Extending the detectability of phosphoglucomutase in old bloodstains and seminal stains by immunofixation with anti-human PGM: a preliminary report.

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Red cell polymorphic enzymes are vitally important in the individualization of dried bloodstains. Unlike those scientists who work exclusively with fresh liquid blood, the forensic serologist is unfortunately often prevented from interpreting phenotypes of particular red cell enzymes simply because the bloodstains are either

a. too small (or too diffuse), or

b. too deteriorated.

A natural question to explore thus emerges: how does the forensic serologist improve the detectability of red cell enzymes in stains whose size and degree of deterioration fall beyond his control?

For the analysis of small or diffuse stains, improved sampling techniques and sample application methods have expanded the capacity of the forensic serologist to determine phenotypes of the enzyme markers successfully. However, when the enzyme markers are too deteriorated (usually from age), there appears to be no solution at the present time.

It would seem the answer rests in locating a satisfactory alternative to the delicate detection procedures employing enzymatic staining currently used for red cell enzyme marker analysis virtually worldwide. In searching for an alternative detection method, it was observed that phosphoglucomutase would be an excellent candidate for such research because of its widespread application in the analysis of body secretion stains as well as bloodstains.

To date, PGM has been analyzed exclusively by electrophoresis or isoelectric focusing methods. The detection method employed to visualize the location of the PGM isozymes on the electrophoresis support media or blotting matrices is currently limited to some variation of a colored dye formation such as MTT, NBT, or meldola blue. The selective staining method unfortunately places a demand on perhaps the most labile property of an enzyme marker, namely its kinetic activity. In to be visualized, an enzyme such as PGM must maintain its conformation and activity at both its substrate bindingsite and its reactive site.

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Any deterioration in the kinetic activity of PGM in stains results in the corresponding loss of detectability of the PGM isozymes, despite the probable migration of PGM isozymes to their usual respective locations in IEF and electrophoresis gels. Eventually, PGM kinetic activity will disappear completely and leave the isozyme bands which are present virtually undetectable.

An alternative detection method for PGM might be found by exploring other binding mechanisms such as potential immunologically reactive sites and enzyme-specific antibodies. That is to say, it is time to view PGM as an antigen as well as an enzyme.

The groundwork for the detection of numerous blood proteins by immunofixation and enzyme linked immune assays is already well-established. Antibody specific for human PGM ought to succeed in immobilizing PGM in a suitable matrix.

Unfortunately, there exists no human PGM antisera that we know of. Therefore, we are endeavoring to produce our own.

Purified human PGM is required as the immunogen. In order to obtain purified human PGM, Dr.Harvey Mohrenweiser, University of Michigan Medical School, Department of Human Genetics, proceeded to isolate approximately 10 mg of PGM from approximately 5 kg of human muscle.

Dr.Mohrenweiser determined by SDS gel electrophoresis that the purification yielded primarily a single band of protein corresponding in molecular weight to rabbit PGM (approximately 70.000 Daltons). He estimated that the end product was quite pure, showing in the gel only one additional faint band of unidentified protein.

The purified PGM was lyophilized and forwarded to two different laboratories for antisera production.
Dr.Robert Myers, Michigan Department of Public Health, immunized two rabbits. Dr. Henry Carwile, Bethyl Laboratories, immunized one sheep.

Both laboratories initiatd immunization employing Freund's Complete Adjuvant mixed with a solution of PGM consisting of 100 µg dissolved or suspended in 1.0 ml of saline. The animals were further stimulated according to their respective immunization protocols. Test bleedings from all three animals demonstrated antibody titers of no better than 1/4 by double diffusion against human hemolysate. The rabbits were hyperimmunized after six weeks raising the antibody titer to 1/8 and subsequently exsanguinated.

Samples of the rabbit sera were applied to ULAGIF gels containing PGM from fresh human hemolysates and it was attempted to visualize PGM by conventional immunofixation in cellulose acetate membranes, washed with saline and water, and stained with Coomassie blue R250. No discrete bands were discernable.

Dale Dykes, Memorial Blood Center, Minneapolis, Minnesota, tested the rabbit sera by passive immunoblotting of ULAGIF gels with nitrocellulose membrane treated with enzyme-conjugated goat anti-rabbit IgG. At dilutions of the rabbit sera up to 1/250, faint bands were discernable on the nitrocellulose, but unfortunately, they did not correspond to the mobility of standard PGM locus 1 or locus 2 isozymes. In fact, there were a multiplicity of faint bands throughout the lanes of sample hemolysates.

There are a number of possible causes for the disappointing preliminary results of the experimentation thus far. However, numerous alternative courses of action remain to be pursued.

If recent history of the improvements made in protein detection enhancement by immunological methods is any indication, it should be obvious that the red cell enzyme markers traditionelly of forensic interest must soon be visualized by alternative detection methods in order to match the sensitivity now observed in serum protein analysis.