TECHNICAL NOTE

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A New Method for Typing Haptoglobin in Bloodstains Using Immobilized Allo A Lectin

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ABSTRACT: Allo A lectin from the beetle, which is β-D-galactose specific, reacts to haptoglobin but not to hemoglobin. The use of allo A-Sepharose for typing haptoglobin in bloodstains helped eliminate hemoglobin from the bloodstain extract and presented highly resolved haptoglobin patterns by disc gel electrophoresis. This method is simple and rapid for typing haptoglobin in bloodstains and can be easily used in forensic science laboratories.

KEYWORDS: forensic science, blood, haptoglobin, bloodstains, allo A, lectin

In forensic science, typing haptoglobin (Hp) in bloodstains is valuable because of its stability. However, Hp typing in older bloodstains is disturbed by excess hemoglobin (Hb) that masks the Hp patterns. Numerous methods have been published describing techniques to remove or avoid the products of Hb. Among these methods are the use of immunoprecipitation [*1*], the classical biochemical techniques of gel filtration and ion-exchange column chromatography [*2*], isoelectric focusing technique [*3*], and chloroform or methylene chloride extraction procedures [*4,5*]. But all of them are not always appropriate for routine analysis of Hp from bloodstains in forensic science laboratories because of their complicated techniques and dilution of bloodstain extracts.

Allo A lectin from the beetle, *Allomyrina dichotoma,* which is β-D-galactose specific [*6*], has been found to react to Hp which contains β-D-galactopyranosyl residue but not to Hb [*7*]. In this paper, a new procedure using allo A-Sepharose is described for typing Hp in bloodstains.

Materials and Methods

Of whole blood from persons with known Hp types, 10 μL were applied to 5- by 5-mm cotton cloth and allowed to dry at room temperature for four weeks. Allo A-Sepharose was

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prepared by coupling 30 mg of allo A lectin(Cosmo Bio Co., Tokyo) to 10 mL of Sepharose 4B(Pharmacia, Uppsala) by the cyanogen bromide method [7].

Each bloodstain sample was extracted with 50 μL of distilled water for 30 min in a tube, and then the tube was centrifuged at 10 000 rpm for 5 min. The supernatant was mixed with about 30 μL of allo A-Sepharose for 15 min. The protein-binding allo A-Sepharose was washed twice with phosphate-buffered saline (PBS) to eliminate nonbinding proteins. The proteins absorbed by allo A-Sepharose, including Hp, were eluted by mixing the protein-binding allo A-Sepharose and 15 μL of 0.5*M* lactose for 10 min, and then the gel mixture was centrifuged at 1000 rpm for 1 min. All procedures were performed at room temperature.

For analysis of Hp type from the eluted proteins, 10 μL of the supernatants were applied to the cathodal end of the discontinuous polyacrylamide gel (60 by 84 by 1 mm) for electrophoresis. The electrophoretic technique was carried out according to Davis [8]. Because Hp in bloodstains is already fully saturated with Hb released in copious amounts from erythrocytes, development of the Hp patterns was accomplished by placing the gel plate in a staining solution (3% acetic acid: 25 mL; 2,7-diaminofluorene dihydrochloride: 25 mg; 30% hydrogen peroxide: 10 μL) for 1 to 2 min after electrophoresis.

Results and Discussion

Figure 1*a* shows the separation of Hp types in bloodstain extracts treated with allo A-Sepharose. The Hp patterns in bloodstains were as sharp, distinct, and clear as those in fresh serum samples (Fig. 1*b*). By use of the allo A-Sepharose, we could eliminate the tailing dark streaks caused by the products of Hb deterioration in the electrophoretic analysis of Hp in bloodstain without dilution of Hp. The results presented here demonstrate that allo A-Sepharose is a very effective affinity adsorbent for rapid separation and concentration of Hp, and the use of allo A-Sepharose for Hp analysis from forensic science samples helps to eliminate Hb from bloodstain extract without loss of Hp. Thus, a simple and rapid method for Hp typing in bloodstain has been developed.

Conclusion

This allo A-Sepharose method for typing Hp in bloodstains is a simpler and faster procedure than any other methods that have been reported and can be easily used in forensic science laboratories.

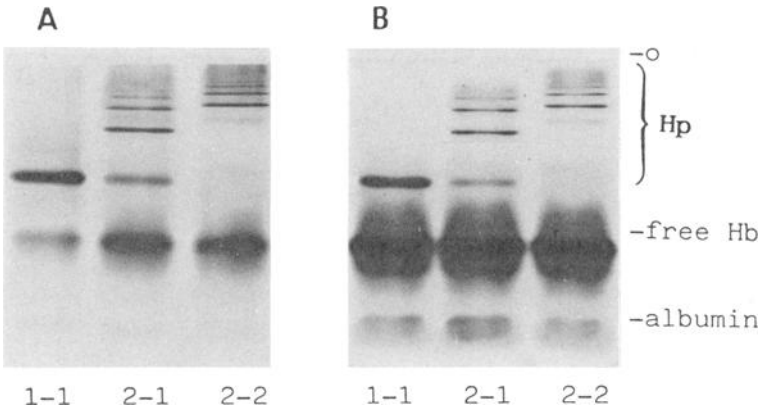

FIG. 1—*Disc gel displaying Hp phenotypes from bloodstains and serum. Cathode at top.* (a) *Bloodstains stored at room temperature for 4 weeks and* (b) *fresh serum samples.*

Of course, this method is not always useful for typing other genetic markers such as transferrin, alpha 1-antitrypsin, and alpha 2-HS glycoprotein in bloodstain, because their patterns must be generally demonstrated by the immunological methods and the products of Hb in bloodstain do not always disturb their typings.

References

[1] Hilgermann, R., "Vergleichende Untersuchungen zur Empfindlichkeit der Haptoglobintypenbestimmung in verschiedenen Medien unter besonderer Berücksichtigung gealterter Blutproben und von Blutspuren," *Zeitschrift für Rechtsmedizin,* Vol. 71, No. 3, 1972, pp. 222-234.

[2] Oya, M. and Takabe, M., "Eine verbesserte Methode zur Darstellung der Haptoglobintypen aus Blutspuren," *Zeitschrift für Rechtsmedizin,* Vol. 77, No. 2, Feb. 1976, pp. 105-107.

[3] Gazaway, S. D., "A New Method for Haptoglobin Typing in Older Bloodstains," *Forensic Serology News,* Vol. 2, No. 4, Aug. 1976, pp. 1-3.

[4] Stolorow, M. D. and Wraxall, B. G. D., "An Efficient Method to Eliminate Streaking in the Electrophoretic Analysis of Haptoglobin in Bloodstains," *Journal of Forensic Sciences,* Vol. 24, No. 4, Oct. 1979, pp. 856-863.

[5] Budowle, B. and Chow, G., "Discontinuous Polyacrylamide Gel Electrophoresis for Typing Haptoglobin in Bloodstains," *Journal of Forensic Sciences,* Vol. 30, No. 3, July 1985, pp. 893-897.

[6] Umetsu, K., Kosaka, S., and Suzuki, T., "Purification and Characterization of a Lectin from the Beetle *Allomyrina dichotoma,*" *Journal of Biochemistry,* Vol. 95, No. 1, Jan. 1984, pp. 239-245.

[7] Umetsu, K., Ikeda, N., Kashimura, K., and Suzuki, T., "Affinity Chromatography of Human Serum Proteins Using Immobilized Lectin from *Allomyrina dichotoma,*" *Biochemistry International,* Vol. 10, No. 4, April 1985, pp. 549-552.

[8] Davis, B. J., "Disc Electrophoresis. II. Method and Application to Human Serum Proteins," *Annals of New York Academy of Sciences,* Vol. 121, Dec. 1964, pp. 404-427.

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