QUANTITATIVE HISTOCHEMISTRY OF THE PRIMATE SKIN.

IV. \( \alpha \)-GLYCEROPHOSPHATE DEHYDROGENASE*

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In contrast to glyceraldehyde-3-phosphate dehydrogenase, which is one of the key enzymes in the Embden-Meyerhof glycolytic pathway, the physiological role of \( \alpha \)-glycerophosphate dehydrogenase in mammalian tissues has remained largely unexplored. The main role of \( \alpha \)-glycerophosphate dehydrogenase in mammalian tissues may be to serve \( L \)-\( \alpha \)-glycerophosphate for the synthesis of phospholipids (glycerophosphatides) as well as participating indirectly in the anaerobic glycolytic pathway.

This study reports the quantitative distribution of \( \alpha \)-glycerophosphate dehydrogenase in skin. The enzyme catalyzes the interconversion of \( \alpha \)-glycerophosphate and dihydroxyacetone phosphate.

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L(\text{--})-\alpha \text{-glycerophosphate} + \text{NAD}^+ \rightarrow \text{Dihydroxyacetone phosphate} + \text{NADH} + \text{H}^+
\]

MATERIALS AND METHODS

The materials and general analytical methods have been described in some detail in a preceding communication (1). The assay method for \( \alpha \)-glycerophosphate dehydrogenase was essentially the same as that of Lowry and Passonneau (2). Since this enzyme catalyzes NADH-coupled reaction with dihydroxyacetone phosphate as substrate, one of the products, NAD\(^+\), may be measured with a sensitive fluorometer. Dihydroxyacetone phosphate was obtained commercially as monobasic acid; pCMB p-chloromercuribenzoate.

The stoichiometry of this reaction was tested to validate the test system for \( \alpha \)-glycerophosphatide synthesis of phospholipids (glycerophosphatides).

RESULTS

The rate of reduction of dihydroxyacetone phosphate at different pH values is shown in Figure 1. The pH activity curve has an optimum at pH 7.6 (in Tris-HCl buffer), consisting of a sharp peak, so that the activity at pH 7.0 is only 65% of the optimal activity. Figure 2 shows the effect of substrate concentration. An optimal reaction was attained at 0.25 mM dihydroxyacetone phosphate, and no significant substrate inhibition was demonstrated up to 2 mM of final concentration. The effect of NAD\(^+\) was also determined (Fig. 3). The optimal reaction was obtained between 0.5 and 4 mM of NAD\(^+\) concentration. Figure 4 shows the time course of the reaction. A linear reaction was observed up to nearly 11 \( \mu \)moles of NAD\(^+\) production per reaction tube. A linear reaction was also obtained with different levels of epidermal homogenate concentration within the limit of 12 \( \mu \)moles of NAD\(^+\) formation per tube.

The stoichiometry of this reaction was tested to validate the test system for \( \alpha \)-glycerophosphatide.

1 Abbreviations used are: NAD\(^+\) and NADH = nicotinamide adenine dinucleotide, oxidized and reduced form; EDTA = ethylenedinitrilotetraacetic acid; pCMB = p-chloromercuribenzoate.

2 Dihydroxyacetone phosphate, NAD\(^+\), NADH and crystalline \( \alpha \)-glycerophosphate dehydrogenase (muscle, 75 \( \mu \)moles/mg protein/min at pH 7.4 at 25° C) were purchased from Sigma Chemical Company.
Fig. 1. pH activity curve. The reaction mixture as described in the text at different pH values. The ordinate represents the fluorescence due to NAD, which is the microammeter reading after subtracting the blank.

Fig. 2. Effect of dihydroxyacetone phosphate (DHAP) concentration. The assay condition as described in the text except for different substrate concentrations.

Fig. 3. Effect of NADH. The test system as for Figure 2 with dihydroxyacetone phosphate held at 0.5 mM.

Fig. 4. Time course of the reaction. The optimal assay condition as described in the text, with 5% epidermal homogenate.

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Phosphate dehydrogenase. The disappearance of both dihydroxyacetone phosphate and NADH (300 and 290 μmole/ml) corresponded well with the formation of NAD+ (270 μmole/ml).

The effect of some activators and inhibitors was examined under the optimal test system described above. EDTA (1 mM) caused approximately a 10% increase in activity; mercaptoethanol (1 mM) did not change its activity. Notwithstanding the report that pCMB at 0.1 mM completely inhibited the purified enzyme (4), pCMB (0.1 mM) inhibited only 25% of the original (control) activity. In our study, the crude epidermal homogenate as well as the bovine plasma albumin added to the test medium possibly combined with pCMB, resulting in only 25% inhibition.

When a reversal reaction was tested according to van Eys et al. (4), no α-glycerophosphate dehydrogenase activity was assayed. Enzyme activity was not detected, even when it was measured fluorometrically with increased substrate levels (up to 1 mM α-glycerophosphate and 1 mM NAD+ at pH 7.2 and 9.6).

**TABLE I**

<table>
<thead>
<tr>
<th>α-Glycerophosphate dehydrogenase activity* of various skin structures</th>
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<tr>
<td>Scalp</td>
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<td>Epidermis</td>
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<td>Hair follicle</td>
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<td>Eccrine gland</td>
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<td>Apocrine gland</td>
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<td>Sebaceous gland</td>
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<td>Dermis</td>
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<tr>
<td>Lip</td>
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<tr>
<td>Mucous membrane, upper</td>
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<td>Mucous membrane, lower</td>
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<td>Sebaceous gland</td>
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*α-Glycerophosphate dehydrogenase activity is expressed as μmole of the substrate decomposed per hour per kilogram of dry weight sample (μmole/hr/kg dry wt.). Each figure represents the mean of five determinations.
The α-glycerophosphate dehydrogenase activities in various parts of the skin of rhesus and stump-tail macaques are summarized in Table I. Each value represents the mean of 5 determinations. Enzyme distribution is generally similar in the rhesus and stump-tail macaque with a few exceptions, such as in the eccrine glands and in the mucous membrane of the lip. The most characteristic feature of this enzyme distribution is that the glandular structures, such as sweat and sebaceous glands (800 to 1100 mmoles/hr/kg dry wt.) contain 10 times as much activity as the epidermis (approximately 100 mmoles/hr/kg dry wt.). Also the activity of this enzyme in various epidermal layers of the sole and mucous membrane of the lip is found to be only one-fifth that of hexokinase and fructoaldolase activities in the same tissues of the same animals (cf. ref. 1, 3). Only small amounts of activity were found in the keratin layer (sole) and in the dermis.

**DISCUSSION**

Among the few enzymes thus far studied quantitatively-histochemically, α-glycerophosphate dehydrogenase in primate skin has a distinctive feature: it has relatively high activity in glandular structures and extremely low activity in the epidermis. When the distribution of this enzyme activity is compared with that of glyceraldehyde-3-phosphate dehydrogenase (5), which is located at approximately the same level in the glycolytic pathway, their differences are conspicuous. The epidermis and mucous membranes contain approximately 50 times more active glyceraldehyde-3-phosphate dehydrogenase than α-glycerophosphate dehydrogenase. Since a considerable amount of triose isomerase activity is present in the epidermis of the rhesus monkey (6), dihydroxyacetone phosphate may be easily converted to glyceraldehyde-3-phosphate, which subsequently will be catalyzed by the highly active glyceraldehyde-3-phosphate dehydrogenase. Therefore, it appears that, in normal epidermis or mucous membrane, α-glycerophosphate dehydrogenase does not participate actively in glycolysis. The physiological role of this enzyme in the epidermis might be limited to the synthesis of glycerophosphatides.

It is speculated that relatively active α-glycerophosphate dehydrogenase in the sebaceous and sweat glands might be related to the physiological role of this enzyme in the synthesis of phospholipids and neutral fat. At the same time, it is possible that this fairly active α-glycerophosphate dehydrogenase participates in the anaerobic glycolytic pathway in such a fashion as to compete for a common substrate (dihydroxyacetone phosphate) with triose isomerase, and/or competing for a common coenzyme (NADH) with lactic dehydrogenase.

**SUMMARY**

α-Glycerophosphate dehydrogenase activity in the skin and appendages is generally much lower than other enzymes participating in glycolysis. The epidermis has approximately 100 mmoles/hr/kg dry wt. of activity, which is one-fifth that of hexokinase or fructoaldolase activity and one-fiftieth that of glyceraldehyde-3-phosphate dehydrogenase activity of the same epidermis. It is possible that the main physiological role of this enzyme in the epidermis and mucous membrane may be concerned with the synthesis of glycerophosphatides, and not with anaerobic glycolysis.

This enzyme’s activity is relatively high in sebaceous and sweat glands, i.e. 850 to 1100 mmoles/hr/kg dry wt. The values are of the same order of magnitude as compared with hexokinase or fructoaldolase activities, and one-fourth to one-sixth those of glyceraldehyde-3-phosphate dehydrogenase activities in glandular tissues. In sebaceous and sweat glands, this enzyme may participate in glycerophosphatides synthesis as well as in the anaerobic glycolytic pathway.

**REFERENCES**