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Erythrocyte Acid Phosphatase in Human Hair Root Sheaths

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ABSTRACT: Erythrocyte acid phosphatase (EAP) was observed in human hair root sheaths where hair sheaths and blood samples were collected from 150 donors. In each case, the hairs were typed first for EAP and then confirmed by typing the blood from the same donors. Of the 150 donors checked, EAP activity was found in 135 of the samples; the other 15 samples showed no activity because they contained no sheath cells. Of the 135 samples showing activity, 108 of those were typed accurately in EAP. The remaining 27 samples had EAP activity but with inconclusive findings. In this study, all six common phenotypes were encountered.

KEYWORDS: pathology and biology, genetic typing, hair, phosphatases, hair root sheaths, erythrocyte acid phosphatase, criminalistics

The typing of hair has been tried in many systems beginning with attempts of typing hair in the ABO system, but studies related to this technique proved it to be unsuitable for courtroom testimony, although ABO types could sometimes be obtained.

New techniques in identification have now led to alternative methods of hair root sheath typing based on polymorphic enzyme systems. The enzyme typing of hair root sheaths is now well documented with hair root sheath showing activity in these systems: phosphoglucomutase (PGM), adenosine deaminase (ADA), adenylate kinase (AK), and esterase D (EsD) [*1*]. Other studies also showed activity concerning the enzyme, 6-phosphogluconate dehydrogenase (6-PGD), and glyoxalase 1 (GLO) [*2-4*].

One of the genetic markers present in nearly all human body tissues is erythrocyte acid phosphatase (EAP) [5]. The EAP system has three common allelic autosomal genes and has six common phenotypes for use in identification [*6*]; thus EAP has excellent discriminatory power. Hair root sheaths have been reported to contain acid phosphatase activity but it does not conform to the electrophoretic patterns associated with EAP [*4*]. The reason for this could be that the activity is a mixture of lysosomal and erythrocyte acid phosphatase. The lysosomal and erythrocyte types of acid phosphatase can be distinguished; the former is inhibited by tartrate acid, the latter by formaldehyde [*7*]. These inhibitors can be used in gel staining solutions to distinguish the patterns given by the two types of acid phosphatases.

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In the present study, when tartrate is included in reaction solutions, clear EAP patterns are seen. Utilizing this technique of electrophoresis, phenotypes of hair root sheaths in EAP were interpretable with no incorrect results.

Method

The method used in this study is a modification of the Wraxall and Emes technique of electrophoresis [8]. The tank buffer consists of 0.245*M* sodium dihydrogen phosphate and 0.10*M* trisodium citrate pH 5.9. The gel buffer is a 1 to 100 dilution of the tank buffer solution. The gel is a 10% (w/v) starch gel which is poured and scraped to a 1-mm thickness. An origin is cut with a slot cutter 6 cm from the cathode end of a 20-cm gel plate. To each slot, 2 μL of 0.05*M* dithiothreitol is allowed to react with the EAP in the root sheaths for approximately 10 min before electrophoresis. The hair root sheaths are then electrophoresed for 4.5 h at 400 V. At the completion of electrophoresis, the plate is removed and blotted with 3MM® Whatman chromatographic paper. The reaction buffer is a mixture of 0.05*M* citric acid and 0.05*M* L-(+)- tartaric acid[4] adjusted to pH 5.0 with 0.10*M* sodium hydroxide. A mixture containing 4 mg of 4-methylumbelliferyl phosphate (4-MUP) and 10 mL of the reaction buffer, warmed to 37°C, is soaked onto 3MM Whatman chromatographic paper and placed in an area from the origin to 6 cm anodal to the origin for development. (The 4-MUP is dissolved in reaction buffer just before its use.) The gel plate is then placed into an incubator for 30 min to allow for development. After the development period, the reaction blotter is removed and replaced with one saturated with 10 mL of 0.06*M* sodium hydroxide for 2 min. The plate is then viewed under long wave ultraviolet light and the results recorded.

Results and Discussion

In the study, 150 hair samples were collected from donors of both sexes and subjected to EAP electrophoresis. One-hundred-and-thirty-five hair samples showed activity, while fifteen did not. The 15 samples that showed no activity had no sheaths attached, indicating the EAP activity in hair roots resides in the sheath. Of the 135 samples showing activity, 108 were typed in EAP in all 6 common phenotypes (Fig. 1); in the absence of tartrate the EAP phenotypes were indistinct and could not be demonstrated reliably in hair root sheaths.

Our results indicate that we were able to type all of the hairs that had sheaths, so it is suggested strongly hairs be viewed stereoscopically for the presence of sheaths before subjecting them to electrophoresis. It was also observed that the reducing agent should be allowed to react with the root sheath for 10 min in the gel before applying the current.

When tartrate is not present in the stain solution, activity bands appear in the "A" region, whereby a B homozygous sample could be mistyped as a heterozygous BA. This activity is due to the mixture of lysosomal and erythrocyte acid phosphatase in sheath cells because, when tartrate is used in the reaction mixture, the smeared patterns were cleared. This inhibiting effect of L-(+)- tartaric acid gave excellent results and EAP electrophoresis should not be attempted on root sheaths without this inhibitor in the reaction buffer. The reaction buffer should be warmed to 37°C before being applied to the gel. Experience showed better results were obtainable by using the reaction buffer at 37°C than room temperature, especially for EAP from root sheaths. Because the method is nondestructive, the hairs can be removed after electrophoresis and subjected to comparison microscopy for further identification. In fact, the hairs can be removed from the gel as soon after the initiation of electrophoresis as the enzyme has left the sheath and entered the gel.

[4]G. F. Sensabaugh, associate professor, University of California, Berkeley, CA, personal communication, March 1982.

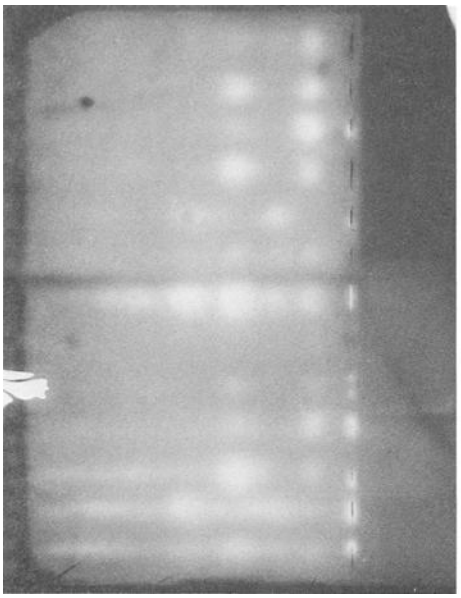

FIG. 1—*Erythrocyte acid phosphatase in human hair root sheaths, with (Samples 1 to 6) and without (7 to 13) tartrate treatment.*

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