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Transferrin Variants Designated by their Relative Mobilities in High-Voltage Agarose Gel Electrophoresis*

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Summary: A unified nomenclature for the designation of transferrin variants, based on their relative mobilities in a standardized high-voltage agarose gel electrophoresis, is proposed. This is aimed at improving terminology of Tf variant typing results with reference to the relatively common variant Tf B2.

Zusammenfassung: Nach Einführung der Untersuchung des C3 - Polymorphismus in der Agarosegel-Hochspannungselektrophorese werden die seltenen Transferrin-Varianten als "Nebenprodukt" beobachtet. Da eine einheitliche Nomenklatur der Transferrine bisher fehlt, wird ein Vorschlag zur Bestimmung der relativen Wanderungsstrecke unter Bezugnahme auf die am häufigsten beobachtete Variante Tf B2 unterbreitet.

Key word: Transferrin nomenclature, variant designation

In recent years, high-voltage agarose gel electrophoresis (HVAGE) has proved to be a reliable and inexpensive technique, particularly for the separation of proteins which are characterized by minute charge differences. The application of HVAGE led to the discovery of minor, but distinct differences between the polymorphic forms of the third component of human complement (ALPER and PROPP (2), RITTNER (6)). Subsequently, the C 3 polymorphism has gained increasing interest in cases of disputed paternity in West Germany (9) as in other European countries (12). As a by-product of this application of HVAGE, transferrin variants can be easily recognized. For various reasons, however, the correct designation of a given variant could not established. The difficulties to type Tf variants correctly have been recently reviewed by BEARN and PARKER (3) and GIBLETT (4). These authors have pointed out that 'new' Tf variants should be carefully compared with known variants. However, even if the corresponding variant is at hand, "this procedure does not ensure the variant's identity, because amino acid substitution is not invariably accompanied by a change in electrical charge" (4).

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Even more relevant is the fact that some of the known variants have only been found once and, in addition, have not been compared with the stock of hitherto known Tf variants. This led us to ask if a systematic nomenclature based on comparison of a given Tf variant with a standard variant according to their relative mobilities - as was agreed upon for the C 3 polymorphism (6) - is to be preferred to the 'classical' nomenclature which merely indicates the variants moving faster or slower than Tf C except for some bands where also the order of migration difference - B2, B 1, BO-1-is indicated.

In this paper, an attempt is made to designate Tf variants according to their relative mobility in HVAGE compared to the 'standard' variant B2. This may enable other workers to classify their own variant sera provided identical electrophoretic conditions are used.

MATERIAL AND METHODS

In this study, a set of 16 rare Tf variant sera has been compared with known Tf variants kindly supplied by Dr. SUTTON, Austin, Texas, and Dr. TEISBERG, Oslo, Norway. High-voltage agarose gel electrophoresis was used under conditions essentially as described by TEISBERG (11) for the determination of the C 3 polymorphism. To reveal even minute migration differences, the time of electrophoresis was prolonged to 5-6 hrs at 20 V/cm in an electrophoretic apparatus cooled with running tap water. Immunofixation was performed with specific antitransferrin serum purchased from Behringwerke, Marburg/Lahn, as described by ALPER and JOHNSON (1).

RESULTS

The results of a routine HVAGE run are shown in Fig. 1. As can be noted, gross migration differences can easily be distinguished (slot 4, 6, 7 and 12). However, more discrete deviations from the common B2C variant (slot 1, 3, 5, 8, 9, 11) as in the variants in slot 2 and 10, can only be suspected from a routine high-voltage gel electrophoresis. Therefore, prolonged runs were performed as shown in Fig. 2. The sample in slot 2, Fig. 1, migrated in slot 4 (BO.83), the sample in slot 10, Fig. 1, is filled in slot 1 (BO.88), respectively. To verify, that there is no overlap with other protein bands, Tf band can be fixed by subsequent precipitation with specific anti-Tf serum as is documented in Fig. 3.

In Table 1, the results of ten determinations of each serum in prolonged HVAGE are summarized. For practical reasons, the 'common' B2C variant has been set to 0.7 as the reference variant. In the last column, the standard deviation is indicated. The variant B2C exhibited the least variation between different runs in our hands.

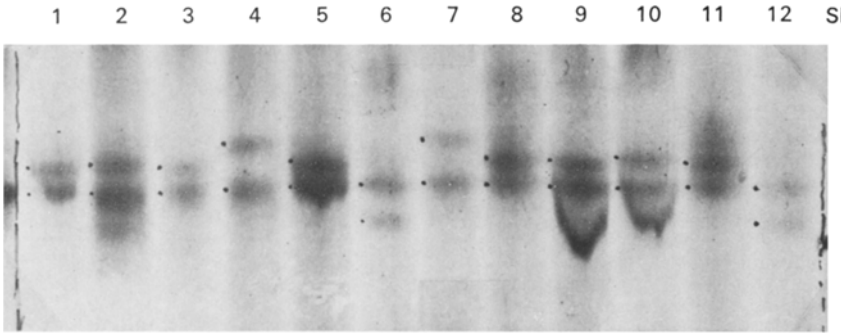

Fig. 1. Transferrin variants in routine high-voltage agarose gel electrophoresis. Irregular bands in the Tf region of slot 5,9 and 10 = low density lipoproteins

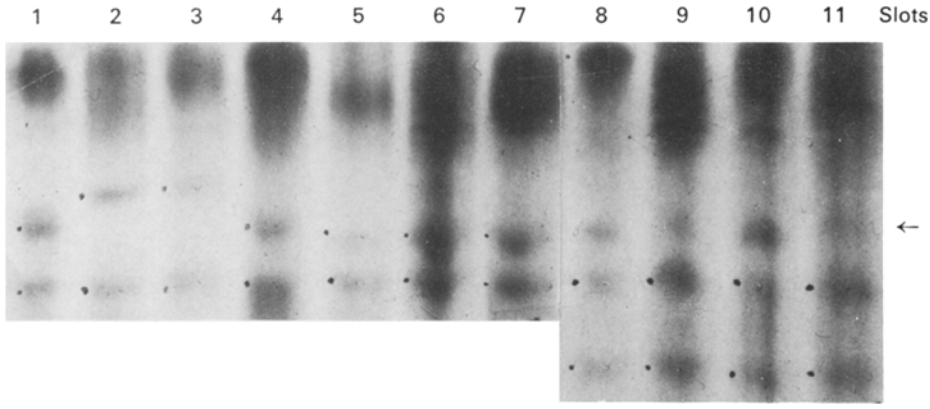

Fig. 2. Transferrin variants in prolonged high-voltage agarose gel electrophoresis (6-hr-run). From left to right: B 0.88C, B 1.20C, BO-1 (1.24)C, B 0.83 C, B2 (0.7)C, B2C, B2C; D1C, D1C, DChiC. Arrow: Minor transferrin bands

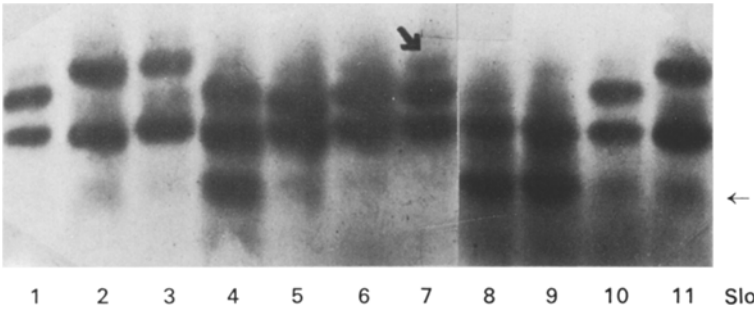

Fig. 3. Transferrin variants in high voltage agarose gel electrophoresis after immunofixation with anti-Tf serum. From left to right: B 0.88C, B 1.20C, BO-1 (1.24)C, B 0.83C, B2(0.7)C, B2C, B2C, D1C, D1C, B 0.88C, B 1.20C. Band in D1 position in slot 4 = storage effect. Arrows: Minor transferrin bands

Table 1. *Results of designation of Tf variant sera according to relative mobilities. SD=Standard deviation, mean of 10 determinations. Underlined: reference variant B2.*

Group	No. of sera tested	"Classical" Tf design.	Design. acc. to rel. mob.	SD
1	10	B2	B 0.7	± 0.001
2	1	?	B 0.78	± 0.005
3	1	?	B 0.83	± 0.07
4	3	?	B 0.88	± 0.04
5	1	?	B 1.20	± 0.06
6	1	BO-1	B 1.24	± 0.06
7	3	D1	D 1.06	± 0.07
8	1	DChi	D 1.06	± 0.07

It emerges from Table 1 that the classical D 1 variant does not fit exactly to one charge unit if B 2 is set to 0.7. We feel, however, that this should be accepted in view of the fact that B2 is found approximately ten times more often than D1 in Caucasian populations. The variant D1, in addition, is indistinguishable from variant DChi in HVAGE, even in prolonged runs, as already noted by TEISBERG (13). According to GIBLETT (4), this was also the case in starch gel electrophoresis prior to PARKER's method of ultra-long starch gel electrophoretic runs.

The outlines of the proposed nomenclature according to relative mobilities are given in Fig. 4. Reference is made in the left scale to an approach by SUTTON (10) to designate variants by relative mobilities in polyacrylamide gel electrophoresis (PAGE). Only those parts of SUTTON's scale are shown which refer to the migration of variants described in this paper. The right-hand scale represents the system of numerical description of Tf variants according to their relative mobilities in HVAGE when B2 is set to 0.7 charge units. + and - signs refer to anode and cathode, resp. It is apparent at the first sight that a number of B variants, only slightly distinct from each other, can be found between 0.7 and 1.0. This demands for a sub-division in the second decimal position, a situation already familiar to those working with the C 3 polymorphism. Further, it can be concluded that 1) the preliminary designation should be based at least on the results of ten determinations, 2) final designation and distinction from similar variants can only be achieved in repeated comparative runs under identical conditions, i.e. on the same gel. This is also a prerequisite for C 3 variant typing (5, 7).

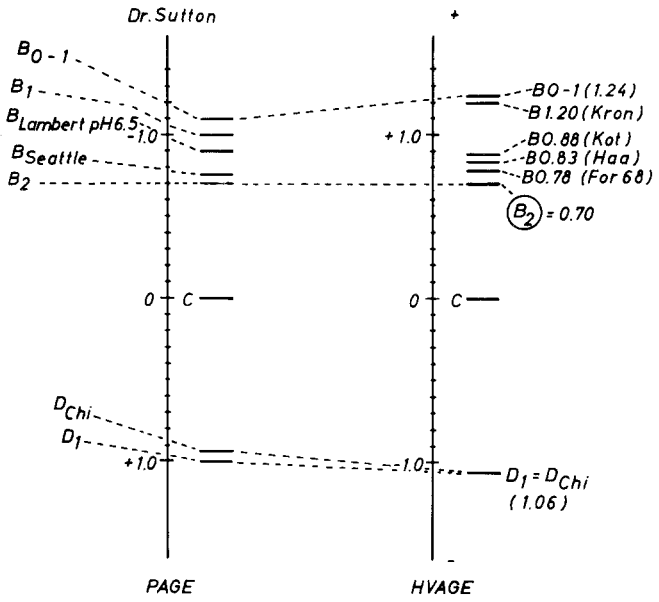

Fig. 4. Schematic representation of Tf variant designation according to relative mobilities in polyacrylaminde gel (PAGE) and high-voltage agarose gel electrophoresis (HVAGE). Dotted lines indicate those variants which have been designated on PAGE and HVAGE

DISCUSSION

Transferrin like some other proteins (C 3, alpha-1-antitrypsin) is characterized by the occurrence of a fairly large number of polymorphic forms which differ from each other by often minute charge differences. However, not all amino acid substitutuions cause changes in the net charge of the protein. Therefore, a common nomenclature can only refer to differences detected in a given test system. From chemical work it has been concluded previously (8) "that classification of the transferrins according to their electrophoretic mobilities....is a fairly reliable indicator of their identity or non-identity at the molecular level".

The 'classical' nomenclature of Tf variants does not seem to fulfill the criteria outlined by WIENER (14) for a common nomenclature. Some designations refer arbitrarily to electrophoretic positions (B1, B 2, BO-1) while others carry names of tribes, cities or countries. Newly disclosed variants could not be easily compared with the hitherto known variants except that reference sera were available.

SUTTON (10) therefore first made an attempt to classify Tf variants according to their relative mobilities in polyacrylamide gel electrophoresis. We made a

similar approach which may be suitable for practical purposes, i.e. routine Tf typing on high-voltage agarose electrophoresis as a by-product of C 3 typing. This classification does not imply any biochemical or population genetical aspects. It may facilitate the exchange of data among workers in various parts of the world. It enables them to compare their own variant sera on a simple descriptive scale provided the conditions used in this paper are applied.

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