

NUCLEIC ACIDS ENZYMES IN HUMAN HAIR ROOTS¹

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Abstract. To investigate the metabolic pathways connected with nucleoprotein and nucleic acid synthesis and degradation in human hair root, several enzymatic activities were investigated. Microbiochemical assays were performed on few hair roots. The reported results indicate a high tissue concentration of deoxycytidylate aminohydrolase, an enzyme known to be connected with DNA synthesis. Among the enzymes involved in the catabolic pathway, ribonuclease, acid deoxyribonuclease, phosphodiesterase, acid phosphatase and nucleoside phosphorylase were also found to be active. Regarding the mechanism of purine degradation, xanthine oxidase was also demonstrated and found very active at pH 8.8. The data reported strengthen the previous hypothesis that high levels of keratin in the hair are related to high concentrations of nucleic acid catabolic enzymes.

Multiplication of the hair matrix cells depends on the active synthesis of nucleoproteins. Accordingly, the bulb cells' content of DNA and RNA is related to mitotic activity and keratin synthesis. During cell differentiation this process is followed by nucleic acid degradation while keratinized structures are built. Thus, in the hair follicle, nuclear material disappears when keratin is synthesized. RNA is present in the hair cortex up

to the limit of the keratogenous zone, whereas the DNA content gradually decreases in the differentiating cells; the latter is in fact detectable up to the keratogenous zone or middle portion of the follicle.

Nuclear material breakdown is seemingly caused by nucleases and related enzymes; histochemical studies have already demonstrated (5, 11, 21) that in the hair root, as also in epidermis, there are detectable activities of enzymes belonging to pathways of nucleic acid degradation. Moreover, the latter have been identified (15) as free bases, nucleosides and nucleotides.

Nevertheless, the degradation pathways have not yet been fully charted and no investigations have been made on the enzymes involved in the synthetic as well as in the catabolic pathways of nucleic acids in hair formation. In particular, the final step in purine oxidation to uric acid is open to question, since the presumably involved enzyme—xanthine oxidase—had not been found at all in human epidermis (6, 20).

In the present work some key enzymes connected with nucleic acid synthesis and breakdown were investigated, with particular reference to xanthine oxidase, whose occurrence would elucidate the manner of uric acid formation in terminal hair.

MATERIAL AND METHODS

All reagents were of analytical reagent grade. dCMP, DNA, RNA, *p*-nitrophenylthymidine-5'-phosphate, hypoxanthine and inosine were purchased from Calbiochem (USA). Hair root extracts were prepared according to a method previously published (15): hairs from the parieto-occipital region of healthy Caucasoid subjects were obtained by manual depilation with specially designed forceps. Growing hairs were used in all experiments. The roots were separated from the hair just above the kerato-

¹ The experiments reported in this paper were communicated at the 1st Annual Meeting of the European Society for Dermatological Research, Noordwijk-aan-zee, 1971.

Abbreviations:

ADP	= adenosine diphosphate
ATP	= adenosine triphosphate
dCMP	= deoxycytidine monophosphate
7,8-DHF	= 7,8-dihydrofolate
DNA	= deoxyribonucleic acid
DNase	= deoxyribonuclease
dUMP	= deoxyuridine monophosphate
dYTP	= deoxyadenosine, deoxycytidine, deoxyguanosine and thymidine triphosphates
5,10-me-THF	= 5,10-methylenyltetrahydrofolate
PP	= pyrophosphate
RNA	= ribonucleic acid
RNase	= ribonuclease

Table I. Specific activities of the enzymes tested

Enzyme	Specific activity (U/mg protein)
A. Deoxycytidylate aminohydrolase	1.9
B. RNase	0.35
C. DNase II	570
D. Phosphodiesterase	0.5
E. Acid phosphatase	4
F. Nucleoside phosphorylase	18
G. Xanthine oxidase	800

genous zone under a dissecting microscope. In each experiment approximately 200 pooled hair roots were used. Buffers were chosen according to the enzyme to be tested. Homogenization was performed in conical glass microhomogenizers (17). Centrifugation was carried out in a MISCO microcentrifuge. Determinations were made on 5 to 20 μ l aliquots of extracts.

dCMP aminohydrolase was determined according to Scarano et al. (19); ribonuclease according to Kunitz (8); acid deoxyribonuclease according to Bernardi et al. (2) and phosphodiesterase according to Razzel & Khorana (12) using as substrates *p*-nitrophenylphosphate (pH 8.0) and *p*-nitrophenyl-thymidine-5'-phosphate (pH 9.2). Nucleoside phosphorylase was determined by the method of Kalckar (7) and xanthine oxidase according to a procedure modified from Roussos (13).

Absorbances were determined on a Beckman DK2A recording spectrophotometer equipped with a microattachment and a thermostatic chamber. Proteins were estimated by the method of Lowry et al. (9) using crystalline bovine serum albumin as standard.

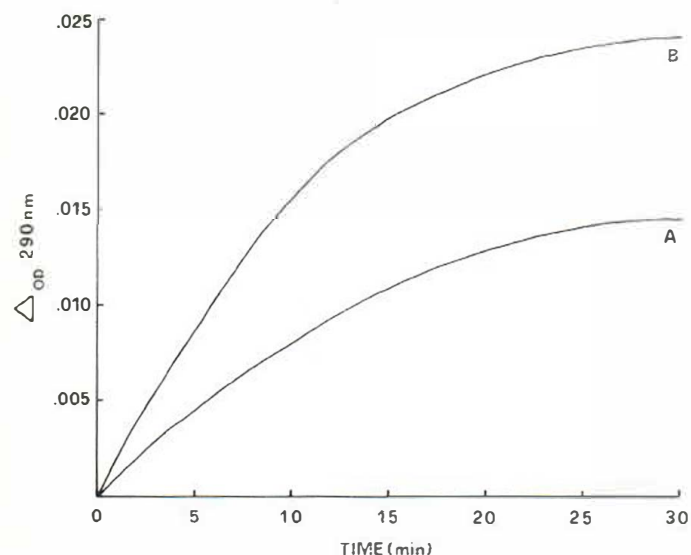


Fig. 1. Spectrophotometric determination of xanthine oxidase activity in hair root. The assay solution had the following composition: 60 μ l glycine buffer 1 M, pH 8.8; 10 μ l hypoxanthine solution (2.58 mM in glycine buffer 0.05 M, pH 8.8); 5 μ l (A) or 10 μ l (B) enzyme in 140 μ l of bovine serum albumin (0.7 mg/ml); metal-free water to 250 μ l. The solution was incubated at 37°C and the enzyme added at 0 time. Increase in absorbance was followed at 290 nm. One unit of enzyme is defined as that amount which, under the above conditions, catalyses an increase in absorbance of 0.001 per 30 minutes.

RESULTS

The results reported in Table I represent the average values obtained from three separate experiments. Enzymatic activities were measured according to the reported procedures. Only xanthine oxidase activity was determined with a procedure especially developed from the method of Roussos (13): enzyme activity determination following an increase in absorbance at 290 nm upon the aerobic oxidation of hypoxanthine to uric acid (Fig. 1). The incubation mixture contained only metal-free water. Albumin was added in order to minimize the inactivation of the enzyme at low protein concentrations.

DISCUSSION

Fig. 2 summarizes the hypothesized pathway of nucleic acid catabolism (14). Specific activities of these enzymes in the hair root are indicated in Table I; here too is reported the specific activity of dCMP aminohydrolase (EC 3.5.4.5.), which appears to play an important role in the regulation of the pool of deoxynucleotides in higher organisms (Fig. 3).

Data set out in Table I suggest the existence of an active metabolism of nucleic acids in hair roots. In our experiments, the specific activity of deoxycytidylate aminohydrolase was very high. This enzyme catalyses the deamination of dCMP

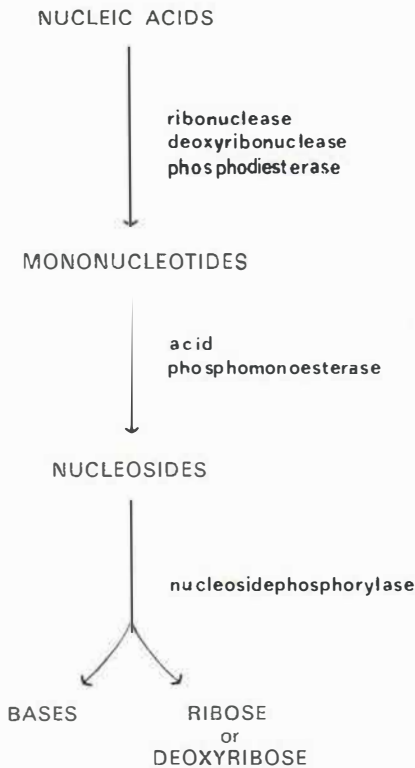


Fig. 2. Scheme of nucleic acid catabolism.

to dUMP (18), thus providing the substrate for TMP synthesis (Fig. 3). It is subject to a mechanism of feedback regulation by the end-products of the nucleotide metabolic pathway, thus playing a physiologically central role in DNA synthesis, and is therefore abundant in actively multiplying tissues. The specific activity of deoxycytidylate aminohydrolase in human hair root is roughly

twice that previously demonstrated (10) in the epidermis.

RNase and DNase II also showed values which appeared slightly elevated, when compared with those found in human epidermis (16), though assayed by a different method.

The exonuclease phosphodiesterase was also found to be active, but its activity was apparently not as high as that of the other tested enzymes.

Nucleoside phosphorylase and acid phosphatase were also found to be quite active; these enzymes catalyse the final stage of the catabolic pathway of nucleic acid derivatives formed by nucleases. Epidermal acid phosphomonoesterase has, as a matter of fact, a strong affinity towards mononucleotides (14).

Xanthine oxidase was also demonstrated and found very active at pH 8.8; no activity was detectable in Tris-HCl buffer at pH 7.2 nor in phosphate buffer at pH 7.0.

We previously found (15) in terminal hair (by thin layer chromatography technique) various catabolic products of nucleoproteins. Besides amino acids, ribose and deoxyribose, we identified nucleotides, nucleosides, pyrimidines, purines and their catabolic products, i.e. hypoxanthine, xanthine and uric acid. Enzymatic data presented in this paper show how these compounds are formed. It can be inferred that, during hair keratinization, nucleic acid degradation follows the catabolic routes accepted for other tissues, including epidermis. However, nucleic acids are only partly degraded to bases.

As far as purines are concerned, hypoxanthine, xanthine as well as uric acid are detectable in the keratinized structures. The results reported

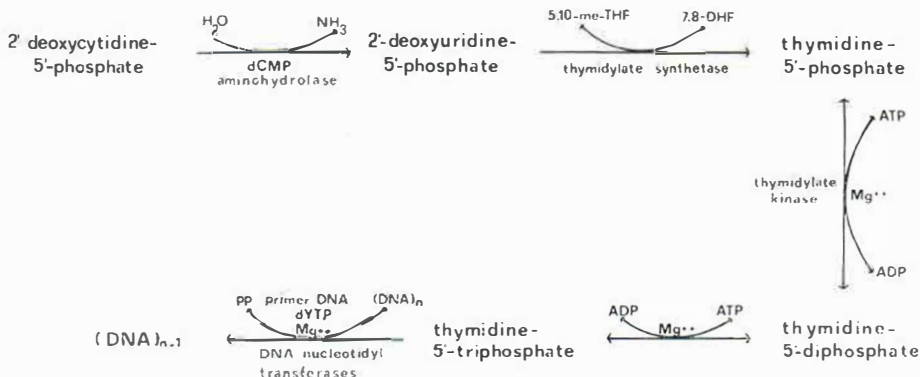


Fig. 3. Scheme of dCMP pathway to DNA.

allow us to state that uric acid formation is accounted for by xanthine oxidase in hair roots. This enzyme had been already found in the skin of some animals (1, 3, 20), but not at all in human epidermis (6, 20), even with experiments using labeled hypoxanthine. On the basis of these data it had been suggested that uric acid in hair could derive from the general pool, in agreement with a supposed excretory mechanism of uric acid through the hair (4).

The occurrence also in man of xanthine oxidase, together with the finding that uric acid content in keratinized structures is in the order of magnitude of precursors (15), finally explains, in our opinion, uric acid formation in the hair. This, furthermore, is in agreement with the general catabolic pathway of nucleic acids.

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Received March 21, 1973

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