Large scale isoelectric focusing analysis of the non-histone proteins of the nucleus: Isolation of components with alkaline isoelectric points

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Summary. After large scale isoelectric focusing of rat liver non-histone protein in polyacrylamide gel, pH range 4–8.6, the only protein material found outside the gradient was present in the cathode solution (20 mM NaOH). This was low mol. wt protein material (approximately 10,000) with an acidic amino acid composition. It bound 5–6 times its own weight of basic ampholine carrier ampholytes to give a complex with a pI of 8.82. This could be dissociated by dialysis against 1 M NaCl.

Since we described isoelectric focusing (IEF) in polyacrylamide gel[2] for the separation and analysis of the important but complex heterogenous mixture of proteins, loosely called the nuclear non-histone proteins (NHP)[3], this method has been extensively used by many workers. For example, a 2 dimensional combination of IEF followed by SDS gel electrophoresis has been shown to be a technique of high resolving power in NHP analysis[4,5]. However, with the use of IEF the possibility exists that proteins will be present with pI's higher or lower than the limits of the pH gradient finally established. Normally these would merely pass unnoticed into the electrode solutions (base at cathode, acid at the anode).

In our studies on the NHP of rat liver nuclei (and other tissues) we have performed large scale separations of NHP (up to 1 g of protein analyzed) in polyacrylamide gel using a Quickfit preparative (PAGE) electrophoresis unit without the usual elution chamber. The gel anulus used had an external diameter of 4 cm and an internal diameter (cold finger) of 1.7 cm, being 10 cm high. The gel mixture employed was a scaled version of that described in our original recipe[2] i.e. 5% polyacrylamide containing 8 M urea, 2% (w/v) ampholine carrier ampholytes pH 3.5–10 (LKB Produkter Ltd). The gel was polymerized and run at 15 °C with 60 ml of 20 mM NaOH at the cathode (top) and 250 ml of 10 mM H_3PO_4 at the anode (bottom). The voltage was raised in steps so that the power did not rise above 3 W and the run terminated after approximately 26 h; usually a final current of 8 mA at a constant 200 V (15 °C).

The material used for isoelectric focusing was an NHP mixture prepared by extracting rat liver nuclei with 8 M urea 50 mM phosphate pH 7.6. This removes 70±5% of the total nuclear protein but no histone or DNA. After large scale IEF of this mixture no protein material was found to be present in the anode solution at the termination of the run; this was checked using radiolabelled NHP. The pH at the top of the gel was usually about 8.5 and 4.0 at the bottom.

However, when the cathode solution (20 mM NaOH) was dialysed exhaustively against distilled water a solid precipitated out which was very soluble in 1 N acetic acid. As the pI of this solid was greater than 8.5 it was thought possible that histones might be present. Electrophoresis using the polyacrylamide gel system of Panyim and Chalkley[6] (a standard histone analytical system), gave one heavily stained band running slightly ahead of H4. Despite the fact that this material stained like a histone it later became apparent that it was in fact alkaline ampholine carrier ampholytes! The latter could easily be dissociated from the protein material by dialysis against 1 M NaCl. This revealed that only 16% of the cathode precipitated material was indeed protein. We confirmed this finding by IEF analysis of ^{3}H-leucine labelled NHP. The ampholines interfered with both the Folin Lowry and Biuret protein estimations but the latter gave about $\frac{1}{5}$ of the values obtained by the former.

When the protein material (free of ampholine) was re-run on rod polyacrylamide gel IEF it gave a single band of pI 8.82. In a large scale IEF run, 2.0±0.5% of the total weight of NHP applied to the gel appeared in the cathode solution. On SDS electrophoresis[7] the material gave a major and minor band with mol. wt around 10,000 (too low for accurate mol. wt determination in this system).

Amino acid analysis (table) of the cathode protein revealed an acidic composition. Obviously its capacity for binding basic molecules leads to a material of high pI, especially with conventional IEF. As it is known that the nucleus contains 2 important classes of basic molecules, histones and polyamines, the material we have isolated could play an important role in nuclear metabolism. This possibility is being investigated further but other preliminary results have indicated that the basic amino acids of this protein(s) are alkylated after administration of low doses of the carcinogen diethylnitrosamine to rats.

Amino acid composition (in nanomoles percent) of cathode solution non-histone protein from rat liver nuclei

	Sample 1	Sample 2
Asp	9.0	8.8
Thr	6.0	6.3
Ser	7.5	7.4
Glut	11.1	11.1
Pro	6.1	5.4
Gly	11.8	10.1
Ala	8.7	8.4
Val	5.9	6.5
Cys	-	-
Meth	2.2	2.0
Isoleu	5.0	6.0
Leu	6.8	8.1
Tyr	0.5	0.5
Phe	2.8	2.4
Lys	7.2	7.5
Hist	2.6	3.2
Arg	6.8	6.3
Ratio $\frac{\text{acidic}}{\text{basic}}$	1.21	1.17

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