Anne M. Gambel,[1] *B.A.; Bruce Budowle,*[1] *Ph.D.; and Linda Terrell,*[1] *M.S.*

Glyoxalase I Typing and Phosphoglucomutase-1 Subtyping of a Single Hair

REFERENCE: Gambel, A. M., Budowle, B., and Terrell, L., **"Glyoxalase I Typing and Phosphoglucomutase-1 Subtyping of a Single Hair,"** *Journal of Forensic Sciences,* JFSCA, Vol. 32, No. 5, Sept. 1987, pp. 1175-1181.

ABSTRACT: A technique is described for the typing of glyoxalase I (GLO I) and the subtyping of phosphoglucomutase-1 (PGM-1) from the root sheath cells of a single forcibly removed hair. This procedure does not require sample preparation and does not alter the morphological characteristics of the hair. The combined discrimination probability (DP) of the two markers taken together is 0.90 for whites and 0.89 for blacks. GLO I can be typed after four weeks, and PGM-1 can be typed after eight to fifteen weeks in hairs maintained at room temperature. Hairs mounted with Permount® showed loss of enzyme activity and loss of band sharpness.

KEYWORDS: forensic science, hair, phosphoglucomutase, glyoxalase, electrophoresis, isoelectric focusing, sheath cell analysis

The examination of hair has long been performed by the microscopical examination of morphological features and characteristics. However, genetic marker proteins also have been shown to be present in the hair sheath and can provide valuable information. ABH blood group substances are present in the hair shaft [*1,2*] and can be typed by adsorption-elution assay or a mixed agglutination method [*3*]. Likewise, keratin proteins in the shaft can be separated electrophoretically [*4-6*], although it is a destructive technique, and the probability of discriminating (DP) between individuals is low. Many genetic marker proteins found in blood are also present in the follicular sheath cells associated with the root. Phosphoglucomutase-1 (PGM-1), esterase D (EsD), glyoxalase I (GLO I), adenylate kinase (AK), 6-phosphogluconate dehydrogenase (6-PGD), adenosine deaminase (ADA), and phosphohexose isomerase (PHI) have been detected electrophoretically [*7-10*].[2]

Given the limited material available in the root sheath cells of a single hair sample, sensitivity of assay and DP of the genetic marker system is of primary importance in the selection of the marker or markers for electrophoretic analysis of hair root. EsD produces weak patterns [*7,9*] in fresh hair root samples, as do AK [*7,8*] and ADA [*7*]. EAP has proven unreliable, yielding indistinct or altered patterns [*7,11*]. In contrast, GLO I and PGM-1 patterns

This is Publication 86-9 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation. Received for publication 15 Aug. 1986; revised manuscript received 31 Oct. 1986; accepted for publication 3 Nov. 1986.

[1]Scientist, research chemist, and scientist, respectively, Forensic Science Research and Training Center, Laboratory Division, FBI Academy, Quantico, VA.

[2]C. Bosley, R. M. Burgess, J. G. Sutton, and P. H. Whitehead, "Enzymes in Human Hair Sheath Cell—A New Means of Characterizing Hair," Home Office Central Research Establishment Report 359, Aldermaston, U.K., 1980.

are intense, distinct, and have high DPs [*15*]. To take advantage of this, several systems have been developed which can type either GLO I or PGM-1 [*7,9,10,12*]. A conventional system, Group I, has been used to type GLO I, PGM-1, and EsD of the root sheath simultaneously [*13*], although the EsD is weak. Alternatively, the sheath may be sectioned with a microtome and each section analyzed separately for a different marker [*14*]. The DP of the Group I conventional system described above is 0.88 for whites and 0.84 for blacks [*15*]. However, isoelectric focusing for PGM-1 subtyping can resolve ten common phenotypes of PGM-1 rather than three by conventional electrophoresis. The DP of PGM-1 subtyping and GLO I typing yields values of 0.90 and 0.89 for whites and blacks, respectively. Thus, a simple procedure which could successfully type GLO I as well as subtype PGM-1 from the sheath of a single forcibly removed hair would be of value.

A simple technique has been developed for the analysis of GLO I and PGM-1 from a single forcibly removed hair containing sheath cells. No sample preparation was necessary. Stability of these markers at room temperature was examined, and because morphological hair analyses frequently requires that a hair be mounted or fixed upon a slide for microscopical examination, the effect of Permount®, a common mounting medium, upon enzymatic activity of the markers was examined.

Materials and Methods

Double Enzyme Typing From One Hair

Freshly plucked head hairs with root sheath cells were used in two sets of experiments to determine if one hair could be typed for two different polymorphic enzymes. In the first set of experiments, the hair was analyzed for GLO I and then PGM-1. A 5-mm portion of the root end of the hair, containing sheath measuring 0.5 to 2 mm, was dipped in 50m*M* dithiothreitol (DTT, Sigma) and inserted into a 1-mm-thick agarose gel 1 cm from the cathode for 3 min. It was then removed and stored in a test tube at 4°C. Gel preparation and electrophoresis was performed for GLO I according to the method of Budowle [*16*]. The hair was subsequently typed for PGM-1 by isoelectric focusing according to the method of Budowle [*16,17*]. The root end of the hair was wetted with distilled water and placed directly on the gel surface for sample application. In the second set of experiments the sequence of analysis was reversed; PGM-1 was typed first followed by analysis for GLO I. Recipes for GLO I tank and gel buffers and assay solutions (modified from the procedure of Kompf et al. [*18*] are given in Table 1. Recipes for PGM-1 gel and reaction overlay are given in Table 2.

Stability Study

Twelve head hairs bearing sheath cells (0.5 to 2 mm in length) were plucked from each of twenty donors of known PGM-1 and GLO I types at the FBI Academy and maintained at ambient temperature and humidity. Hairs were analyzed first for GLO I and subsequently for PGM-1. The initial typing of the hairs was done one day after they were plucked. Analysis was repeated using one hair from each donor during Weeks one, two, four, eight, and fifteen.

Analysis of Head Hairs Fixed in Permount

Six head hairs were plucked from each of five donors and mounted on glass slides in the following manner. Each hair was positioned on a glass slide and saturated with a drop of xylene. Excess xylene was absorbed with a cotton swab. Three drops of Permount (Fisher) were placed on the hair, and a glass coverslip was overlaid in a manner to avoid air bubble formation. Slides were stored at room temperature. Analysis was performed using one hair

TABLE 1—*Recipes for GLO I buffers.*

Buffer Type (Total Volume)	Ingredient	Amount of Ingredient
Tank buffer (4 L)	distilled water	3500 mL
	Trizma base	48.44 g
	maleic acid	46.48 g
	EDTA disodium, dihydrate	11.68 g
	magnesium chloride 6H2O	8.12 g
	sodium hydroxide	24.00 g
	pH 7.4, with NaOH, qs to 4 L	
Gel buffer: 1 : 14 dilution of tank buffer		
Phosphate buffer	distilled water	450 mL
	NaH2PO4	3.060 g
	Na2HPO4	3.480 g
	pH 6.8, qs to 500 mL	
Tris buffer	Tris	6.055 g
	distilled water	500 mL
	do not adjust pH	
Reaction Mixture I	reduced glutathione	0.032 g
	magnesium chloride, 6H2O	0.025 g
	methyl glyoxal	130 μL
	phosphate buffer	10 mL
Reaction Mixture II	methyl tetrazolium dye (MTT)/Tris buffer	20 mg/10 mL
	Dichlorophenol-indophenol/Tris buffer	6 mg/10 mL
	2% agarose (400-mg/20-mL H2O)	20 mL

TABLE 2—*Recipes for PGM-1 gel and assay solutions.*

Gel or Solution	Ingredient	Amount of Ingredient
Acrylamide stock solution	acrylamide	29.1 g
	bis acrylamide	0.9 g
	distilled water	50 mL
	filter and qs to 100 mL	
Gel solution	stock solution	1.83 mL
	distilled water	7.70 mL
	ampholine pH 5 to 7 (LKB)	1.10 mL
	EPPS (Sigma)	130 mg
	ammonium persulfate	7.5 mg
	degas 1–2 min	
	TEMED	7.5 μL
Assay buffer	distilled water	400 mL
	Trizma base	18.0 g
	magnesium chloride 6H2O	2.0 g
	histidine monohydrochloride	1.0 g
	pH 8.0 w/HCl, qs to 500 mL	
Reaction solution	assay buffer	10 mL
	glucose-1-phosphate	35 mg
	NADP	6 mg
	methyl tetrazolium dye	6 mg
	Meldola Blue solution (5-mg/10-mL H2O)	200 μL
	Glucose 6 phosphate dehydrogenase,	150 μL (75U)
	2% agarose (200-mg/10 mL H2O)	10 mL

TABLE 3—*Stability study[a] of PGM-1 and GLO I in hair root sheath cells from 20 individuals.*

	Number Conclusive Calls[b]				
Marker	Week 1	Week 2	Week 4	Week 8	Week 15
GLO I	20	20	20	0	0
PGM-1	20	20	20	20	5

[a]Hairs were maintained at ambient temperature and humidity.
[b]Numbers indicate conclusive calls. All others were inconclusive or negative results.

from each donor after one, two, four, eight, twelve, and fifteen days. Before analysis, xylene was pipetted under an edge of the coverslip, and the cover slip was carefully removed. The root was dipped in five separate 100-μL aliquots of xylene and washed for 1 min in distilled water. Immediately before electrophoresis for GLO I, the hair was dipped in 50m*M* DTT. Before isoelectric focusing, the hair was wetted with distilled water to facilitate protein migration.

Comparison of Hair Morphology Before and After Electrophoresis

Six head hairs were plucked from each of five donors. Three samples from each donor were mounted, and their microscopic characteristics were observed by a Federal Bureau of Investigation Microscopic Analysis examiner. The remaining three samples from each donor were treated as described above for marker analysis. After electrophoresis and isoelectric focusing, the samples were mounted and compared directly with the controls.

Results and Discussion

Figures 1 and 2 show that the procedure described in this paper for the analysis of GLO I and subsequent analysis of PGM-1 from the hair follicular sheat cells yields highly resolved patterns. Zymograms for both genetic marker protein systems were identical to those in blood. If the sample was left on the gel for the duration of the electrophoretic run of GLO I, streaking patterns and aggregate formations were observed (Fig. 1). The 3-min application of the hair root in the agarose without any applied field strength across the gel deposited sufficient protein for GLO I analysis without depleting the sample for subsequent PGM-1 analysis and yielded well-defined bands. The same effect was observed in isoelectric focusing of hair. Samples left on the gel for more than the alloted 10-min sample application period exhibited streaking patterns which were poorly defined.

Because the sample application procedure of PGM-1 required electrophoretic elution, there was not sufficient protein left for analysis of GLO I; therefore, GLO I was analyzed first. However, in some cases it was observed that PGM-1 subtyping could be performed twice upon the same sample. Extraction of the sheath proteins was found to be unnecessary and should be avoided because use of the hair sheath itself for sample application gave better results with less chance of losing or diluting the sample. Additionally, use of the hair is easier because it requires no sample preparation. The hair should be wet upon application, because this facilitated protein migration out of the sheath and improved contact with the gel. A dry application yielded weaker patterns.

The stability study of unmounted hairs containing 0.5 to 2.0 mm of root sheath indicated that GLO I could be typed up to about four weeks at room temperature. After that time,

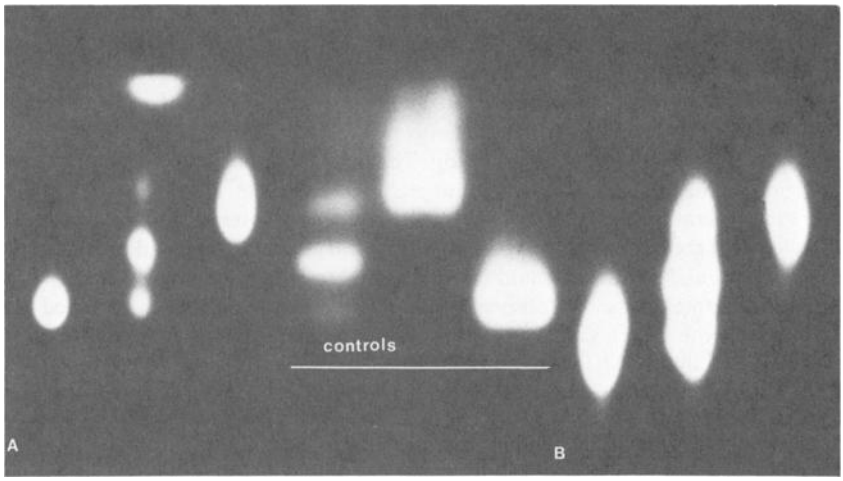

FIG. 1—*GLO I typing of hair. Hair phenotypes shown are:* (a) *hair removed after 3 min—1, 2—1, 2;* (b) *hairs left on the gel during the electrophoretic run—1, 2—1, 2. The anode is at the top.*

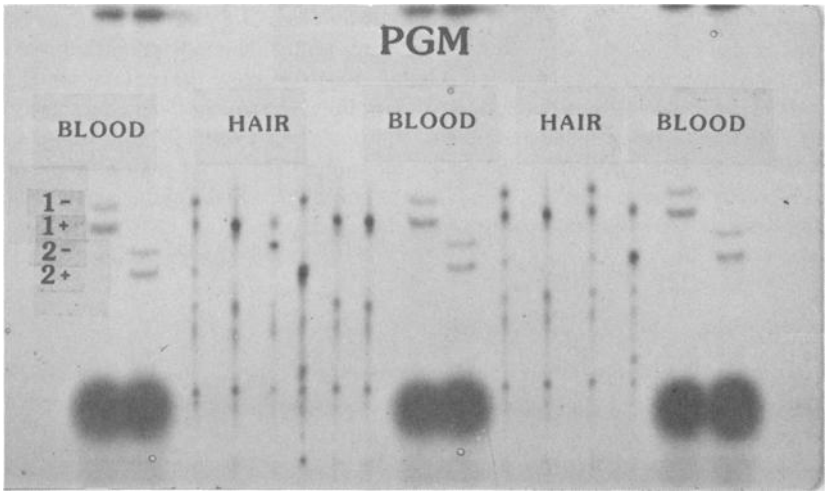

FIG. 2—*PGM-1 subtyping of hair by isoelectric focusing. Hair phenotypes shown are: 1—1+, 1+, 1+2—, 1—2+, 1+, 1+, 1—1+, 1+, 1—1+, 1+2+. The cathode is at the top.*

activity was detectable, but no defined patterns were observed. PGM-1, however, was typed in all samples up to eight weeks. At fifteen weeks, five samples (25%) still could be typed. In all cases both the GLO I and PGM-1 phenotypes determined from the hair roots matched the known phenotypes of the donors.

The above results were obtained using unmounted hairs; however, it is standard practice in many forensic science laboratories to mount the hair on a slide using the commercial

preparation Permount, in conjunction with xylene. Analysis of hairs after only one day in Permount demonstrated substantial loss of activity and band definition in GLO I for all donors and loss of PGM-1 activity. Because of the marked loss of GLO I activity in these samples as compared with unmounted hairs after only one day, GLO I analysis was abandoned and PGM-1 run exclusively. Hairs of only two donors were able to be typed for PGM-1 between eight and twelve days after mounting. The three other donors showed reduced activity at four days and produced no patterns at eight or more days.

There were several possible causes for the difficulties encountered in analysis of mounted hairs. Removal of the hair from a viscous medium could cause loss of sheath material. Treatment with xylene and Permount could be detrimental to enzyme activity. Further, washing could result in protein loss. We observed that coarse hairs were able to be typed longer, possibly because the sheath was less easily removed. Therefore, the best results can be obtained for GLO I and PGM-1 from an unmounted hair.

Hairs were examined microscopically to compare their structures before and after electrophoresis and isoelectric focusing. No changes in shaft or root morphology were observed, thus confirming the observation of Bosley et al.[2] Therefore, these enzymes may be analyzed before microscopical examination of the hair without altering the morphological characteristics of the sample.

In conclusion, there are several points to consider when using the above described method. Intact sheath is associated with anagen, or actively growing, hair. Anagen hair specimens have been forcibly pulled or possibly brushed out, and may be found at the scene of violent crime. When intact sheath is found on a hair specimen (although this comprises a small percentage of hairs submitted for forensic science examination), typing of GLO I and PGM-1 can be attempted using existing technology. The method is simple, requires no sample preparation, and uses standard electrophoretic and focusing procedures already widely used for bloodstain analysis. Thus, it does not place undue demands upon the time or resources of the laboratory. Additionally, it is unnecessary to remove the root portion from the hair shaft because electrophoretic elution will not affect the microscopic characteristics of the hair. The sample is not consumed, and its morphology is preserved. If possible, electrophoretic analyses should be performed before mounting the specimen, since the mounting process has been observed to be detrimental to the enzymatic activity of the genetic markers. This procedure is currently undergoing additional validation studies for possible use at the FBI Laboratory.

Acknowledgment

The authors wish to express their appreciation to Special Agent Andrew G. Podolak, Microscopic Analysis Unit, Laboratory Division, FBI, for microscopical comparison of hair samples.

References

[1] Yada, S., Okane, M., and Sano, Y., "Blood Grouping of Aged and Formalin-Fixed Hairs," *Acta Criminologiae et Medicinae Legalis Japonica,* Vol. 32, 1966, pp. 92-95.

[2] Kimura, W. and Yokoyama, M., "ABO Blood Groups in Human Hair," *Hawaii Medical Journal,* Vol. 28, No. 5, May-June 1969, pp. 384-386.

[3] Lincoln, P. J. and Dodd, B. E., "Mixed Agglutination as a Method for the Determination of A, B and H Blood Groups of Hair," *Medicine, Science and Law,* Vol. 8, 1968, pp. 38-40.

[4] Schechter, Y., Landau, J. W., and Newcomer, V. D., "Comparative Disc Electrophoresis of Hair Kerateines," *Journal of Investigative Dermatology,* Vol. 51, 1969, pp. 57-62.

[5] Baden, H. P., Lee, L. D., and Kubilus, J., "A Genetic Electrophoretic Variant of Human Hair," *American Journal of Human Genetics,* Vol. 27, 1975, pp. 472-477.

[6] Lee, L. D., Ludwig, K., and Baden, H. P., "Matrix Proteins of Human Hair as a Tool for Identification of Individuals," *Forensic Science,* Vol. 11, 1978, pp. 115-121.

[7] Twibell, J. and Whitehead, P. H., "Enzyme Typing of Human Hair Roots," *Journal of Forensic Sciences,* Vol. 23, No. 2, April 1978, pp. 356-360.

[8] Sutton, J. G., Bosley, C. M., and Whitehead, P. H., "Polymorphic Enzyme Systems in Human Hair Sheath Cell," *Journal of the Forensic Science Society,* Vol. 22, 1982, pp. 199-202.

[9] Yoshida, H., Abe, T., and Nakamura, F., "Studies on the Frequencies of PGM_1, PGM_3 and EsD Types from Hair Roots in Japanese Subjects and the Determination of These Types from Old Hair Roots," *Forensic Science International,* Vol. 14, 1979, pp. 1-7.

[10] Oya, M., Ito, H., Kido, A., Suzuki, O., Katsumata, Y., and Yada, S., "Phosphoglucomutase (PGM) and 6-Phosphogluconate Dehydrogenase (PGD) Types in the Human Hairbulb," *Forensic Science Journal,* Vol. 11, 1978, pp. 135-138.

[11] Budowle, B. and Davidson, L., "Selecting Genetic Markers for Analyses of Forcibly Removed Hairs" in *Proceedings of An International Symposium on Forensic Hair Comparison,* FBI Academy, 1985, in press.

[12] Burgess, R. M. and Twibell, J., "The Typing of Human Hair Sheath Cells in the Glyoxalase System Using Cellogel Membranes," *Journal of Forensic Science Society,* Vol. 19, 1979, pp. 283-286.

[13] Montgomery, D. and Jay, B. W.-H., "Multiple Enzyme Systems Grouping of Human Hair Root Sheaths," *Canadian Society of Forensic Science Journal,* Vol. 15, No. 1, 1982, pp. 1-10.

[14] Lawton, M. E. and Sutton, J. G., "Multiple Enzyme Typing of the Sheath Cells Associated with the Root of a Single Human Head Hair," *Journal of the Forensic Science Society,* Vol. 22, 1982, pp. 203-209.

[15] Budowle, B., Sundaram, S., and Wenk, R., "Population Data on the Forensic Genetic Markers: Phosphoglucomutase-1, Esterase D, Erythocyte Acid Phosphatase and Glyoxalase I," *Forensic Science International,* Vol. 28, 1985, pp. 77-81.

[16] Budowle, B., "An Agarose Gel Electrophoretic Method for Typing Phosphoglucomutase-1, Esterase D, or Glyoxalase I," *Journal of Forensic Sciences,* Vol. 30, No. 4, Oct. 1985, pp. 1216-1220.

[17] Budowle, B., "Phosphoglucomutase-1 Subtyping of Human Bloodstains on Ultrathin-layer Polyacrylamide Gels," *Electrophoresis,* Vol. 5, No. 3, 1984, pp. 165-167.

[18] Kompf, J., Bissbort, S., Gussman, S., and Ritter, H., "Polymorphism of Red Cell Glyoxalase I (E.C.: 4.4.1.5). A New Genetic Marker in Man," *Humangenetik,* Vol. 27, 1975, pp. 141-143.

Address requests for reprints or additional information to
A. M. Gambel
Forensic Science Research and Training Center
Laboratory Division
FBI Academy
Quantico, VA 22135