Journal of Chromatography, 171 (1979) 497–499

CHROM. 11,540

Note

Separation of polyamines by polyacrylamide gel exchange chromatography

JULIO J. LUDOWIEG and LAURENCE J. MARTON*

Brain Tumor Research Center, Department of Neurological Surgery, University of California, San Francisco, Calif. 94143 (U.S.A.)

(First received September 13th, 1978; revised manuscript received October 18th, 1978)

We have separated putrescine (Pu), spermidine (Sd) and spermine (Sp) from macromolecules present in the plasma by using Bio-Gel P-2 column chromatography. The polyamine elution order, Pu first followed by Sd and Sp, suggests that the gel can be used as both a sieve and a cation exchanger.

Bio-Gel P-2 is a highly cross-linked polyacrylamide gel frequently used in molecular sieve chromatography as a neutral gel. We have measured the ion-exchange capacity of a series of Bio-Gel P-2 lots by titration with hydrochloric acid in gel samples which were first made neutral with sodium hydroxide[1]. Our results showed that many of the commercially available gels were not entirely neutral and that lot-to-lot differences occur. A recently purchased gel preparation was essentially neutral (0.005 mequiv./g) (Bio-Rad P-2, lot 166663). Other lots from the same vendor (Bio-Rad Labs., Richmond, Calif., U.S.A.) showed an average ion-exchange capacity in the range 0.26–0.29 mequiv./g.

EXPERIMENTAL

The chromatographic method used for the separation of polyamines was routinely carried out in a glass column (21 × 1.5 cm I.D.) with adjustable headspace eliminators, which was refrigerated at 5°. An upwards flow-rate of 0.37 ml/min, comparable to a downward flow-rate that would be obtained under gravity, was maintained with a low pulsating peristaltic pump. A single 0.15 *M* ammonium formate buffer (pH 5.4) was used for the entire chromatographic run.

RESULTS AND DISCUSSION

Fig. 1 shows the results obtained with a neutral Bio-Gel P-2 (0.005 mequiv./g) as it was obtained from the manufacturer. Both Pu and Sp were found to co-elute from the column using 0.15 *M* ammonium formate buffer. Fig. 2 shows the separation of polyamines achieved when the chromatographic elution was repeated using a gel having acidic properties (0.3 mequiv./g).

The analysis of plasma containing radiolabeled polyamines is presented as an

* Also a member of the Department of Laboratory Medicine.

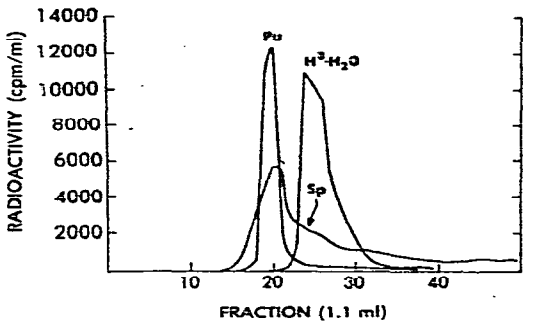

Fig. 1. Chromatography of a solution mixture containing [^{14}C]polyamines in a neutral Bio-Gel P-2 column. Elution with 0.15 *M* ammonium formate buffer (pH 5.4).

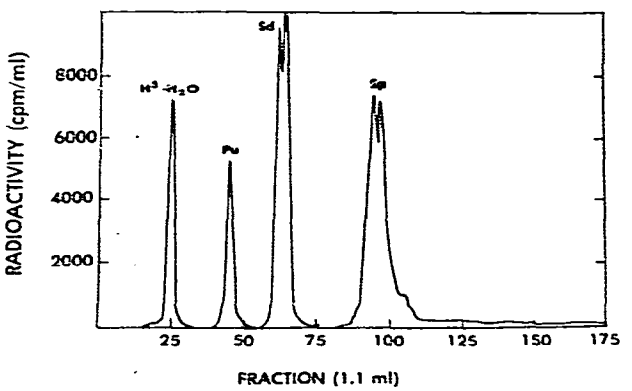

Fig. 2. Chromatography of a solution mixture containing [^{14}C]polyamines in an acidic Bio-Rad P-2 gel (0.3 mequiv./g) column. Elution with 0.15 *M* ammonium formate buffer (pH 5.4).

example of the utility of the technique. For this experiment, plasma samples were obtained from a male rat which has been injected i.v. with 0.7 ml of a solution mixture of ^{14}C-labeled polyamines in saline. Fig. 3 shows the results of the chromatography of plasma specimens collected 2 and 60 min after injection. It is evident that the amounts of all three polyamines decreases significantly by 60 min (note the different radioactivity scales in the two parts of Fig. 3). Also evident is the presence of a new peak(s), shown as X in the bottom part of Fig. 3, which elutes prior to Pu and which is radiolabeled.

The elution profiles of a freshly packed column (Fig. 2) and of the same column after a number of runs (Fig. 3) were different. After several runs the gel was maximally compacted and elution profiles were consistent.

We have observed that essentially the same chromatographic profile has been obtained with several lots with different exchange capacities (0.26–0.36 mequiv./g). One of these preparations was made in the laboratory (0.35 mequiv./g) by treating a neutral gel with sodium hydroxide[1].

Earlier and closer elution peaks for the three polyamines were obtained by increasing the concentration of the ammonium formate buffer to 0.3 *M*. Minor shifts in the positions of the elution peaks were also obtained by changing the buffer pH. Tailing of Sp has been consistently observed under the specific conditions used in the chromatographic procedure.

As a gel exchanger, Bio-Gel P2 with acidic properties may not perform as well

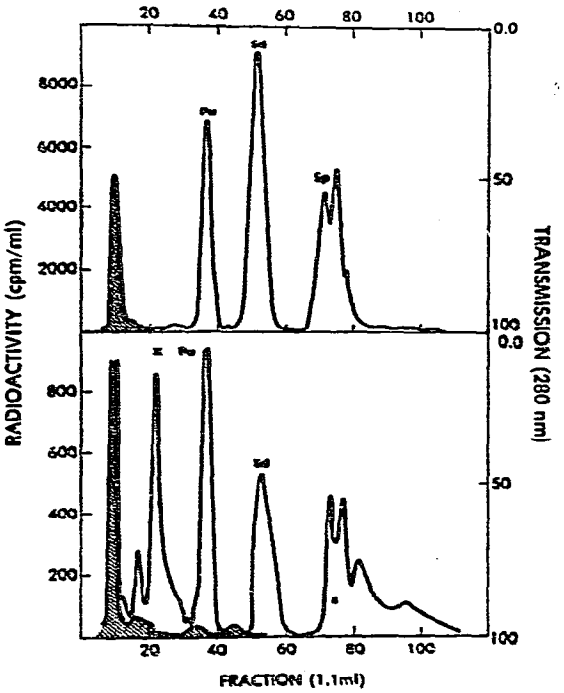

Fig. 3. Separation of the [^{14}C]polyamines in rat plasma after i.v. injection of an undiluted mixture of [^{14}C]Pu (0.198 μmole), [^{14}C]Sd (0.26 μmole) and [^{14}C]Sp (0.45 μmole) in 0.7 ml of saline. The total activity in the 0.7 ml of injected solution was $89 \cdot 10^6$ cpm. [^{14}C]Pu, 63 mCi/mmole; [^{14}C]Sd, 55.5 mCi/mmole; [^{14}C]Sp, 35.71 mCi/mmole. The counting efficiency was approximately 90%. The shaded area corresponds to non-radioactive plasma components. Top: 0.1 ml of frozen rat plasma, 2 min after injection. Bottom: 0.2 ml of frozen rat plasma, 1 h after injection.

as ion-exchange resins used in high-pressure chromatographic columns[2]. On the other hand, the use of Bio-Rad P-2 for the isolation of polyamines from fresh plasma samples is clearly advantageous inasmuch as the elution buffer (ammonium formate) is volatile and can be eliminated by lyophilization. In this respect, Bio-Gel P-2 also appears to be an improvement over common cation-exchange resins used in low-pressure column chromatography[3]. Further, the method may also prove useful for the examination of polyamines and derived metabolites in other biological fluids and tissue specimens.

The recovery of radiolabeled polyamines from the column was about 90% and 80% for Pu and Sd, respectively, and about 60% for Sp. Essentially the same recoveries were obtained by using a solution mixture of radiolabeled polyamines in plasma.

ACKNOWLEDGMENTS

This work was supported by NCI Grants CA-15515 and CA-13525 and gifts from the Margaret M. Anton and Tim Grutman Memorial Funds. L. J. M. is a recipient of NCI Research Career Development Award CA-00112.

REFERENCES

1 J. K. Imman, *Methods Enzymol.*, 34B (1974) 30.
2 L. J. Marton and P. L. Y. Lee, *Clin. Chem.*, 21 (1975) 1721.
3 M. G. Rosenblum and D. H. Russell, *Cancer Res.*, 37 (1977) 47.