Short Communication

Comparison of Peptidylarginine
Deiminases of Various
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The enzyme which converts arginine residues to citrulline residues has been newly identified in guinea pig hair follicles by Rogers and his co-workers.^{1,2)} It catalyzes the deimination of arginyl residues in several proteins including trichohyaline of hair follicles.^{1,2)} Previous papers from this laboratory also reported the occurrence of a similar enzyme in the epidermis of newborn rats.³⁾ We proposed that this enzyme be named "Peptidylarginine deiminase" based on its substrate specificity.⁴⁾ Recently, Kubilus *et al.*⁵⁾ also showed some properties of a similar enzyme from bovine snout. While studying the activity of peptidylarginine deiminase in several tissues other than epidermis, we found that the activity of rabbit skeletal muscle was much higher than that of epidermis.⁶⁾ In addition some properties of the enzyme from skeletal muscle, *e.g.* molecular weight and substrate specificity, were different from those of the epidermal enzymes.^{4,6)} These observations prompted us to compare the activities and properties of peptidylarginine deiminases from various tissues of rabbits. This paper indicates marked tissue differences in activities but little variation in some properties of the enzymes prepared from several tissues.

Male albino rabbits (2~3 kg) were decapitated, and skeletal muscle, kidney, brain, lung,

thymus, liver, spleen, stomach and heart were quickly removed. Each tissue was then homogenized in 5 volumes of 20 mM Tris-HCl, pH 7.6, containing 1 mM EDTA and 10 mM 2-mercaptoethanol with an Ultra Turrax homogenizer at 0°C. The homogenate was filtered through nylon gauze and the filtrate was centrifuged at 10,000 × *g* for 30 min at 4°C. The supernatant was used as the enzyme solution after being dialyzed against the homogenizing buffer. To compare some properties of peptidylarginine deiminase from skeletal muscle, kidney, brain and lung, the enzymes were partially purified by the following methods. Saturated ammonium sulfate solution was added to the crude extract and the precipitate between 0.3~0.5 saturation was collected by centrifugation. This fraction was dissolved and dialyzed against 20 mM Tris-HCl, pH 7.6, containing 10 mM 2-mercaptoethanol, 1 mM EDTA and 0.1 M NaCl, and was applied to a DEAE-Sephacel column previously equilibrated with the same buffer. The column was washed with the buffer, and elution was conducted with a linear gradient of 0.1~0.5 M NaCl in the buffer. In all cases, the enzyme activity emerged as a single peak at about 0.2 M NaCl. The active fractions were combined and used for the studies. The activity of the enzyme was determined by the colorimetric method^{4,6)} using protamine or arginine derivatives as substrates. One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmol of citrullyl residues from arginine residues per hours at 52°C. Protein concentrations were determined by the method of Read and Northcote⁷⁾ using bovine serum albumin as a standard.

Peptidylarginine deiminase activity of various tissues from rabbit, when protamine was used as substrate, are shown in Table I. The enzyme activities were apparent in skeletal muscle, kidney, brain and lung but there was very little or no activity in the other tissues. Among the tissues thus far tested, the activity

TABLE I. PEPTIDYLARGININE DEIMINASE ACTIVITY IN VARIOUS TISSUES

Crude extracts were obtained from male albino rabbit tissues as described in the text. The incubation mixture was composed of 100 mM Tris-HCl (pH 7.5), 10 mM CaCl_2 , 10 mM DTT, 1 mg protamine sulfate and the enzyme (0.2~1.8 mg of protein). The activities and proteins were measured as described in the text. Values are means of the experimental values obtained for 5 rabbits with standard errors.

Tissue	Units/g of fresh tissues	Units/mg of protein
Muscle	3.06 ± 0.68	0.102 ± 0.018
Kidney	2.07 ± 0.63	0.059 ± 0.016
Brain	0.86 ± 0.23	0.043 ± 0.012
Lung	0.19 ± 0.06	0.004 ± 0.001
Liver	<0.01	<0.001
Thymus	<0.01	<0.001
Spleen	0	0
Stomach	0	0
Heart	0	0

of the enzyme solution from skeletal muscle exceeded that of any other tissue.

The enzymes from skeletal muscle, kidney, brain, and lung required calcium ions for their activity, and the maximal activity was observed at more than 2 mM. Furthermore, in the presence of DTT, the stimulation of the activity of the enzyme from these tissues was observed with a maximal activation of several-fold at 10 mM. The optimum pH was 7.5 and the maximal activity was observed at 55°C. These properties are also common to the peptidylarginine deiminases from hair follicles and the epidermis.

Peptidylarginine deiminases from the tissues of rabbit showed similar molecular weights, which were calculated to be about 130,000 by gel filtration. These values were much higher than those for the epidermal enzymes (48,000~69,000).^{3,5)}

Enzymatic activities towards various arginine derivatives were tested with the peptidylarginine deiminases from skeletal muscle, kidney, brain and lung. As shown in Table II, all the enzymes preferably catalyzed the formation of citrulline derivatives from arginine derivatives in which both the amino and the

TABLE II. COMPARISON OF SUBSTRATE SPECIFICITY OF THE ENZYMES TOWARDS SYNTHETIC ARGININE DERIVATIVES

The incubation mixture was composed of 100 mM Tris-HCl (pH 7.5), 10 mM CaCl_2 , 10 mM DTT, 10 mM substrate and the partially purified enzyme (27~36 μg of protein). The activities were measured as described in the text. Relative activity was calculated on the basis of the activity towards Bz-L-Arg-O-Et.

Substrate	Muscle	Kidney	Brain	Lung
Bz-L-Arg-O-Et	100	100	100	100
Bz-L-Arg-NH ₂	50	54	55	71
Bz-L-Arg-O-Me	58	73	81	109
Tos-L-Arg-O-Me	68	74	77	99
Ac-L-Arg-O-Me	39	54	53	76
Bz-L-Arg	17	19	27	24
Boc-L-Arg	2	3	0	23
Ac-L-Arg	5	4	13	11
L-Arg-O-Me	2	2	9	11
L-Arginine	8	6	3	10

carboxyl groups were substituted. On the other hand, lower or negligible activities were observed towards either an α -amino-free or α -carboxyl-free arginyl derivative, and towards L-arginine. These results show that substrate specificities of the enzymes from skeletal muscle, kidney, brain and lung were different from that of the epidermal enzyme from newborn rats. The epidermal enzyme catalyzed the deimination of an α -carboxyl-free arginine derivative as well as arginine derivatives which were substituted on both sides.⁴⁾ Table II also shows that the substrate specificity of the enzyme from the lung is somewhat different from those of other tissues. The reaction rate of the enzyme from the lung was slightly affected by the character of substituted group in the arginyl derivatives.

Thus, peptidylarginine deiminases are distributed in several tissues of the mammal, although the levels of these activities are considerably different. Our present survey also confirms the previous finding that rabbit skeletal muscle possessed a large amount of peptidylarginine deiminase activity. To date the enzymes found in various tissues can apparently be divided into two major groups by substrate specificity; epidermal *versus* that of

most other tissues. Elucidation of further details of these differences may ultimately lead to better understanding of the function of the enzymes in these tissues. Experiments along this line are currently in progress in this laboratory.

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