CHARGE-SHIFT ELECTROPHORESIS OF APOLIPOPROTEINS FROM NORMAL HUMANS AND PATIENTS WITH TANGIER DISEASE

Gerd UTERMANN and Ulrike BEISIEGEL
Institut für Humangenetik der Universität Marburg, 3550 Marburg/Lahn, Bahnhofstraße 7 A, FRG

Received 6 November 1978

1. Introduction

Integral membrane proteins are amphipathic molecules and contain hydrophobic domains in their primary structure which anchor these proteins to the membrane by interaction with the hydrocarbon interior of the lipid bilayer [1]. A charge shift electrophoresis has been introduced [2] as a simple method to differentiate integral membrane proteins from hydrophilic proteins. The method is based on the fact that amphipathic membrane proteins bind large amounts of neutral detergents that cluster in a micellar like structure around the hydrophobic segments of the proteins whereas hydrophilic proteins bind little or no neutral detergent [3,4]. When mixed micelles of neutral detergent and anionic or cationic detergent are bound to these proteins negative or positive charges are introduced into the detergent–protein complex. This will result in a corresponding shift in the electrophoretic mobility of the protein.

Another group of proteins that specifically interact with lipids are the apoproteins of plasma lipoproteins. Sequence data show that the A- and C-group apoproteins do not contain continous hydrophobic regions in their primary structure but probably contain segments forming amphipathic helices with a hydrophilic and a hydrophobic face [5]. Two types of interactions have been demonstrated between Apo A-I and Apo A-II and amphiphilic or hydrophobic ligands: Both A-apoproteins have discrete non-interacting binding sites for amphiphiles that are primarily hydrophobic in character and both undergo a cooperative conformational change in the presence of free amphiphile in excess of that required for binding of the discrete sites for hydrophobic ligands [6,7]. The interaction of the B protein from LDL with detergents has been less intensively studied but it is known that this protein also binds large quantities of neutral detergent [3]. No detergent binding studies have yet been performed with other apolipoproteins. We here have used charge shift electrophoresis to demonstrate the amphipathic nature of various apolipoproteins and to analyse whether the A-I and A-II proteins from patients with Tangier disease are able to bind detergent by hydrophobic interaction.

Abbreviations: LDL, low-density lipoproteins; HDL, high-density lipoproteins; TX, Triton X-100; NaDOC, sodium deoxycholate; CTAB, cethyltrimethylammoniumbromide; SDS, sodium dodecylsulfate

2. Materials and methods

Sera were obtained from fasting control subjects, from 4 patients with Tangier disease (O.M.[8]; R.E. [9]; J.Si. and E.Ga. [10]) and from individuals with post-prandial hypertriglyceridaemia. Lipoprotein fractions were isolated by sequential ultracentrifugation at +4°C according to standard procedures [11]. Lipoproteins were dialysed against 0.85% NaCl 0.05% EDTA pH 7.0 and delipidated by 5 successive extractions with acetone: aethanol, 1 : 1 (v/v) at −20°C. Apolipoproteins A-I and A-II were isolated according to [12,13] and apoproteins E and A-IV by preparative SDS–polyacrylamide gel electrophoresis as detailed [14,15]. Apo B was isolated from LDL by column chromatography in the presence of Triton X-100 (TX) according to [16]. Antisera against apoproteins were raised in rabbits by routine methods.

Charge shift electrophoresis according to [2] and charge-shift crossed immunoelectrophoresis were performed following the modifications [17].

3. Results and discussion

Four of the well characterised apolipoproteins from normal human plasma, Apo A-I, A-II, B and E all demonstrated a marked positive and negative charge shift upon electrophoresis in the presence of mixed TX + NaDOC or TX + CTAB micelles, respectively, when compared to their mobility in TX alone (fig.1,2). This is in agreement with the known detergent binding properties of apolipoproteins A-I, A-II and B and demonstrates that also Apo E does

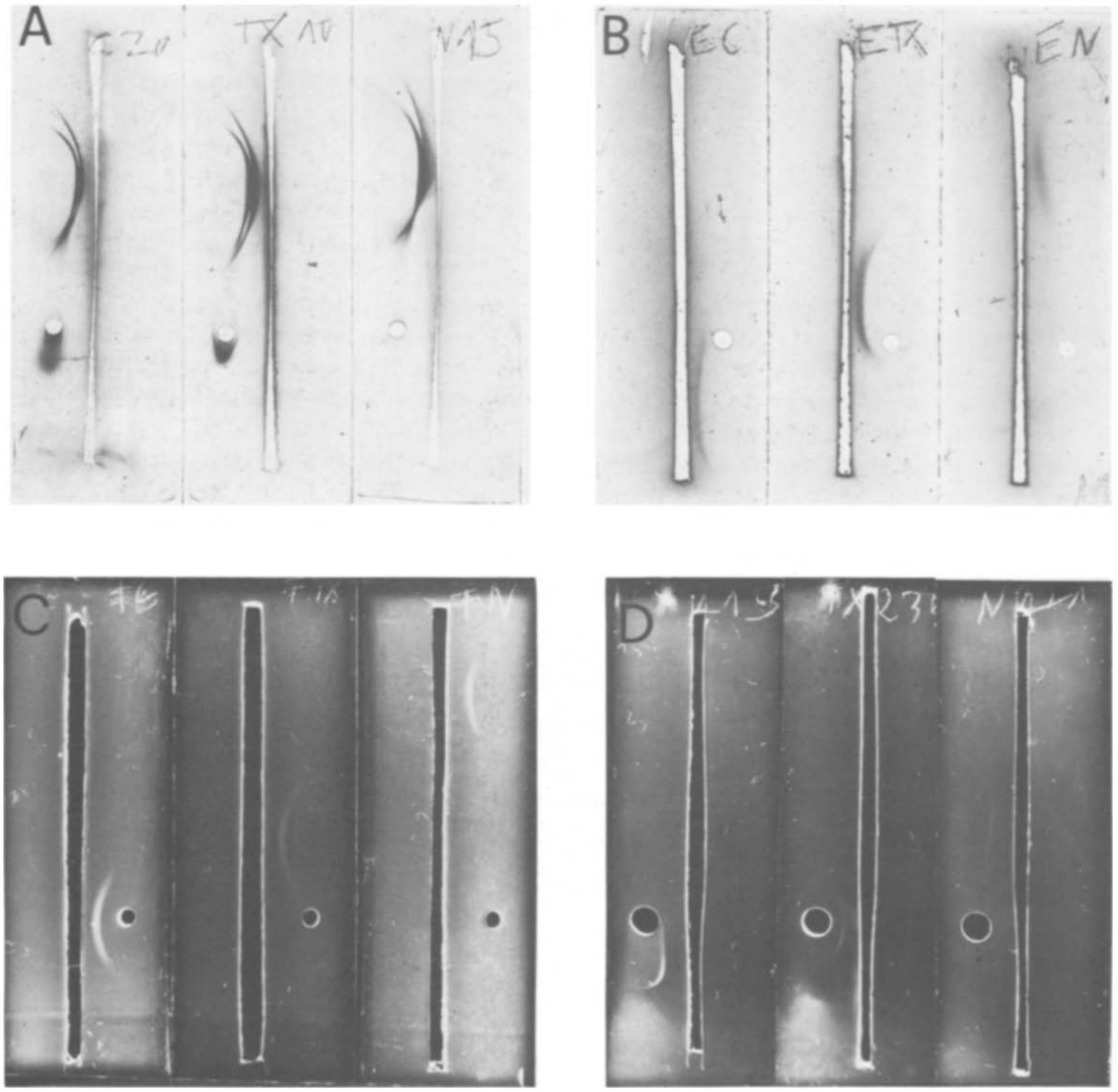

Fig.1. Electrophoresis of human serum (A), apolipoprotein E (B), apolipoprotein A-IV (C) and apolipoprotein B (D) in the presence of TX (middle plates), TX + CTAB (left plates) and TX + NaDOC (right plates). Troughs contain: (A) anti-Gc + anti-α_2-macroglobulin; (B) anti-Apo E; (C) anti-Apo A-IV; (D) anti-Apo B. Anode at the top.

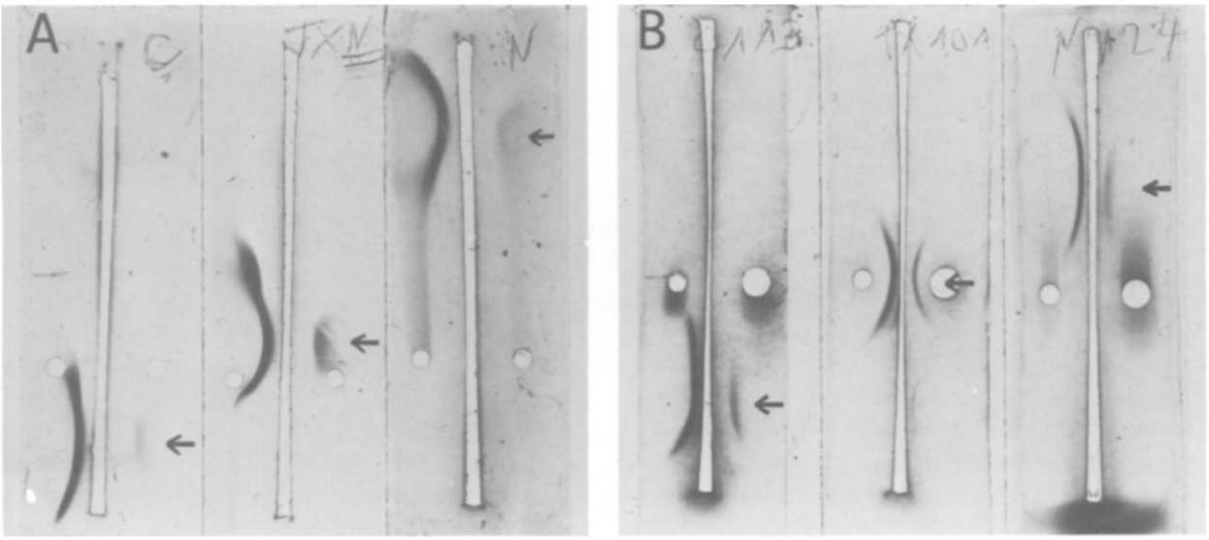

Fig.2. Comparison of electrophoretic mobility of apolipoproteins A-I (A) and A-II (B) from control sera (left lanes) and Tangier sera (right lanes) in the presence of TX (middle plates), TX + CTAB (left plates) and TX - NaDOC (right plates). (A) Electrophoresis of purified Apo A-I from control HDL (left) and fraction $d > 1.21$ g/ml from Tangier plasma (right). Trough contains monospecific anti-Apo A-I. (B) Electrophoresis of a control serum (left) and Tangier serum. Trough contains monospecific anti-Apo A-II.

bind detergents by hydrophobic interaction. Identical charge shifts were obtained when the purified apolipoproteins were analysed or when whole sera or the $d > 1.21$ g/ml bottom fraction (for Apo A-I) were subjected to charge shift electrophoresis.

A further protein of M_r 46 000 (Apo A-IV) that has been isolated recently in our laboratory from triglyceride-rich plasma lipoproteins of non-fasting humans and from chylomicrons of chylos ascites also exhibited a significant positive and negative charge shift (fig.1 and table 1). Apo A-IV is removed easily from lipoproteins by ultracentrifugation and may exist in free form unassociated with the main lipoprotein fractions in human plasma [15]. Therefore it was not clear whether this protein is an integral lipoprotein constituent or only unspecifically adsorbed to TG-rich lipoproteins. The results from charge-shift electrophoresis however clearly indicate that Apo A-IV does bind detergent by hydrophobic interaction and thus has to be considered a true amphipathic apolipoprotein.

Since the A-I and A-II polypeptides from normal HDL both exhibited a significant charge shift it was of special interest to analyse the respective apolipoproteins from patients with Tangier disease. This rare autosomal recessive disorder is characterised by deficiency of HDL in plasma. The concentration of Apo A-I, the main structural protein of HDL, (and to a lesser degree that of Apo A-II) is grossly reduced and most of the residual Apo A-I (90–95%) is not

Table 1
Detergent-induced changes in electrophoretic migration of human apolipoproteins and control plasma proteins

Apolipoprotein	Cathodic shift (CTAB) mm	Anodic shift (NaDOC) mm
A-I	15	24
A-II	17	13
A-IV	9	18
B	4	12.5
E	16	8
Albumin	3	5
α_2-Macroglobulin	0	2
Gc-Protein	0	2.5

Hemoglobin migration was 5 mm in all cases

associated with lipoproteins but resides in the $d > 1.21$ g/ml fraction [10,18]. Therefore it has been suggested that Tangier Apo A-I does not normally associate with lipids [18]. It has however been demonstrated that Apo A-I is synthesised by intestinal epithelial cells from Tangier patients and is released from the cells into the collecting lymph vessels [19]. One possible reason for the HDL deficiency than might be a mutation affecting the lipid binding properties of Apo A-I resulting in an inability of Apo A-I to direct the formation of a stable HDL complex. Since the concentration of Apo A-I in Tangier plasma is very low it is difficult to obtain sufficient amounts of pure protein to perform detergent binding studies by classical methods. Charge-shift electrophoresis however allow the analysis of small amounts of protein even in complex mixtures as demonstrated here for Apo A-I from control sera and should indicate a gross disturbance of the proteins binding capacity for amphiphiles. Apo A-I and Apo A-II from 4 patients with Tangier disease showed positive and negative charge shifts to the same extent as did their counterparts from control sera (fig.2). Hence these proteins apparently have preserved the property to bind amphiphiles by hydrophobic interaction.

Previous studies from our laboratory have shown that the Apo A polypeptides from control and Tangier plasma have identical molecular weights as judged by SDS-PAGE and have the same pI values in analytical isoelectric focusing [8]. Also the proteins from both sources are indistinguishable in their amino acid composition and immunochemical properties [10,20,21]. It thus seems unlikely that a structural mutation in one of the Apo A polypeptides is the underlying defect in Tangier disease. This is also supported in [19] where an enhanced catabolism of normal HDL and a preferential loss of normal Apo A-I from HDL in the plasma compartment of a patient with Tangier disease is demonstrated.

In conclusion the present investigation shows that human apolipoproteins A-I, A-II, B, E and A-IV exhibit significant bidirectional charge shifts, indicating that they bind with detergents by hydrophobic interaction. Charge-shift electrophoresis may be applied in cases were the apolipoprotein nature of a protein is disputed and to determine in lipoprotein disorder states whether individual apolipoproteins have preserved their capacity to bind amphiphiles.

Acknowledgements

The expert technical assistance of Mr Wilfried Weber is gratefully acknowledged. We thank Dr Lorenz Neuburger, Bern and Dr Gerd Assmann, Münster for providing serum samples from their patients with Tangier disease. This work was supported by a grant from Deutsche Forschungsgemeinschaft to G.U.

References

[1] Singer, S. J. and Nicolson, G. L. (1972) Science 175, 720–731.
[2] Helenius, A. and Simons, K. (1977) Proc. Natl. Acad. Sci. USA 74, 529–532.
[3] Helenius, A. and Simons, K. (1972) J. Biol. Chem. 247, 3656–3661.
[4] Makino, S., Reynolds, J. A. and Tanford, Ch. (1973) J. Biol. Chem. 248, 4926–4932.
[5] Segrest, J. P., Jackson, R. L., Morrisett, J. D. and Gotto, A. M. (1974) FEBS Lett. 38, 247–253.
[6] Makino, S., Tanford, Ch. and Reynolds, J. A. (1974) J. Biol. Chem. 249, 7379–7382.
[7] Stone, W. L. and Reynolds, J. A. (1975) J. Biol. Chem. 250, 3584–3587.
[8] Utermann, G., Menzel, H. J. and Schoenborn, W. (1975) Clin. Genet. 8, 258–268.
[9] Kummer, H., Laissur, J., Spiess, H., Pflugshaupt, R. and Bucher, U. (1968) Schweiz. Med. Wschr. 98, 406.
[10] Assmann, G., Smootz, E., Adler, K., Capurso, A. and Oette, K. (1977) J. Clin. Invest. 59, 565–575.
[11] Havel, R. J., Eder, H. A. and Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345–1353.
[12] Kostner, G. and Alaupovic, P. (1971) FEBS Lett. 15, 320–324.
[13] McConathy, W. J. and Alaupovic, P. (1973) FEBS Lett. 37, 178–182.
[14] Utermann, G. (1975) Hoppe-Seyler's Z. Physiol. Chem. 356, 1113–1121.
[15] Beisiegel, U. and Utermann, G. (1979) Europ. J. Biochem. in press.
[16] Helenius, A. and Simons, K. (1971) Biochemistry 10, 2542–2547.
[17] Bhakdi, S., Bhakdi-Lehnen, B. and Bjerrum, O. J. (1977) Biochim. Biophys. Acta 470, 35–44.
[18] Henderson, L. O., Herbert, P. N., Fredrickson, D. S., Heinen, R. J. and Easterling, J. C. (1968) Metabolism 27, 165–174.
[19] Assmann, G. (1978) in: High Density Lipoproteins and Atherosclerosis, (Gotto, A. M., jr, Miller, N. E. and Oliver, M. F. eds) pp. 77–89, Elsevier/North-Holland Biomedical Press, Amsterdam, New York.
[20] Assmann, G., Herbert, P. N., Fredrickson, D. S. and Forte, T. (1977) J. Clin. Invest. 60, 242–252.
[21] Lux, S. E., Levy, R. I., Gotto, A. M. and Fredrickson, D. S. (1972) J. Clin. Invest. 51, 2505–2519.