New Variation in Low-Sulfur Keratins Detected by Hybrid Isoelectric Focusing (HIEF)

M. S. Rodriguez-Calvo, I. Muñoz, A. Carracedo

Institute of Legal Medicine. University of Santiago de Compostela. Galicia. Spain

INTRODUCTION

Variability in non-carboxymethylated keratins by IEF in the presence of 6M urea, 1.5% Nonidet P40 and correlation with SDS-PAGE patterns has been recently demonstrated (Rodriguez-Calvo et al. 1990). Using the IEF technique, the variability could only be detected by the silver staining method, nevertheless with SDS-PAGE the patterns could be seen after Coomassie staining, so variability in other parts of the gels with higher concentration of proteins should exist. Further investigation on the variability of hair keratins applying HIEF in various pH ranges is reported.

MATERIAL AND METHODS

Treatment of samples

1. Washing: Petroleum ether (X3), ethanol (X2) and distilled water (X2)
2. Drying
3. Cutting into small pieces
4. Extraction: according to Marshall and Gillespie (1982) but without carboxymethylation (Carracedo et al. 1985).

3 cm of hair/15 µl of solution: 0.05M TRIS, 0.05M DTT and 8M urea (pH 9.3).

HIEF procedure

1. Formation of IPGs: according to Bjellqvist et al. (1982) (260x100x0.5mm)

Table 1.- Composition of the acidic and basic components of the gradient mixer

Components	Acidic (dense) solution	Basic (light) solution
Immobiline volumes	see Table 2	see Table 2
Acrylamide (29.1%) + bis-acrylamide (0.9%)	1.25	1.25
Glycerol 87%	2.10	0.00
H_2O	X	Y
Total volume	7.50	7.50
TEMED (10 ml%)	0.01	0.01
Ammonium persulfate (10g%)	0.01	0.01

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Table 2.- Specific 0.2M Immobiline volumes (µl)

pH range	Immobiline(pK)	Acidic sol.	Basic sol.
4.7-5.6	3.6	252.90	-
	4.6	313.15	431.50
	6.2	431.50	431.50
	9.3	5.25	52.50
4-6	3.6	284.50	195
	4.6	49.50	260.50
	6.2	219.50	138
	9.3	-	361

2. HIEF

- Polimerization: 15 min at room temperature + 1 h at 50°C
- Washing: 6x10 min with distilled water
  1x30 min with 2% Glycerol
- Drying
- Rehydratation (mold method): at least 3 h
  *IPG 4.7-5.6 with1.5% Ampholine 4-6, 6M urea,
  1.5% Nonidet P40 and 0.05M DTT
  *IPG 4-6 with 1% Ampholine 4-6, 6M urea,
  1.5% Nonidet P40 and 0.05M DTT

3. Running conditions

- 7W, 4mA, 3000V, for 5 h at 14°C
- Electrode solutions: 10mM NaOH for the cathode and 10mM glutamic acid for the anode
- Sample application: 0.5x0.5 with Whatman 3 MM, in the cathode.

3. Staining

Silver staining method according to Carracedo et al. (1983).

RESULTS AND DISCUSSION

IEF patterns of non-carboxymethylated keratins in the pH range 2.5-8, with 6M urea and 1.5% Nonidet P40 (Fig. 1) has been recently demonstrated (Rodríguez-Calvo et al. 1990).

Additional variation was explored with HIEF in various pH ranges: first in a wide pH range to determine the pI of interest, then in a narrow pH to analyze the variability.

With this technique, bands are sharper and more clearly defined so the interpretation of the patterns is easier. The different keratin phenotypes were clearly distinguished in the pH range 4.7-5.6 (Fig. 2) and their correlation with the variability previously described by IEF has been proved. The reproductibility and insensitivity of salts makes HIEF the method of choice for the study of the keratins in areas of the gel with high concentration of proteins.

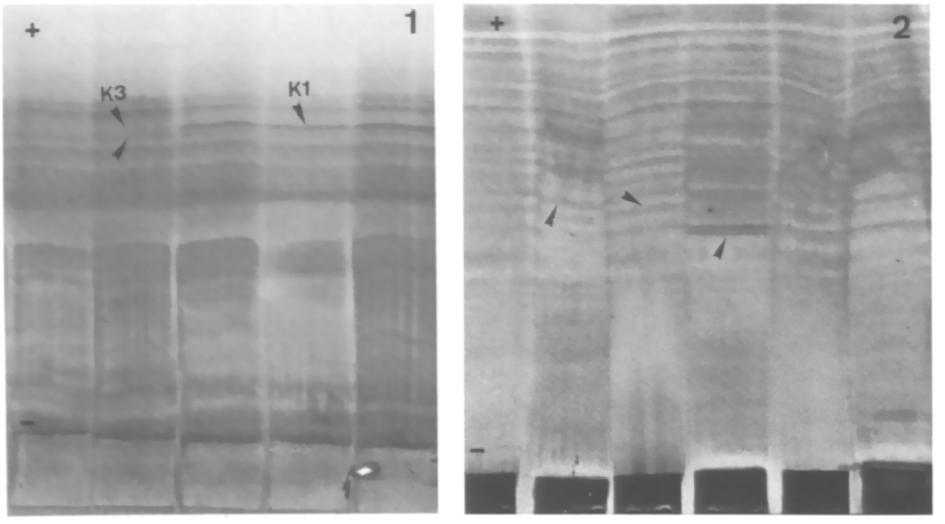

Fig. 1: IEF keratin patterns (I=4.65%; C=3.2%; pH 2.5-8) in the presence of 6M urea and 1.5% NP40

Fig. 2: Variability of hair keratins detected by HIEF (pH range 4.7-5.6)

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