Two-dimensional gel electrophoresis of apolipoprotein C-III and other serum glycoproteins for the combined screening of human congenital disorders of O- and N-glycosylation

Arnaud Bruneel, Tiphaine Robert, Emilie Loncle, Amel Djedour, Geneviève Durand and Nathalie Seta.

AP-HP, Service de Biochimie Métabolique & Cellulaire, GH Bichat - Claude Bernard, Paris, France.

INTRODUCTION

Congenital disorders of glycosylation (CDG) are inherited diseases that can affect not only the N-glycan but also the O-glycan biosynthesis pathway. In absence of specific clinical symptoms, there is a need for a reliable biological screening of these two groups of CDG. In this study, 2-DE and immunoblotting were applied to the separation and simultaneous detection of the isoforms of the O-glycosylated protein apolipoprotein C-III (apoC-III) and of four N-glycosylated proteins, namely alpha antitrypsin (AAT), alpha-1 acid glycoprotein (AGP), haptoglobin (Hpt) and transferrin (Trf). This technique will be further applied to the combined biological screening of human congenital disorders of O- and N-glycosylation.

MATERIALS & METHODS

- Samples: 1 µL to 5 µL of human serum / plasma
- 2-DE : IPG 3-10 / SDS-PAGE 15 %
- Transfer : 100 Volts for 50 min
- Anti-Trf (1/2500), anti-AAT, anti-Hpt, anti-AGP(1/5000) from Dade Behring
- Anti-apoC-III (1/5000) from Biodesign International
- Neuraminidase from Sigma, cat. no. N-8271, ratio 1:3 for 1 h. at 37°C
- ECL revelation & Image Master Platinium software from Amersham Biosciences

RESULTS

Figure 1A shows that, under saturating exposure times, apoC-III was separated into 3 protein fractions i.e. 2 major spots with p*I* 4.3-4.5 (spot 2) and 4.5-4.7 (spot 1) and one vertical cluster of minor spots with p*I* 4.8-5.0 (spots 0). Based on experimental p*I*/Mw values, spot 2 and spot 1 respectively matched with the known disialyl (apoC-III_2) and monosialyl (apoC-III_1) glycoforms of apoC-III. The apoC-III_0 fraction could be separated into up to 4 spots (0a, 0b, 0c and 0d) sharing identical p*I* but distinct Mw values; while spots 0a and 0b respectively matched with apoC-III_0 substituted with GalNAc and with Gal-GalNAc, spots 0c and 0d could not be evidently assigned to other known asialylated isoforms of the protein. Neuraminidase treatment induced the nearly complete disappearance of spots 2 and 1 confirming their identifications as apoC-III_2 and apoC-III_1, respectively; concomitantly, neuraminidase induced the increase of spot 0b in accordance with its identification as apoC-III_0-Gal-GalNAc (**fig. 1B**).

Under these conditions and after non-saturating exposure times, the mean (SD) percentage (%) values of apoC-III isoforms determined from the analysis of 24 plasma samples from healthy adult donors were as following: apoC-III_0, 1.5 (2.0)%, apoC-III_1, 52.8 (9.0)% and apoC-III_2, 45.7 (9.6)%. Lastly, the determination of the mean (SD) percentage values of apoC-III isoforms separated from the same control plasma on six different days gave the following results: apoC-III_0, 0.5 (0.1)%, apoC-III_1, 54.2 (1.2)% and apoC-III_2, 45.3 (1.3)%. Up to now, more than 50 samples were analyzed and 3 among them showed abnormal O-glycosylation patterns.

We were also able to specifically detect on the same gel and at the same time the isoforms of up to four major circulating N-linked glycoproteins i.e. AAT, AGP, Hpt and Trf (**fig. 2**). Thus, the 2-DE patterns of plasma N-glycosylated proteins from patients with CDG type Ia (deficiency in phosphomannomutase), all revealed unequivocal p*I*/Mw differences by comparison with controls (**fig.3**).

CONCLUSIONS

This method which improves the resolution of the protein separation over IEF or SDS-PAGE alone, could be dedicated to different purposes depending on antibodies used. It can be routinely applied with only anti-apoC-III antibody for the specific screening of disorders of O-glycosylation. With the five described antibodies, it will also be employed as a secondary technique to get an accurate overview on N- and/or O-glycan abnormalities in patients suggestive of CDG on the basis of abnormal routine screening test, i.e. IEF of transferrin. Lastly, since this method is easily adaptable to mini-gel electrophoresis equipment offering relatively "high throughput" capabilities, we may speculate that it could be shortly applied to the overall biological screening of both O- and N-glycosylation congenital disorders

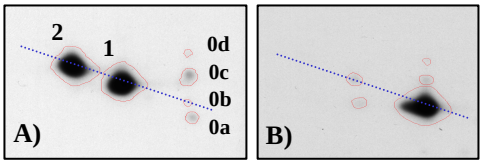

Figure 1:
A) 2-DE pattern of apoC-III isoforms.
B) 2-DE pattern obtained after neuraminidase treatment.

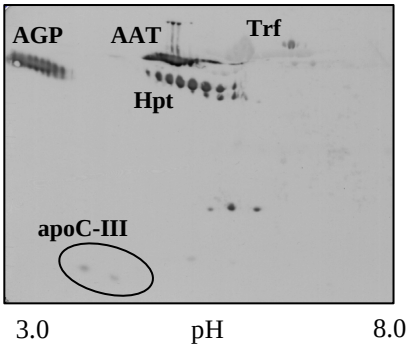

Figure 2: 2-DE pattern obtained after simultaneous detection of apoC-III, AGP, AAT, Hpt and Trf.

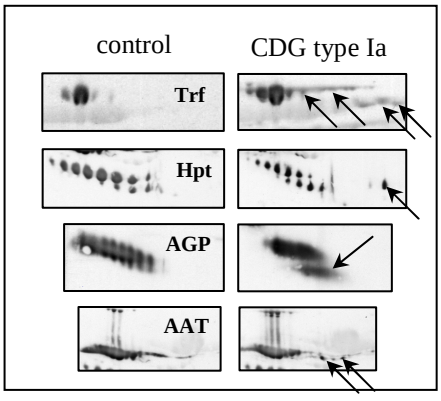

Figure 3: enlarged areas showing N-glycan abnormalities in one plasma from a patient with CDG-Ia (right; arrows) by comparison with control (left).