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Adenosine Deaminase in Human Epidermis from Healthy and Psoriatic Subjects

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Summary. Adenosine deaminase, which catalyzes the irreversible hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively, plays an important role in the degradation of adenine nucleotide and purine nucleotide salvage pathway metabolism. We investigated human epidermal adenosine deaminase activity using a radiochemical method, which enabled us to measure the adenosine deaminase activity of protein samples as small as several micrograms. We measured adenosine deaminase activity of microdissected pure epidermis of the healthy skin and the psoriatic affected and unaffected skin. It was shown that psoriatic affected epidermis had increased adenosine deaminase activity compared with the healthy epidermis ($P < 0.05$) and the unaffected epidermis ($P < 0.01$). There was no difference in enzyme activity between healthy and psoriatic unaffected epidermis. The increased adenosine deaminase activity in the psoriatic affected epidermis may reflect the accelerated salvage pathway of the nucleic acid metabolism probably associated with the hyperproliferative condition of the psoriatic epidermis.

Key words: Adenosine deaminase – Human epidermis – Psoriasis

Introduction

Adenosine deaminase catalyzes the irreversible hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. This enzyme plays an important role in the degradation of adenine nucleotides. In human tissue adenosine cannot be directly degradated to adenine in contrast to guanosine and inosine, which are degradated by direct deribosylation resulting in the formation of guanine and hypoxanthine respectively. Adenosine is first catalyzed to inosine by adenosine deaminase. Because of the irreversibility of this reaction and the relatively low enzyme activity, this enzyme reaction seems to be one of the rate limiting steps of adenosine degradation [1, 2]. The product, inosine, is then converted to hypoxanthine, which is either converted to IMP by hypoxanthine-guanine phosphoribosyl transferase [2], a final rate limiting enzyme of the purine nucleotide salvage pathway, or catalyzed to xanthine and uric acid by xanthine oxidase [3–5].

From the investigation of severe immunodeficiency disease which is defective in adenosine deaminase [6], it has been suggested that detoxification of adenosine and deoxyadenosine might be another significant function of adenosine deaminase. The cytotoxic and cytostatic effects of adenosine and deoxyadenosine are generally potentiated by inhibitors of adenosine deaminase. Several mechanisms of toxicity have been proposed. First, adenosine or deoxyadenosine causes dATP accumulation, which is a strong inhibitor of ribonucleotide reductase and causes inhibition of DNA synthesis [7]. Second, access of deoxyadenosine inactivates S-adenosylhomocysteine hydrolase. Absence of this enzyme causes interference with the critical methylation-dependent processes such as the synthesis, maturation or function of DNA [8].

It has been reported that in adenosine deaminase-defective lymphocytes cyclic AMP level is increased [9]. Since adenosine is known to stimulate the adenylate cyclase system of epidermis [10], adenosine deaminase might be significant for the regulation of cell proliferation through the cyclic AMP system. Adenosine deaminase also plays an important role in cell maturation such as human monocyte [11].

These findings suggest that adenosine deaminase might play a significant role in the nucleic acid metabolism of the epidermis where the significance of salvage

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pathway has been emphasized recently [12, 13]. These findings also suggest that adenosine and deoxyadenosine metabolism in the epidermis is important for cell proliferation and maturation. However, because of the low enzyme activity in the epidermis [14, 15], the nature and activity of the enzyme in skin disorders remain unknown at present. Although Kalcker's method of adenosine deaminase determination, which employs the decrease in OD at 265 nm, is simple and popular [16], the sensitivity is relatively low and we cannot detect the adenosine deaminase activity of small biopsy samples.

In this report we attempt to characterize the biochemical nature of the epidermal adenosine deaminase using the microanalytical method. Then we determine the adenosine deaminase activity of normal and psoriatic pure epidermis obtained by microdissection technique.

Materials and Methods

Chemicals

(2-^{3}H) adenosine was purchased from New England Nuclear (Boston, MA, USA). TLC plates cellulose F were obtained from Merck (Darmstadt, FRG). Coformycin was a generous gift from Dr. Umezawa of the Institute of Microbial Chemistry, (Tokyo, Japan) [18–21]. Adenosine, inosine, AMP, hypoxanthine, adenine and reagents for scintillation were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other reagents used were of the highest purity commercially available. All chemicals were freshly prepared before each experiment.

Preparation of the Enzyme

Healthy human epidermis was obtained from intermediate split thickness skin obtained by free hand dermatome under systemic anesthesia or 0.2 mm thickness by Castroviejo keratome.

Two slices of 5 mm square epidermis were homogenized in glass homogenizer in 1 ml 50 mM phosphate buffer, pH 7.1. The homogenate was centrifuged at 1,500 *g* for 10 min and the supernatant was used for the enzyme source. All procedures were performed at 4° C.

Adenosine Deaminase Assay

Adenosine deaminase activity was quantitated by measuring the formation of ^{3}H-inosine. Adenosine deaminase was assayed by mixing 10 µl enzyme source and reaction mixture which contained 340 µM ^{3}H-adenosine and 50 mM phosphate buffer, pH 7.1. Incubation was carried out for 30 min at 37° C, and the reaction was stopped by 8 N formic acid. The sample was then developed by thin layer chromatography and the radioactivity of the inosine fraction was measured by liquid scintillation counter. Protein was determined by the method of Lowry et al. [22] using bovine serum albumin as a standard.

Thin Layer Chromatography

All samples were analyzed by ascending chromatography on TLC plates cellulose F. The plates were developed at room temperature with the two systems of solvents for the separation of the purine analogs. The first system of the chromatogram was developed with 1 M ammonium acetate [23], which separated inosine, AMP, adenosine plus hypoxanthine and adenine. The second system was developed with a mixture composed of 60 parts butanol, 20 parts methanol, 20 parts water and 1 part ammonium hydroxide, which separated adenosine, adenine, inosine plus hypoxanthine and AMP [24]. An appropriate mixture of standard materials and 1 µl of each sample were chromatographed and the plates were examined under ultraviolet light. The developed chromatograms of the experimental samples were cut at right angles to the direction of migration. Each fraction cut out was put into the scintillation vial and mixed with 100 µl 1 M LiCl, and radioactivity was measured by liquid scintillation counter. The first system of the chromatogram required about 1.5 h and it sharply separated each purine analog, but hypoxanthine was developed on the same fraction as adenosine. Adenosine was catalyzed to inosine by adenosine deaminase and then inosine to hypoxanthine by purine nucleoside phosphorylase. If the concentration of purine nucleoside phosphorylase is high, hypoxanthine is produced in incubation mixture, and adenosine deaminase activity cannot be measured correctly. The second system required 4 h, and inosine and hypoxanthine were developed on the same fraction. In these two systems, the counts of the adenine fraction were not increased as compared with the blank control. It seems that there is no conversion of adenosine to adenine in epidermis. The conversion of adenosine to AMP was only about 5% in these systems. In keratome sliced healthy epidermis the difference in the counts of inosine between these two systems was only about 15% under the assay condition described. The difference might represent the conversion of inosine to hypoxanthine, which was larger in the psoriatic epidermis. We used the second system for studying the enzyme nature and for comparing the psoriatic epidermal and healthy epidermal adenosine deaminase activity.

Preparation of Microdissected Pure Epidermis

The skin samples were obtained by 7 mm punch from affected and unaffected areas of psoriasis under local anesthesia with 0.5% lidocaine. The healthy skin samples were provided by plastic skin surgeons. The pure epidermis was obtained as described previously [25]. Each sample washed with saline was placed in and frozen on the cryostat adaptor in less than 10 s. The samples were cut 20 µm thick. After lyophilization the epidermis and dermis were dissected under a stereomicroscope and the keratin layers were removed. The pure epidermal tissues weighing approximately 30 µg were used for each assay.

The statistical significance was determined by Student's *t*-test.

Results

Using epidermal homogenate as an enzyme source we investigated the biochemical nature of adenosine deaminase and established the assay conditions. The time course showed that the activity linearly increased for 60 min, and then gradually decreased (Fig. 1). The boiled enzyme had no adenosine deaminase activity. The tissue dependency curve showed that the enzyme activity was proportional to the amount of tissue (Fig. 2). Consequently, the incubation in the following experiments was 30 min and about 7 µg protein was used as the enzyme source.

The K_m value for adenosine was 3.3×10^{-5} M, which is almost identical to the values reported by several investigators [26, 27] (Fig. 3). Optimum pH was around 7 (Fig. 4.). Coformycin [18–21], a tight binding inhibitor of adenosine deaminase, inhibited adenosine

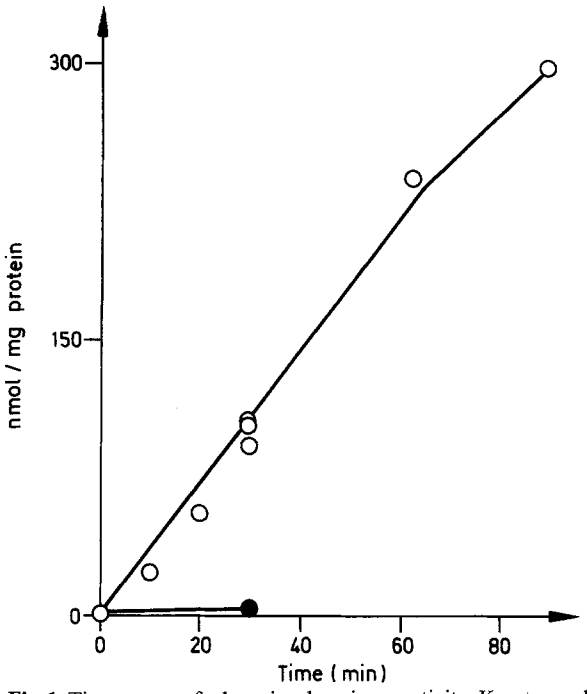

Fig. 1. Time course of adenosine deaminase activity. Keratome sliced epidermis was homogenized and centrifuged and used as the enzyme source. Samples of 7.8 μg protein were assayed at 37°C. The adenosine deaminase activity was determined as nmol/mg protein (○). The boiled extract was assayed for adenosine deaminase activity at 37°C for 30 min (●)

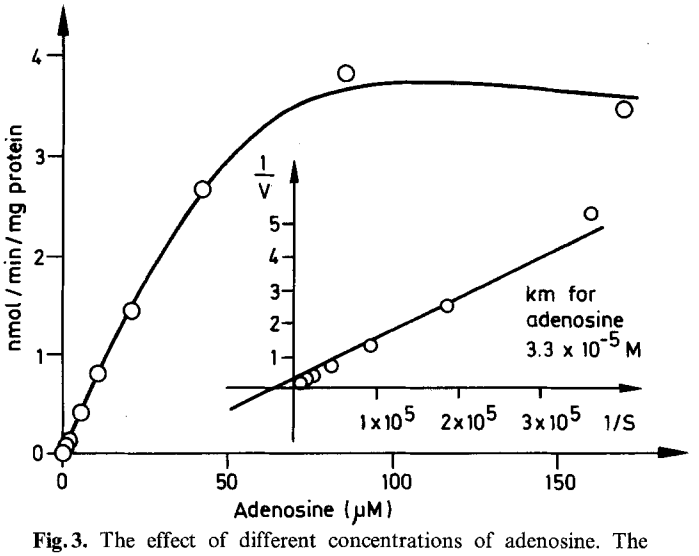

Fig. 3. The effect of different concentrations of adenosine. The homogenate was used as the enzyme source. Adenosine deaminase activity was measured as described in 'Materials and Methods'

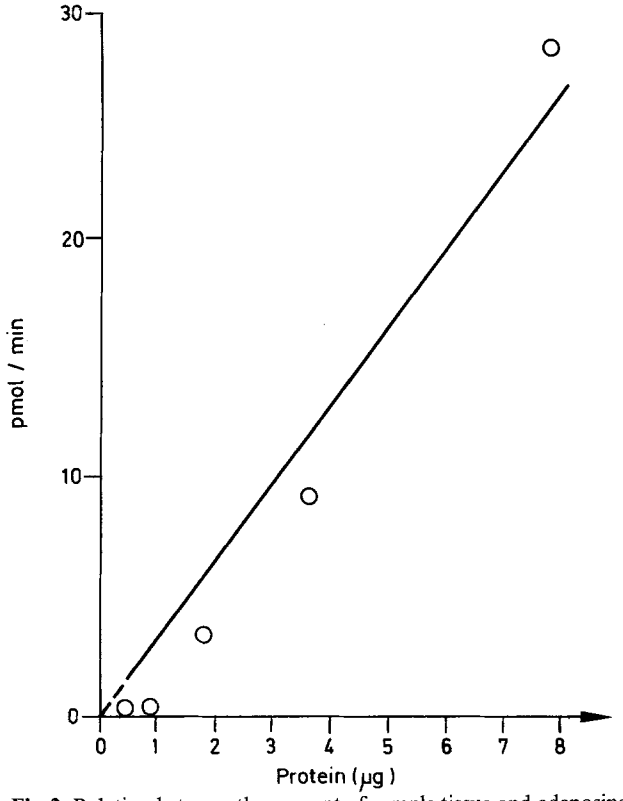

Fig. 2. Relation between the amount of sample tissue and adenosine deaminase activity. The homogenate was used as the enzyme source. The protein of the enzyme source was determined, and the enzyme source was diluted with 50 mM phosphate buffer, pH 7.1. The enzyme activity was measured

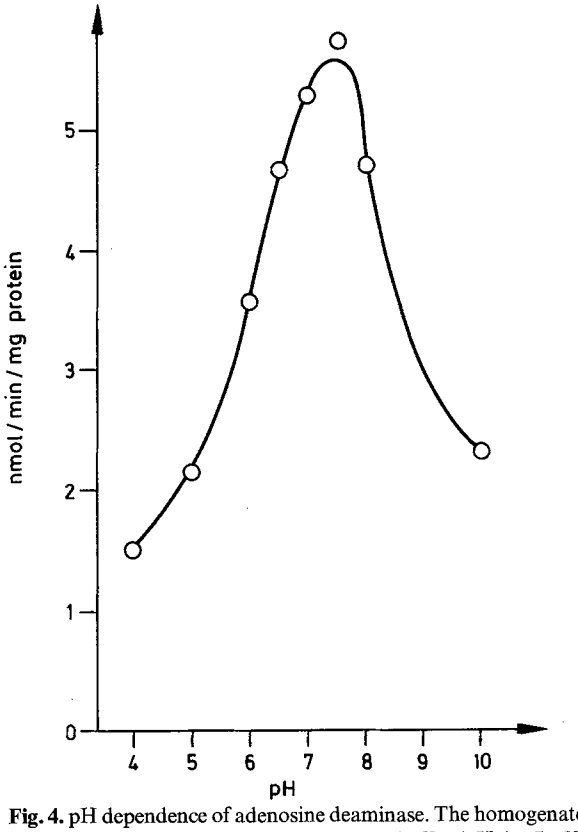

Fig. 4. pH dependence of adenosine deaminase. The homogenate was used as the enzyme source. 50 mM acetate buffer (pH 4–5), 50 mM phosphate buffer (pH 6–8), 50 mM borate-NaOH buffer (pH 9–10) were used

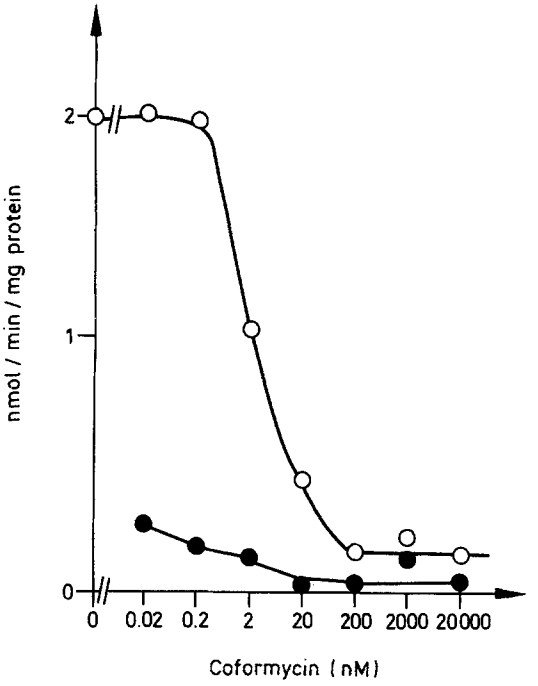

Fig. 5. Inhibition of adenosine deaminase by coformycin. Coformycin was added in the reagent mixture simultaneously with adenosine and skin extract (○). Coformycin and enzyme were preincubated for 30 min at 37°C and the enzyme activity was assayed (●). Final concentration of adenosine for the enzyme assay was 170 μM

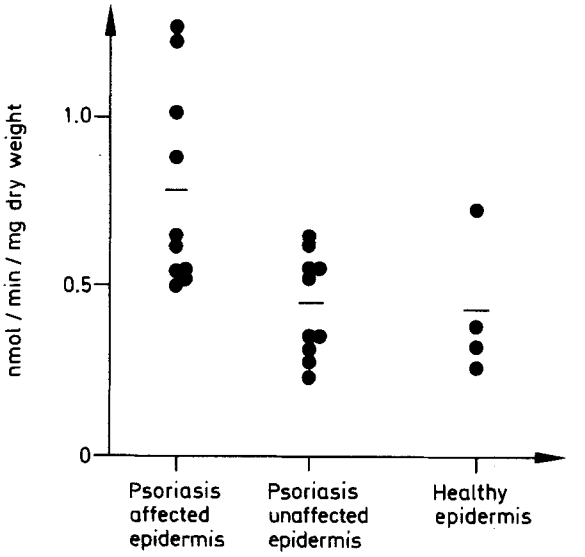

Fig. 6. Adenosine deaminase activity in the microdissected pure epidermis of ten psoriatic patients and four healthy controls. Adenosine deaminase assay was started by mixing approximately 30 μg dry weight pure epidermis with 10 μl of the reagent mixture containing 340 μM (2-^{3}H) adenosine in 50 mM phosphate buffer, pH 7.1, and incubated for 30 min at 37°C. Vertical bars indicate mean adenosine deaminase activity

deaminase activity markedly (Fig. 5), and 50% inhibition of the enzyme activity was obtained at the concentration of 2×10^{-9} M of coformycin when substrate, coformycin and the enzyme were mixed simultaneously. However, after 30 min preincubation of the enzyme and coformycin, almost complete inhibition was obtained at the concentration of 2×10^{-11} M. The data are consistent with the tight binding nature of coformycin to the enzyme [20]. The inhibition constant (K_i) is reported approximately $1 - 10 \times 10^{-11}$ M [21]. In the case of the tight binding inhibitor the association and dissociation reaction may be so slow that proper inhibitory constants can only be obtained after preincubation of the enzyme and the inhibitor.

Thus the microanalytical method of adenosine deaminase assay enables us to determine the adenosine deaminase activity of protein samples as small as a few micrograms. Since a significant amount of dermal component was contaminated in the skin slice samples, we used microdissected pure epidermis and determined the adenosine deaminase activities of the healthy epidermis and the psoriatic unaffected and the affected epidermis. Ten psoriatic patients and four healthy controls were investigated (Fig. 6). The mean adenosine deaminase activity ± 1 SD in the psoriatic affected epidermis was 0.78 ± 0.28 nmol/min/mg dry weight and that of unaffected epidermis was 0.45 ± 0.14 nmol/min/mg dry weight. The difference was statistically highly significant ($P < 0.01$). The mean adenosine deaminase activity in the normal controls was 0.43 ± 0.18 nmol/min/mg dry weight. The difference was also statistically significant ($P < 0.05$) against psoriatic involved epidermis. There was no difference in the enzyme activity between healthy and psoriatic unaffected epidermis.

In summary, it was shown that psoriatic affected epidermis had about 180% increased adenosine deaminase activity compared with the healthy epidermis and the unaffected epidermis.

Discussion

A microanalytical method is now available to determine the adenosine deaminase activity of small protein skin samples. Two systems of thin layer chromatography can be used. However, the ammonium acetate system was not adequate for the samples which have high adenosine deaminase activity such as psoriatic affected epidermis because of the increased conversion of inosine to hypoxanthine due to purine nucleoside phosphorylase. Although it required 4–5 h for development, the butanol-methanol-ammonium hydroxide-water system was better in such cases.

In psoriatic affected epidermis the germinative cell cycle time is shortened [28], and RNA content is increased [29]. Psoriatic scales are also known to contain large amounts of RNA and DNA with RNase and DNase. Several enzyme activities of purine nucleotide salvage pathway are reported to be increased in psoriatic affected epidermis [4, 30]. The increased adenosine deaminase activity reported in this study seems to be another finding of increased enzyme activity of nucleotide metabolism in the psoriatic affected epidermis. The physiological significance of increased adenosine deaminase activity remains unknown at present. Our data indicate that adenosine deaminase activities of healthy and psoriatic unaffected epidermis were about the same (Fig. 6). Previously we reported that adenosine deaminase activity in peripheral lymphocytes of psoriatic patients was not increased [17]. Therefore, the increased adenosine deaminase activity in the psoriatic affected epidermis seems to be a secondary phenomenon, which may reflect the accelerated salvage pathway of nucleic acid metabolism associated with the hyperproliferative state of psoriasis affected epidermis.

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