Evaluation of unusual band patterns in stains of biological interest

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INTRODUCTION

The polymorphic protein systems Gc, A2HS, PLG are now routinely used for individualization in paternity and stain cases. They meet the accepted standard for forensic work and have the advantage that they are generally more stable than enzymes on storage and therefore more suitable for use in stain grouping.

MATERIALS AND METHODS

Blood samples and stains from routine paternity, identification and stain cases were investigated as previously described (Rand et al 1988).

DISCUSSION

The visualisation of protein polymorphisms by immunochemophoresis depends on the presence of an intact antigenic site on the protein and not on the presence of a functionally intact enzyme molecule. The detection and interpretation of the results is not however free from complications and, in the case of stain grouping, false interpretations are possible due to insufficient care or lack of awareness of the problems involved.

The basic problem revolves around the differentiation between true variants and artefacts caused by suboptimal storage conditions. In most protein systems a variety of rare variant alleles have been found. This is particularly true for the Gc-system where at least 35 variants have been described at present. Gc 1 variants have 2 bands whereas Gc 2 variants have only 1 band (Cleve et al 1981).

This can sometimes be confusing when a discrete alteration occurs producing very few bands. A typical variant (2A7)in the Gc-system can be seen in fig.1a lanes 3 and 4 and can be distinguished from an artefact (fig.1b) seen in an old blood sample by comparison of the band concentrations . The presence of a potential third allele product would also arouse suspicion except in cases of mixtures (Rand et al 1989).

The problem can basically be divided into 3 categories:
- the **formation** of complexes
- **removal** of sialic acid or other carbohydrate residues
- and the **breakdown** of the primary structure of the protein molecule.

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In this context the third category usually produces no difficulties as the bands tend to be totally absent.

Proteins with internal disulphide bonds or cysteine residues can form several types of complex:
1.as multiple forms of the same protein eg. **Dimer**
2.combination with **fragments** of the same molecule
or 3. combination with **other** molecules (fig.2).

A good example of the third type is the binding of the Gc protein with actin released from the breakdown of platelets to form larger molecules with altered isoelectric points. In most cases this can be reversed by pretreatment with a strong reducing agent such as 6M urea to restore the original form.

Most proteins have in addition to the amino acid residues a carbohydrate and often sialic acid component. The amount varies from protein to protein and in some cases, as in Gc, between subtypes. The Gc 1 molecule for example possesses sialic residues whereas the Gc 2 protein does not (Kimura et al 1985).

The NANA residues confer a positive charge and during storage the number of residues can be reduced either through passive bond breakage or by bacterial neuraminidase activity. This can result in an irregular distribution of positive charges on the molecules and subsequent band pattern changes. By pretreating with neuraminidase all the sialic acid residues can be removed creating a uniform structure for all molecules. The solution of the problem is obviously not quite as simple because in many cases artefacts appear even after suitable pretreatment.

In the plasminogen system many bands occur cathodally even after appropriate pretreatment with neuraminidase (fig.3). With A2HS no artefacts have yet been demonstrated although without neuraminidase pretreatment bands were very often completely absent. In both cases extended treatment resulted in the disappearence of all bands. Obviously the alteration process described here will not occur in isolation and it is most probable that a complex of reactions occurs to give the resulting band pattern changes.

This problem becomes more acute when evaluating the results from mixtures of blood and other body fluids where a multiple band pattern is to be expected. Here extreme caution must be taken.

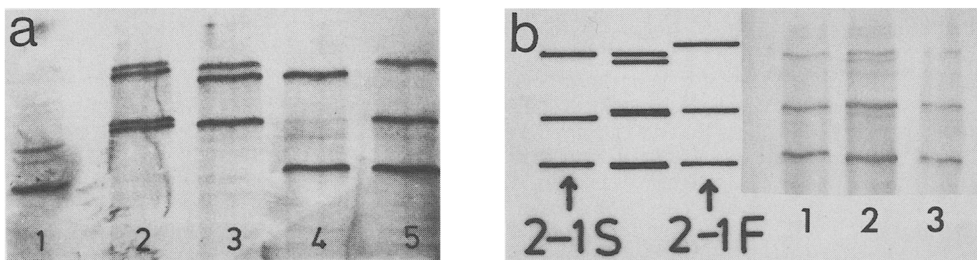

Fig. 1 a)Gc - subtypes after IEF (pH 4.5-5.4) 1)2, 2)1S-1F, 3)1S-2A7, 4)2-2A7, 5)2-1S.
b) 1)2-1S, 2)aged blood sample with extra bands, 3)2-1F.

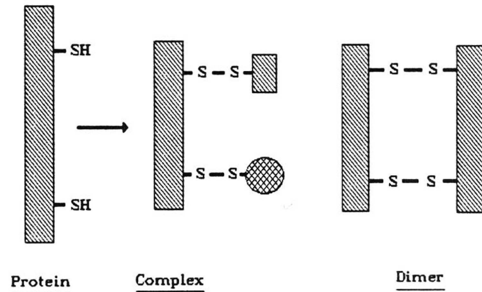

Fig. 2 Diagrammatic representation of possible complex formations.

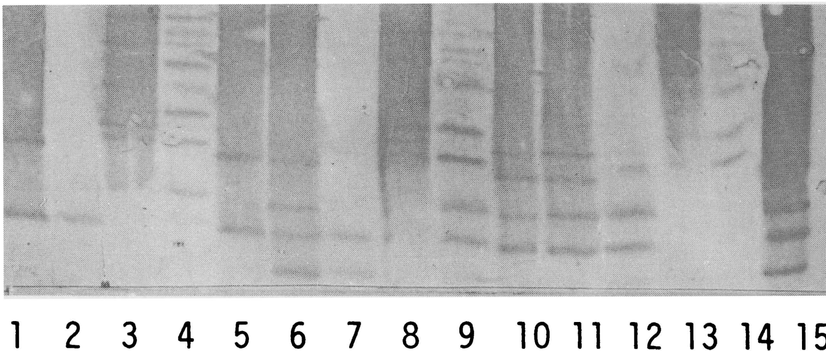

Fig. 3 PLG band patterns with and without neuraminidase pretreatment. Lanes 1, 5, 6, 10, 11 and 15 are treated plasma, 3, 8 and 13 are untreated.
Lanes 2, 7, and 12 are treated blood stains, 4, 9 and 14 are untreated. Controls PLG A: 1,2,5; PLG AB: 6,7,15; PLG A-A3: 10,11

LITERATURE

Cleve H, Constans J, Berg S, Hoste B, Ishimoto G, Matsumoto H, Spees EK, Weber W (1981) Gc revisited: Six further Gc-phenotypes delineated by isoelectric focusing and by polyacrylamide gel electrophoresis. Hum Genet 57: 312-316

Kimura H, Saheki S, Yoshida K, Ohsawa M (1985) Characterisation of purified Group-Specific Components (Gc 1 and Gc 2 proteins) from human plasma. Jpn J Legal Med 39: 113-123

Rand S, Kohfahl A, Ritter P, Brinkmann B (1988) An approach to individualisation of micro-bloodstains using immunochemophoresis. in Adv For Haem 2: 474-477 Ed W Mayr Springer Verlag Berlin Heidelberg

Rand S, Schürenkamp M, Schütte U, Brinkmann B (1989) Zur Bewertung ungewöhnlicher Bandenmuster in der Immunochemophorese. Beitr ger Med XLVII: 159-164 Verlag Franz Deuticke Wien

Shinomiya K, Shinomiya T, Yoshida K, Kimura H (1982) Conversion of a plasma group-specific component (vitamin D binding protein) to a macromolecule by a protein factor in tissue extracts. J Biochem 92: 1163-1171

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