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Differences in the Demonstration of C 3 Polymorphism in High-Voltage Gel Electrophoresis Depending on Agarose Quality

Investigations of Rare C 3 Variants in Behring Agarose AGS 082

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Summary: Different batches of agarose may lead to migration differences in the demonstration of C 3 polymorphism in high-voltage gel electrophoresis. We studied a particular batch (i.e. AGS 082) which caused two basic phenomena concerning mobility and electrophoretic position of C 3 phenotypes:

1. C 3 variants migrate to the cathode, apparently due to a positive net charge of the protein caused by this agarose batch.
2. The relative mobility of slow and fast bands is virtually reversed.

Previously obtained results are controlled and verified by further investigations on rare C 3 variants. The possible causes of the net charge differences of the C 3 proteins in various agarose batches are discussed.

Zusammenfassung: Verschiedene Agarose-Chargen können zu einer uneinheitlichen Darstellung des C3-Polymorphismus in der Hochspannungs-Gelelektrophorese führen.

Speziell die Behring-Agarose AGS 082 bewirkt hinsichtlich der Mobilität und Elektrophoresemuster der C3-Phänotypen zwei grundlegende Phänomene:

1. C3-Varianten wandern kathodisch, offensichtlich eine Folge der positiven Aufladung des Proteins, die durch diese Agarose-Charge bewirkt wird.
2. Die Umkehrung der relativen Mobilität der langsamen und schnellen Bande.

Vorausgegangene Ergebnisse werden anhand weiterer Untersuchungen seltener C3-Varianten überprüft und bestätigt.

Die möglichen Ursachen für diese Agarose bedingten Ladungsdifferenzen der C3-Proteine werden diskutiert.

Key words: Bloodgroups - C 3 polymorphism - variants - gel electrophoresis - agarose quality

INTRODUCTION

Slight differences between continuous and discontinuous electrophoresis systems in the determination of C 3 phenotypes in agarose gel have already been noted

by TEISBERG (1). Technical intricacies of the C 3 determination were a major topic of the Bonn workshop (2). Limitations of available techniques for the standardization of C 3 typing have been recently discussed by PFLUGSHAUPT and co-workers (3), KÜHNL and STROBEL (4) and RITTNER and RITTNER (1974) (5).

We have reported previously that a particular batch of agarose of the Behringwerke, AG, Marburg/Lahn, leads to strongly deviating results when applied for C3 typing (6). In view of the contrasting results with normal C 3 phenotypes in agarose of batch AGS 082, it was interesting to investigate rare C3 variants in order to find out if charge differences between rare variants could be detected.

MATERIAL AND METHODS

Reference sera for rare C3 variants were sent to us from the Reference Laboratory for the C3 polymorphism at Bonn by express mail. The sera arrived in dry ice, still frozen.
The following reference variants were studied:

F variants: F0.5S, F0.6S
S variants: S0.4S, S1.0S, S1.55S, FS0.5, FS0.4

Sera of common types from healthy laboratory personnel were used for comparison.
Horizontal prolonged agarose gel electrophoresis according to TEISBERG (7) and RITTNER and RITTNER (1973) was carried out in three different systems:

1. Shandon High Voltage Electrophoresis, Model Q11: Gel size: 27,5 x 29 cm, 0.945 g agarose in 113.4 ml buffer, 900 V, 70 ma, 3 h.
2. Electrophoresis cell from Hübscher, Hamburg: Gel size: 20 x 20 cm, 0.5 g agarose in 60.0 ml buffer, 570 V, 65 ma, 3 h.
3. DESAGA DE cell, model 121201/10: Gel size: 20 x 20 cm, 0.5 g agarose in 60.0 ml buffer, 400 V, 60-70 ma, 3 h.

In each system, a constant temperature of 12°C was achieved by tap water cooling.

RESULTS AND DISCUSSION

As shown previously, the common types of the third component of complement do not migrate to the anode but to the cathode in a particular batch of agarose from Behringwerke AG (REUTER, 1974).

In agarose AGS 082, in contrast to batches of the F series, the migration distance is about one third, at the same field strength and electrophoresis time, as shown in Fig. 1a and 1b[1]. In addition, the C3 bands appear not as sharply separated as in batches of the F series. Although the absolute migration rate is reduced, the relative mobility of variant bands remained unchanged.

The most striking feature of AGS agarose is the reversal of F and S bands:

[1] The migration rate of other serum proteins like transferrin and albumin is reduced in a similar fashion.

In this system, S is moving faster than F. Therefore, C 3 F bands are closer to the origin than S bands. Rare C3 variants are, in accordance with this behaviour: The variant S1.55, the slowest in usual agarose systems, is the fastest one in agarose AGS. Accordingly, the variant F0.6, usually the fastest among the variants tested, has the slowest migration in AGS agarose (see Fig. 1a and 1b). All other variants studied fit into this reversed fashion.

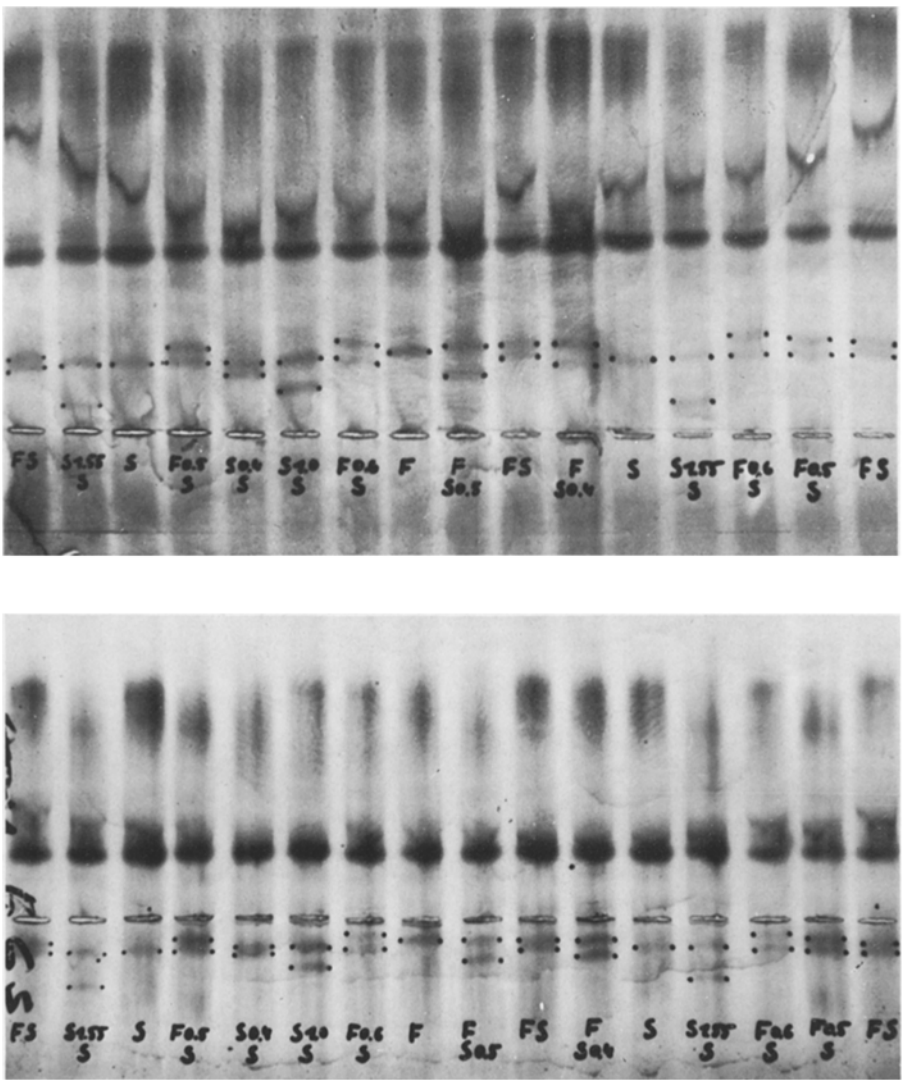

Fig. 1. Original pherograms of C3 variants in Behring agarose F 5388 (1a) and AGS 082 (1b). The common phenotypes S, F and FS are added as standard markings

Fig. 2. is a schematic representation of the distribution of C3 phenotypes in agarose AGS compared with usual agarose batches. The most likely explanation for the complete reversal of the migration direction of C3 in AGS agarose is an electrochemical change of charge of the C3 protein, due to the influence of this particular batch. It appears that every C3 component gets an equal amount of positive charge. Due to the charge balance of different components this additional charge is highest in the case of variant S1.55 and lowest in the case of F0.6. This is depicted in Fig. 3. As an explanation for an altered

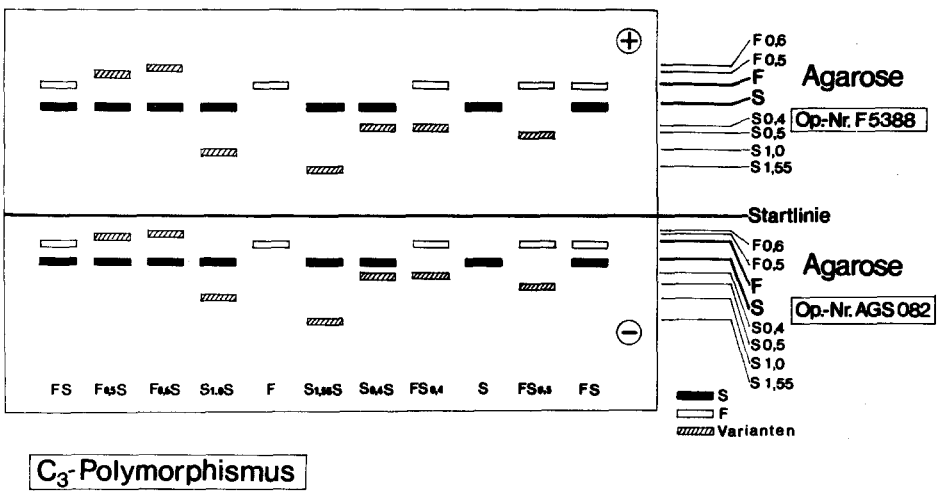

Fig. 2. Diagram of electrophoretic patterns of C3 variants in Behring agarose of the batches F 5388 and AGS 082

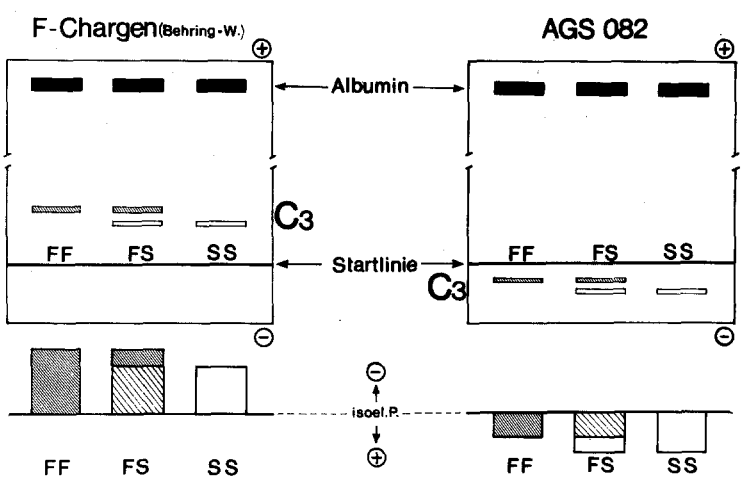

Fig. 3. Reversal of electrophoretic migration and of the relative mobilities of slow and fast C3 phenotypes due to a recharging of the C3 protein

electroendosmosis, possibly increased contents of sulfuric and carboxyl groups was discussed by the Behringwerke company.

In addition, there is an increased ash content in AGS agarose. AGS agarose gels are more solid than F batches, due to a tighter net structure of the gel. The true changes in the quarternary structure of the proteins are completely unknown.

CONCLUSIONS

The demonstration of the C3 polymorphism by agarose gel electrophoresis depends in part on the quality of the agarose used. New batches should be carefully evaluated to avoid changes in the absolute migration rate and/or in the migration direction as shown in the present paper.

Acknowledgement

The author is grateful to Dr. Ch. RITTNER, Reference Laboratory for the C 3 polymorphism, for providing the reference sera of rare C3 variants used in this investigation.

It has since been established that AGS 082 agarose was a faulty product and has now been withdrawn by the manufacture from the list of commercial products. The results of this research can therefore not be reproduced of cause. However the author still has a stock of nearly 40 g of this agarose batch and would be pleased to supply a sample to other interested investigators.

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