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ENZYMES OF AMINO ACID METABOLISM IN NORMAL HUMAN SKIN

II. ALANINE AND ASPARTATE TRANSAMINASES*

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In the preceding paper (1) we described the partial characterization and quantitative histochemical localization of glutamate dehydrogenase in normal human skin. In comparison with glutamate dehydrogenase, which catalyzes reversible oxidative deamination, the transaminases catalyze transfer of amino groups, usually from an amino acid to a keto-acid, without net loss of nitrogen. In other words, transamination represents the intermolecular exchange of amino nitrogen. Transamination plays a significant role in metabolism by providing numerous alternative pathways between amino acids. Obviously, it is a useful process for tissues, by which excessive amounts of certain amino acids can be removed rapidly without storage, and the amino acids of body protein deaminated to serve as energy sources upon oxidation via the citric acid cycle in case of caloric deprivation.

This paper characterizes human epidermal alanine and aspartate transaminases (E.C. 2.6.1.2, L-alanine: α-oxoglutarate aminotransferase and E.C.2.6.1.1, L-aspartate: α-oxoglutarate aminotransferase) and undertakes a quantitative histochemical study of these enzymes in normal skin and its appendages.

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MATERIALS AND METHODS

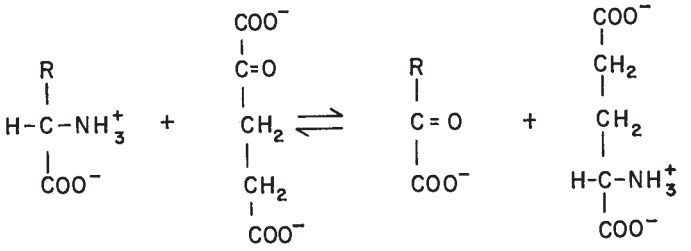
Alanine (or aspartate) transaminase activity was assayed according to the steps shown in Equation 1, with L-alanine (or L-aspartate) and α-ketoglutarate as substrates. Alanine (or aspartate) transaminase catalyzes the formation of pyruvate (or oxaloacetate) and L-glutamate. The resulting pyruvate (or oxaloacetate) is immediately converted to lactate (or malate) in the presence of NADH[1] and purified lactate (or malate) dehydrogenase. The NAD^+ [1] produced was measured fluorometrically (1, 2).

The complete alanine transaminase substrate reagent consisted of 2.5 mM α-ketoglutarate, 100 mM L-alanine (adjusted to pH 7.9), 1 mg% pyridoxal phosphate, 0.5% bovine plasma albumin, 20 mM nicotinamide, 1 mM NADH, and 5 μg/ml reagent mixture of crystalline lactate dehydrogenase in 100 mM Tris-HCl buffer at pH 7.9. The complete aspartate transaminase substrate reagent consisted of 2.5 mM α-ketoglutarate, 20 mM aspartate, 1 mg% pyridoxal phosphate, 0.05% bovine plasma albumin, 20 mM nicotinamide, 2 mM NADH and 1.5 μg/ml reagent mixture crystalline malate dehydrogenase in 100 mM Tris-HCl buffer at pH 8.2.

Human epidermal homogenate was prepared as described in the preceding paper (1), and 5 μl of this homogenate (generally 1%, W/V) were added to 50 μl of the buffered substrate reagent. The mixture was incubated at 37° C for an appropriate period (generally 30 minutes). The resulting NAD^+ was measured fluorometrically in the same way as for glutamate dehydrogenase assay (1).

Frozen and dried sections (0.5 to 5.0 μg) of skin and its appendages were prepared in the manner described by Hershey (3). Skin sections were placed on the bottoms of 2.5 x 50 mm test tubes, and 5 μl of iced buffered substrate added to each tube. After incubating 1 hour at 37° C, the mixtures were returned to the ice bath and 3 μl of 0.6 N HCl added to each to arrest the reaction. A 5 μl aliquot was taken from each tube and the NAD^+ formed determined fluorometrically as previously described. Standards containing 1 and 5 mμmoles of NAD^+ were carried along with the samples. Reagent blanks (reagent

[1] Abbreviations used: NAD^+ and NADH = nicotinamide adenine dinucleotide, oxidized and reduced forms; tris-HCl buffer = tris(hydroxymethyl)amino-methane-HCl buffer.



L – alanine or (L–aspartate) + α keto glutarate ⇌ Pyruvate or (oxaloacetate) + L–glutamate

mixture only) and enzyme blanks (complete assay mixture without substrate) were also run simultaneously.

The lactate dehydrogenase was obtained from the Sigma Chemical Company, St. Louis, Missouri, and was substantially free of transaminase and pyruvate kinase activity. It had a specific activity of approximately 300 μmoles of pyruvate to lactate/min/mg protein at pH 7.5 and 37° C. The malate dehydrogenase (Sigma, Type I) was obtained as a dry preparation and reconstituted with water to make a 1.5 mg/ml suspension. The specific activity was approximately 125 μmoles of oxaloacetate to malate/min/mg protein at pH 7.5 and 25° C.

Protein was measured according to the method of Lowry et al. (4).

RESULTS

The effects of pH on alanine and aspartate transaminase activities are shown in Figure 1. The optimum pH ranges for alanine and aspartate transaminases are 7.6–7.9 and 8.1–8.3 respectively. The pH curves for both enzymes show somewhat flat-topped peaks, and enzyme activities at physiological pH are approximately 80% of those at optimum pH values. Figure 2 shows the effects of various alanine and aspartate concentrations on the respective enzyme activities. Optimum alanine transaminase activity is obtained at L-alanine concentrations above 100 mM and remain optimum with concentrations up to 200 mM, while optimum substrate concentrations for aspartate transaminase activity are 10 to 30 mM; concentrations higher than 50 mM are inhibitory (25% inhibition at 80 mM). The Michaelis constants (Km values) of alanine and aspartate transaminases for their respective amino acid substrates were 1×10^{-2} and

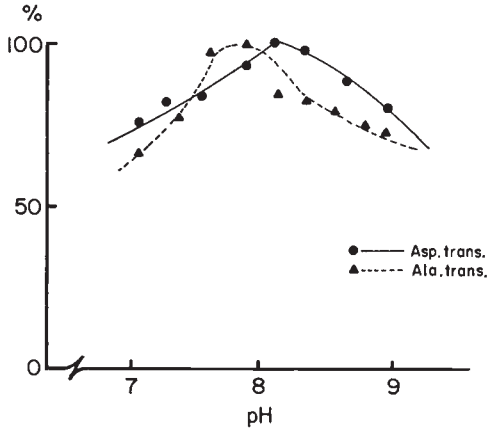


FIG. 1. Effect of pH on alanine and aspartate transaminase activities. The assay conditions are the same as described in the text except for pH. The ordinate shows relative enzyme activities (maximum activity as 100). Homogenate used.

9×10^{-3} respectively. Figure 3 shows the effects of varied α-ketoglutarate concentrations on epidermal alanine and aspartate transaminase activities. It should be noted that excessive amounts of α-ketoglutarate cause marked inhibition. Optimum activities for both enzymes are obtained at 2.5 mM α-ketoglutarate. Alanine transaminase activity is inhibited nearly 50% at an α-ketoglutarate concentration of 8.5 mM, while aspartate transaminase activity is depressed 40% at 30 mM. The Km values of alanine and aspartate transaminases for α-ketoglutarate were 1×10^{-4} and 2×10^{-3} respectively.

Table I shows the effects of omitting each constituent of the test medium. In the absence of any one of the substrates, no transaminase

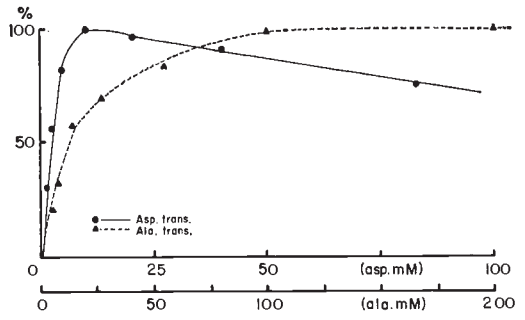


FIG. 2. Effect of L-alanine and L-aspartate concentrations. The reaction systems are as described in the text except for varied substrate concentrations. Homogenate used.

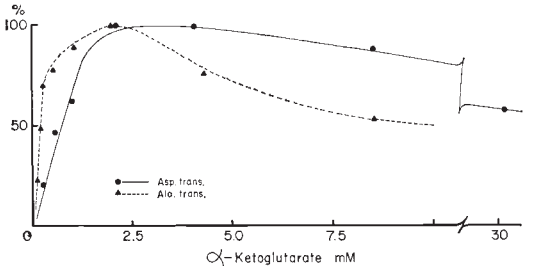


FIG. 3. Effect of α-ketoglutarate concentration. Test systems as in Fig. 1 at optimum pH's. Homogenate used.

activities could be assayed. L-alanine, L-aspartate, α-ketoglutarate, and NADH were essential for the reaction. On the other hand, omission of pyridoxal phosphate caused 25 to 35% reduction of maximal activities. Omission of nicotinamide did not change activities under the assay conditions, while omission of bovine plasma albumin caused 10 to 20% reduction of maximal activity. The latter finding indicates protective action of the plasma albumin against loss of transaminase activities. When their respective auxiliary enzymes were omitted, both transaminase maintained 70–75% of optimum activities. Since malate and lactate dehydrogenases in skin have 10 to 40 times more activity than the transaminases and the respective Km's for oxaloacetate and pyruvate are extremely low, the endogenous malate and lactate dehydrogenases in the homogenate are nearly sufficient for the assay of transaminases.

Figure 4 shows the time course of alanine and aspartate transaminase reactions and the effects of enzyme (epidermal homogenate) concentrations. A linear rate was observed up to 10 mμmoles of NAD^+ production per reaction vessel (55 μl volume) for alanine transaminase and up to 16 mμmoles NAD^+ per tube (55 μl) for aspartate transaminase activity, respectively. Both transaminase activities were di-

TABLE I

Effect of omitting one constituent from the reaction mixture

Constituent omitted	Asp. trans.	Ala. trans.
None*	100*	100*
Aspartate (or alanine)	1.5	3.0
α-Ketoglutarate	2.5	2.1
NADH	0	0.5
Pyridoxal phosphate	64	77
Auxiliary enzyme	71	74
Bovine plasma albumin	83	90
Nicotinamide	100	105

* The complete mixtures with 1.5% (W/V) epidermal homogenate as described in the text were incubated for 30 min. at 37° C. This activity was taken as 100%. Each figure is expressed as % of the maximal activity.

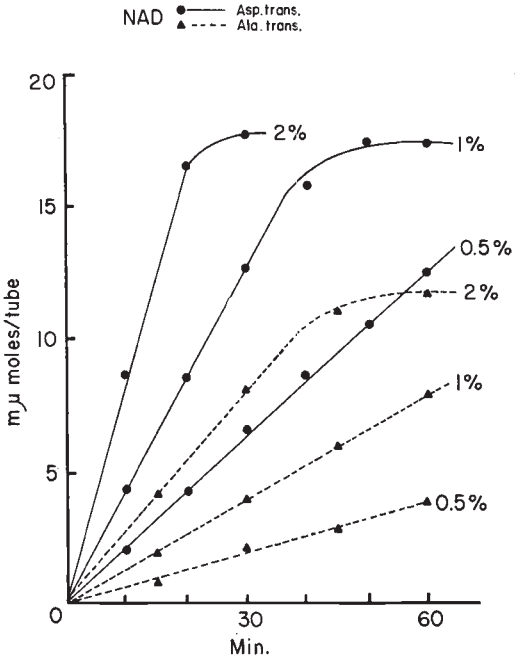


FIG. 4. Reaction rate curves and the effects of enzyme concentrations. Enzyme concentrations as indicated in the figure. Other conditions are the same as described under "methods." Homogenate used.

rectly proportional to the amount of enzyme over a relatively wide range.

Alanine and aspartate transaminase activities of epidermis withstand both freezing and drying. Frozen and dried homogenates stored at −20° C lost no activity after two weeks and less than 20% activity after 1 month of storage. The activities were compared on a protein basis. Both transaminase activities were preserved exceedingly well when fresh skin samples were immediately sectioned in a cryostat and frozen-dried. No loss of transaminase activities occurred even after 3 months of storage at −20° C in vacuum. The frozen-dried sections used for this report were analyzed after less than 2 months of storage.

Alanine transaminase activities in various parts of human abdominal skin are summarized in Table II and aspartate transaminase activities in Table III. The most characteristic feature in the distribution patterns in skin and its appendages is that the sebaceous gland contains extremely high transaminase activities (approximately 4 times more than the epidermis). Variation between individuals is within the expected limits for skin components, except for the hair follicle. This variation may be attributed to the fact that in Cases 2 and 4 no hair follicles at the anagen stage were present. The results of further study on alanine and aspartate transaminase activities in different types of hair follicles are summarized in Table IV. In this experiment human scalp skin samples were obtained 4 hours after the subject's death from an auto accident. Vellus hair follicles were dissected from the frontal region and terminal hair follicles from the temporal region; only external sheaths of hair follicles were dissected as samples. Table IV shows that aspartate transaminase activity does not change significantly in relation to hair follicle types; on the other hand, alanine transaminase activity increases 100% in active hair follicles. Therefore, alanine transaminase may be a sensitive functional barometer reflecting a specific metabolic process.

Alanine transaminase activities in fresh

TABLE II

Alanine transaminase activities in various parts of skin*

Case	Epidermis	Dermis	Hair follicle	Sebaceous gland	Sweat gland
1	.35 ± .01	≦.006	.27 ± .07	1.7 ± .16	.14 ± .02
2	.34 ± .05	≦.009	.08 ± .01	1.3 ± .14	.10 ± .01
3	.27 ± .01	≦.008	.23 ± .03	1.1 ± .14	.13 ± .02
4	.46 ± .05	≦.001	.07 ± .01	—	.10 ± .01
5	.38 ± .05	—	—	—	.40 ± .06
6	.41 ± .05	—	.09 ± .01	—	.13 ± .02
Ave.	.37 ± .04	≦.006	.15 ± .03	1.4 ± .15	.17 ± .02

* Each value is the mean activity of 6 frozen-dried sections weighing 0.5 to 5.0 μg. The activity is expressed as moles of product formed per hour per kilogram dry weight ± standard error. Each case number represents skin from a separate individual.

TABLE III

Aspartate transaminase activities in various skin structures*

Case	Epidermis	Dermis	Hair follicle	Sebaceous gland	Sweat gland
5	1.1 ± .12	≦0.03	2.0 ± .29	5.2 ± .50	3.5 ± .17
6	1.0 ± .08	≦0.02	1.4 ± .12	2.0 ± .17	1.7 ± .07
Ave.	1.1 ± .10	≦0.03	1.7 ± .21	3.6 ± .34	2.6 ± .12

* Expressed as moles/hour/kg dry weight tissue ± standard error. Each figure is the mean of 6 determinations. These determinations were done on frozen-dried sections.

TABLE IV

Aspartate and alanine transaminase activities in different types of hair follicles*

Hair type	Asp. trans.	Ala. trans.
Vellus hair	2.6 ± .31	.22 ± .04
Termnal hair		
Telogen	1.8 ± .15	.21 ± .02
Anagen	2.2 ± .15	.40 ± .05

* Activities are expressed as moles/hour/kg dry weight tissue ± standard error. Average of 8 determinations. These determinations were done on frozen-dried sections.

human epidermis were .14, .15 and .11 moles/kg wet wt/hr. Alanine transaminase activities in the epidermis of frozen-dried sections are approximately 3.3 times more active than those in the epidermal homogenates. This finding corresponds to the dry-over-wet weight ratio of 1:3.5.

DISCUSSION

The present paper is, to our knowledge, the first report of transaminase activities in "normal" human skin. Earlier work has shown alanine transaminase activity in rat epidermis (5) and in pathological human epidermis obtained from two cases of pemphigus (6). In comparison with the data presented in this report, the activities previously shown for human pathological epidermis (6) are approximately 1/5 to 1/10 of the alanine transaminase activity that we observed. Namely, if values for a total nitrogen content of human skin of 4.5% (7) and a temperature coefficient at 25° C of 30% are taken respectively, the 56 and 31 units per 10 mg total nitrogen for the two cases would be expressed as 0.025 and 0.014 moles/kg wet wt/hr, while the data presented in this report showed an approximate activity of 0.13 moles/kg wet wt/hr. These differences in activity may well be due to the pathological status of the pemphigus sample and to the differences in assay conditions. The data previously reported (6) were obtained with a reaction mixture in which no pyridoxal phosphate was present and the α-ketoglutarate concentration was slightly excessive (6.6 mM). Each of these factors alone would reduce the activity by 40%.

Although epidermal transaminases are activated by pyridoxal phosphate, dialysis of the epidermal homogenate does not cause complete loss of these transaminase activities: the homogenate still retains more than 40% of optimum activities without further addition of pyridoxal phosphate. Therefore, we suspect that most of the endogenous pyridoxal phosphate is bound to the transaminases in a physiological state.

Pyridoxal phosphate represents the main biocatalytically active form of vitamin B6. A deficiency of this vitamin causes acrodynia in rats (8) and a condition resembling sicca type dermatitis in man (9). Therefore, vitamin B6 deficiency may well be related to changes in various transaminases and may play an important role in the mechanism involving certain kinds of dermatitis; however, this requires further investigation.

The localization of transaminase activities in skin is somewhat similar to that of isocitrate dehydrogenase (10). In the case of both enzymes the sebaceous gland has the greatest activity. It is interesting to note that the product of the isocitrate reaction is α-ketoglutarate, a common substrate of both alanine and aspartate transaminases. However, glutamate dehydrogenase, which also utilizes α-ketoglutarate, has a different distribution in skin with epidermis having the greatest activity (1). The relative activities of sebaceous glands and epidermis are 3.5:1, 2:1 and 0.2:1 for transaminases, isocitric dehydrogenase, and glutamate dehydrogenase respectively. These different ratios suggest different amino acid metabolisms for epidermis and sebaceous glands.

Changes in various enzyme activities at different stages of the hair cycle have been recognized histochemically (11). However, there has been little work on quantitative enzyme changes in the hair cycle. It appears that the only work reported is that of Carruthers (12), who found that cytochrome oxidase activities of hair follicle preparations from very young mice were considerably higher than those of epidermis. Later Dushoff et al. (13) reported that both QO_2 and 2,3,5-triphenyl tetrazolium reduction decreased as the cycle went from early anagen to the telogen stage. However, in this experiment, hair fol-

licles were not isolated from epidermis or other skin constituents. Qualitatively, the tetrazolium reduction, for example, was observed over the entire hair follicle, especially in the external sheath in early anagen; while in late anagen stage the follicles were only diffusely stained. Our data on alanine and aspartate transaminase activities in different types of hair follicles may be taken as an additional contribution to the understanding of metabolism in the hair cycle.

SUMMARY

1. A sensitive and reproducible micro method for the determination of alanine and aspartate transaminase activities in epidermal homogenates and various parts of skin (0.5 to 5 μg dry weight) is described.

2. Alanine transaminase activities of epidermis, dermis, hair follicle, sebaceous gland, and sweat gland in normal human skin were 0.37, 0.006, 0.15, 1.4 and 0.17 moles/kg dry weight/hr respectively (average of 6 cases). Epidermal homogenates have an alanine transaminase activity of approximately 0.13 moles/kg wet wt/hr.

3. Aspartate transaminase activities of epidermis, dermis, hair follicle, sebaceous gland, and sweat gland in normal human skin were 1.1, 0.03, 1.7, 3.6 and 2.6 respectively (average of 2 cases).

4. Localization patterns of these two transaminases are similar, i.e., sebaceous glands contain the highest activities, 3.5 times more than epidermis. This pattern also is somewhat similar to that of isocitrate dehydrogenase activity previously described. It coincides with a product and substrate relationship among these 3 enzymes.

5. Assay of transaminases in different types of hair follicles showed that alanine transaminase activity is 100% greater in anagen than in telogen or vellus type hair follicles. Alanine transaminase reflects more sensitivity to the functional status of the hair cycle than does aspartate transaminase.

6. Available data suggest the active participation of alanine and aspartate transaminases in various functional statuses of skin and appendages.

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