acid and *N*-acetyl L-phenylalanine, respectively. The elution patterns of these acylases of rat kidneys in DEAE-cellulose column chromatography is shown in fig.1A. Acylase I was separated from acylase II and III. The deformylation activity towards each formyl amino acid was parallel to the corresponding enzyme activity. In the case of the guinea pig enzymes, these 3 acylases were separated from each other (fig.1B). The retention of these enzymes to the column is different from that of the rat enzymes. In this case also, the activities of the deacetylation and deformylation were parallel. No enzyme that is specific to the deacetylation or deformylation alone was observed in both animals. Figure 2 shows the relationship between the substrate concentrations and the deacylations of the acyl derivatives of L-methionine by rat kidney acylase I. The deformylation was more rapid than the deacetylation at the higher concentra-

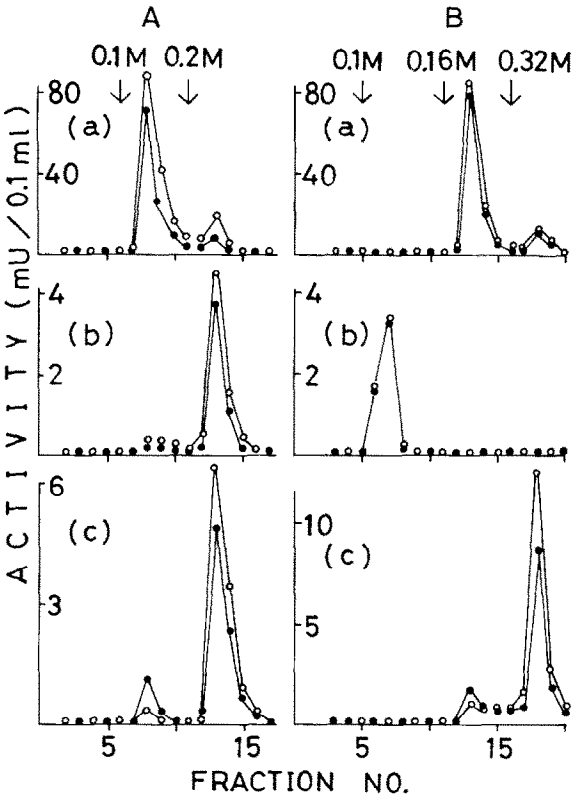

Fig.1. Profiles of deacetylation and deformylation activities in DEAE-cellulose column chromatography. Tissues were homogenized in 10 vol. 0.04 M borate buffer (pH 8). The homogenate was centrifuged (20 000 × g, 20 min) and the supernatant (5 ml) was applied to a DEAE-cellulose column (1 × 13 cm) equilibrated with 0.04 M borate buffer (pH 8). The elution was carried out step-by-step with the borate buffers containing NaCl of different concentrations as indicated. Fractions (6 ml) were collected at a flow rate of about 6 ml/7 min. An aliquot (0.1–0.2 ml) of the eluate was assayed for the activities of deacetylation and deformylation towards each acyl amino acid. Activities are expressed as mU/0.1 ml enzyme solution. (A) Rat kidney; (B) guinea pig kidney. Activities for acetyl (•——•) and formyl (○——○) derivatives of L-methionine, L-aspartic acid and L-phenylalanine are shown in (a), (b) and (c), respectively.

tions of the substrates. The K_m values for *N*-acetyl L-methionine and *N*-formyl L-methionine were 0.8 mM and 20 mM, respectively, indicating that the affinity of *N*-acetyl L-methionine to the enzyme is markedly higher than that of *N*-formyl L-methionine. Similar relationships to those described above were observed with respect to acylase II and III.

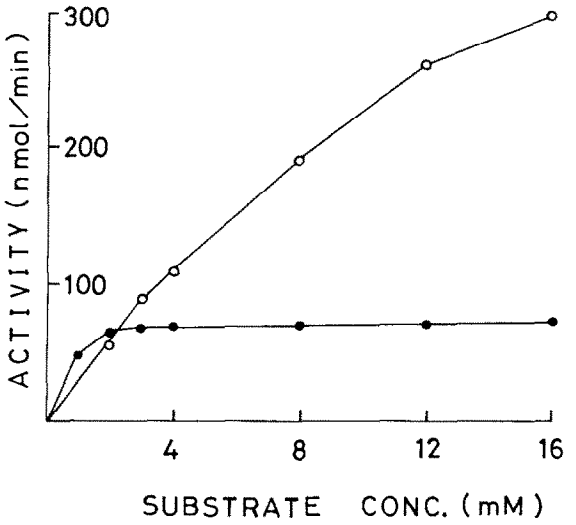

Fig.2. Relationship between the substrate concentrations and the deacylations of the acyl derivatives of L-methionine by rat kidney acylase I. An aliquot (0.1 ml) of acylase I fraction obtained by DEAE-cellulose column chromatography was used as the enzyme solution. (•——•) Deacetylation; (○——○) deformylation.

4. Discussion

This study shows that the deacetylation and deformylation are common properties of the known enzymes acylase I, II and III. No enzyme that is specific to the deacetylation or deformylation was observed. The observation [3] that the deformylase activity in rat tissues is several times higher than the deacetylase activity can be explained by the present result (fig.2).

The enzymes such as formyl-methionyl peptide deformylase [4] and formyl-methionine deformylase [5] in micro-organisms have been suggested to participate in the protein synthesis. The substrate specificities of these deformylases are different from the mammalian acylases I, II and III. Although data are not shown, the deacetylation activities of acylase I and III (per g tissue) did not change significantly in the regenerating rat liver for 0.5–6 days after the hepatectomy. Therefore, the role of these acylases in mammalian tissues appears to be different from the enzymes in micro-organisms described above.

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