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## Note

# Analysis of radiomethylated $N^{\epsilon}$-methyllysines and other methylated amino acids by ion-exchange chromatography with fluorescent detection

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Although several methods for separating and detecting radioactive $N^{\epsilon}$-methyllysines have been described[1–5] they do not provide adequate sensitivity for our research. Accordingly we have developed[6] an analyzer and method for the ion-exchange separation and fluorescent detection of methyl-labeled $N^{\epsilon}$-mono-, di- and trimethyllysine, which requires only a small part of the effluent for fluorometric quantitation of the carrier amino acids. The remainder of the effluent is unaltered and is collected for radioactivity counting.

The analyzer is mainly based on the Dionex (Sunnyvale, CA, U.S.A.) amino acid peptide analyzer kit, which is easily modified for this purpose.

A schematic diagram is shown in Fig. 1. Most of the original design is left intact, except for the following changes:

(1) A Milton Roy positive displacement pump is used for addition of the Fluoropa® (*o*-phthalaldehyde, Dionex) reagent solution to the column effluent. Reagent solution flow-rate is approximately 0.12 ml $min^{-1}$.

(2) Use of a 30 × 0.9 cm preparative column containing Durrum DC-6A (Dionex) cation-exchange resin. Jacket temperature is kept constant at 28°C. Eluent pump pressure is typically 200–300 p.s.i. at an eluent flow-rate of approximately 0.9–1.0 ml $min^{-1}$.

(3) Several resistance coils of 0.6 mm I.D. PTFE tubing are installed at various positions (Fig. 1) to distribute effluent flow so that approximately 10–15% is directed through the fluorometer detection pathway, while the remainder is collected for radioactivity counting. The fluorometric detection pathway consists of a filter fluorometer (Aminco fluoromonitor, with 30-$\mu$l flowcell, 7–51 excitation filter which passes 330–400 nm, and Wratten 2Å emission filter, which passes all wavelengths above 415 nm). The fluoromonitor is connected to a chart recorder. Flow-rate through the fluorometric detection pathway is 0.24 ml $min^{-1}$ (eluent and Fluoropa solution). Column resin, sample injection valve, eluent selection valve, pulse damping column, eluent and reagent solution bottles, programmer, manifolds, tubing, connectors and framework were included in the amino acid analyzer kit (Dionex).

The following eluents and reagent solutions were used: (a) Eluent I: 0.117 *M* sodium citrate, pH 6.9, with 0.1% phenol (0–180 min); (b) Eluent II: 0.233 *M* sodium citrate, pH 8.0, with 0.1% phenol (180–360 min); (c) 0.2 *N* sodium hydroxide (Pierce,

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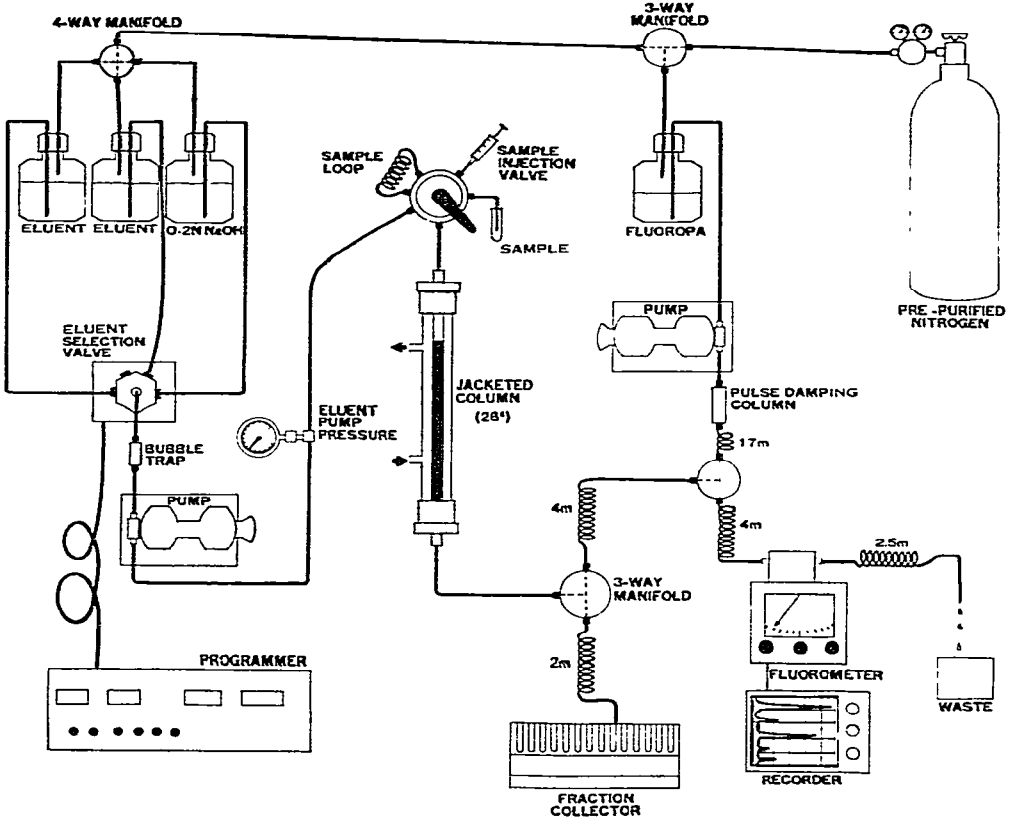


Fig. 1. Schematic diagram of a simple amino acid analyzer modified to separate lysine, methylated lysine derivatives and other basic amino acids, equipped with a streamsplitter in order to monitor radioactivity as well as fluorescence. Note especially the resistance coils (0.6 mm I.D. PTFE tubing) installed at various points, with lengths given in meters.

Rockford, IL, U.S.A.), should be of high purity and preferably stored in a plastic container. (d) Fluoropa solution: Dissolve 500–800 mg Fluoropa in 10 ml ethanol, add to 1 l 0.4 *M* boric acid brought to pH 10.4 with potassium hydroxide, then add 2 ml fresh mercaptoethanol and 3 ml 30% Brij (Pierce).

All eluents should be filtered through a 0.22-$\mu$m Millipore (Bedford, MA, U.S.A.) filter before use, in order to prevent clogging of the resin bed, and kept under positive pressure of 1–2 p.s.i. of prepurified nitrogen to avoid atmospheric contamination.

$N^{\varepsilon}$-Mono-, di- and trimethyllysine, histidine, 3-methylhistidine, arginine and $N^{\varepsilon}$,$N^{\varepsilon}$-dimethylarginine derivatives were obtained from Sigma (St. Louis, MO, U.S.A.). Methyllabeled mono-, di- and trimethyllysines were generated biologically by incubating rat liver nuclei *in vitro* in the presence of S-adenosylmethionine containing labeled methyl, as described previously in ref. 1. Histones were isolated from thus incubated nuclei according to standard methods[1]. Radioactively labeled histones were isolated and hydrolyzed at 110°C for 16 h in evacuated Reacti-Therm tubes (Pierce). During evacuation the tubes were immersed in liquid nitrogen. Hydrolysates

were dried by cryogenic transfer, dissolved in 0.1 *N* hydrochloric acid and applied to the ion-exchange resin bed via the sample injection valve (Fig. 1).

With the aid of non-radioactive, methylated amino acid derivatives a single eluent was found which provided a satisfactory resolution of histidine, lysine and methylated lysine derivatives. For the subsequent elution of arginine and its methylated derivatives a second eluent is required (Fig. 2).

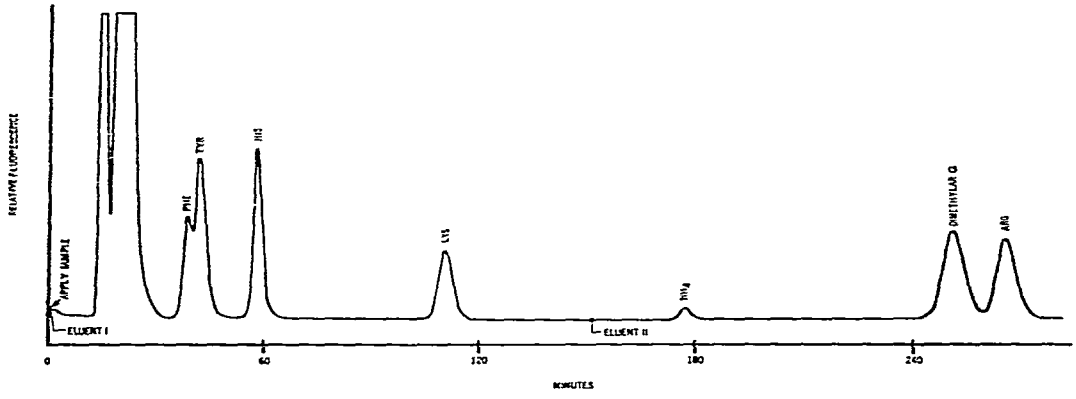


Fig. 2. Chromatogram of a standard amino acid mixture including dimethyl arginine, containing 25 nanomoles of each amino acid. Note change of eluent at 2½ h.

The results of a typical analysis of a hydrolysate of histones from cell nuclei incubated in the presence of S-adenosylmethionine (methyl-$^3$H) containing radiomethylated lysine derivatives, are summarized in Fig. 3.

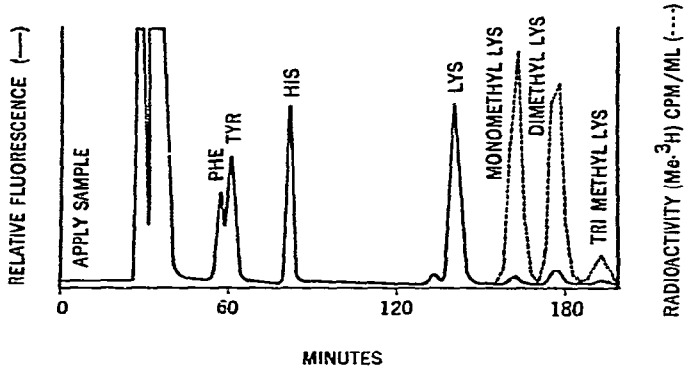


Fig. 3. Chromatogram of rat liver whole histone hydrolysate containing N$^e$-methyl-[$^3$H]-lysine derivatives. Radioactive labeling of methylated lysines was obtained as described in the text. A small peak which eluted immediately preceding lysine, was identified as a trace of tris(hydroxymethyl)aminomethane.

As can be seen in Figs. 2 and 3, amino acids appear as follows: acidic amino acids, neutral amino acids, tyrosine, phenylalanine, histidine, lysine (eluent I), ammonia, dimethylarginine and arginine (eluent II). Radioactivity measurements indicate that following lysine, its methylated derivatives mono-, di- and trimethyllysine are

eluted well separated and in that order. If required, elution can be continued with eluent II after trimethyllysine has emerged*.

When the analysis is completed, the resin bed is stripped with 0.2 *N* sodium hydroxide and equilibrated with eluent I prior to application of the next sample.

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* The optimal separation of histidine and 3-methylhistidine obtainable in this system was elution of the latter slightly prior and on the leading edge of the histidine peak.