ACTA CHEMICA SCANDINAVICA 12 (1958) 641—645

The Fractionation of Rat Liver Supernatant by Salt Gradient Elution from Triethylaminoethyl Cellulose Columns

JOHN GLOMSET

Institute of Medical Chemistry, University of Uppsala, Uppsala, Sweden

Gradient elution from triethylaminoethyl cellulose columns has been employed for the separation of fractions from rat liver containing proteins and nucleotides. The method has proved to be useful for the chromatographic separation of each of these types of compound.

The experiments of Sober and Peterson and others [1-3] successfully employing acidic and basic cellulose derivatives for the chromatographic separation of proteins have aroused a great deal of interest. Many papers describing the utilization of various types of cellulose ion exchange columns have appeared in the literature. Not infrequently columns of DEAE * cellulose prepared according to Peterson and Sober [4] have been employed. Recently Porath [5] has described the preparation of triethylaminoethyl cellulose, and reports of the successful use of this material have appeared [6,7]. In an earlier paper [8] the present author described some experiments utilizing stepwise elution chromatography with columns of TEAE cellulose. The main object of these experiments was to separate and purify phosphoproteins present in the supernatant fraction of rat liver as obtained by centrifugation according to Schneider and Hogeboom [9]. In the present paper the use of a simple saline gradient at constant pH for the fractionation of the same starting material will be described. To further characterize the salt gradient system employed here as a preliminary to later experiments nucleotide extracts have also been chromatographed.

EXPERIMENTAL

Rat liver supernatant was prepared as before [8] except that 0.01 M sodium EDTA, pH 7.4 was included in the initial perfusion saline and omitted from the sucrose. In experiments in which the whole supernatant fraction was to be chromatographed the material was applied to the column immediately after the final centrifugation. When the

* The following abbreviations will be used: DEAE cellulose, diethylaminoethyl cellulose; TEAE cellulose, triethylaminoethyl cellulose; EDTA, ethylenediaminetetraacetic acid; Tris, Tris (hydroxymethyl)-aminomethane; TCA, trichloroacetic acid; ATP, adenosine triphosphate; ADP, adenosinediphosphate.

acetone precipitated proteins of the supernatant were to be chromatographed the pH of the supernatant was first adjusted to 6.5 with 0.1 N acetic acid. Analytical grade acetone which had been pre-cooled to −14°C was then added dropwise with continuous stirring in an alcohol-dry ice bath at −14°C. Precipitates were collected by centrifugation in refrigerated centrifuges at −14°C after addition of acetone to concentrations of 50 and 70 % by volume. The precipitates were washed twice, dissolved in 0.01 N Tris-HCl, pH 7.4, combined, and immediately applied to the ion exchange column.

In one type of experiment a mixture of radioactive mitochondrial nucleotides was prepared in the course of the preparation of the supernatant fraction. The method described by Beyer *et al.*[10] was used except that the sucrose did not contain EDTA and the radioactive phosphate was added to a sucrose suspension of the washed mitochondria. The reaction was stopped after 10 min. at 0°C by the addition of TCA.

TEAE was prepared from DEAE according to Porath [5]. It contained 24.3 mg N per g dry powder. It was used in the chloride form after equilibration with 0.01 N Tris-Cl buffer, pH 7.4. All chromatographic separations were performed at +4°C. The gradient system comprised two steps. The first extended from 0 to 0.2 N NaCl. The second extended from 0.2 to 1.0 N NaCl. The entire system was buffered throughout with 0.01 N Tris-HCl, pH 7.4. The gradients were established as described by Boman [11]. A beaker was used as the mixing flask, and an Erlenmeyer flask of the same volume as the reservoir flask. All that was required in addition was a magnetic stirrer and some plastic tubing. In the different experiments two flask sizes were used, 500 ml or 1 000 ml, depending on whether the column contained 15 or 35 g of the ion exchanger, respectively.

The effluent from the columns was collected in 3−5 ml portions. The chromatographic separation was followed by spectrophotometric readings in the Beckman model DU apparatus, employing cuvettes of 1 cm light path at 260 and 280 mμ. Radioactivity determinations were made on aliquots which had been dried on glass planchets. The chloride content of the effluent was followed by a modification of the method of Bergman and Senik [12] worked out by the Analytical laboratory of AB Pharmacia, Uppsala.

RESULTS AND DISCUSSION

Three different types of experiment were performed. In the first the fresh supernatant, obtained directly after centrifugation of the liver homogenate, was immediately filtered into a column containing 35 g of TEAE, previously equilibrated with 0.01 N Tris-HCl, pH 7.4. After washing the column with the same buffer elution was carried out as described in the Experimental section. Fig. 1 shows the type of chromatogram obtained with this procedure. The first peak consists of compounds not adsorbed to the ion exchanger under these conditions. The succeeding peaks comprise both proteins and nucleotides as will be seen by comparing the relative absorption at 260 and 280 mμ in Fig. 1 with that of Fig. 3. The last peak in Fig. 1 contains most of the RNA of the supernatant. As noted previously [8], however, a significant percentage of the RNA of the supernatant (about 20 %) cannot be eluted from the column under these conditions nor by increasing the salt concentration or reducing the pH. It can be removed, however, using 1 % NaOH. With these reservations this method should be a convenient way to prepare soluble RNA from rat liver homogenates. A cardinal advantage of the method is that no preliminary precipitation steps or lengthy dialysis are needed.

In order to obtain a clearer conception of the behaviour of nucleotide material when chromatographed by salt gradient elution using columns of TEAE cellulose a second type of experiment was performed. Here, various types of nucleotide extracts were chromatographed. Fig. 2 shows a chromatogram of a TCA extract of rat liver mitochondria.

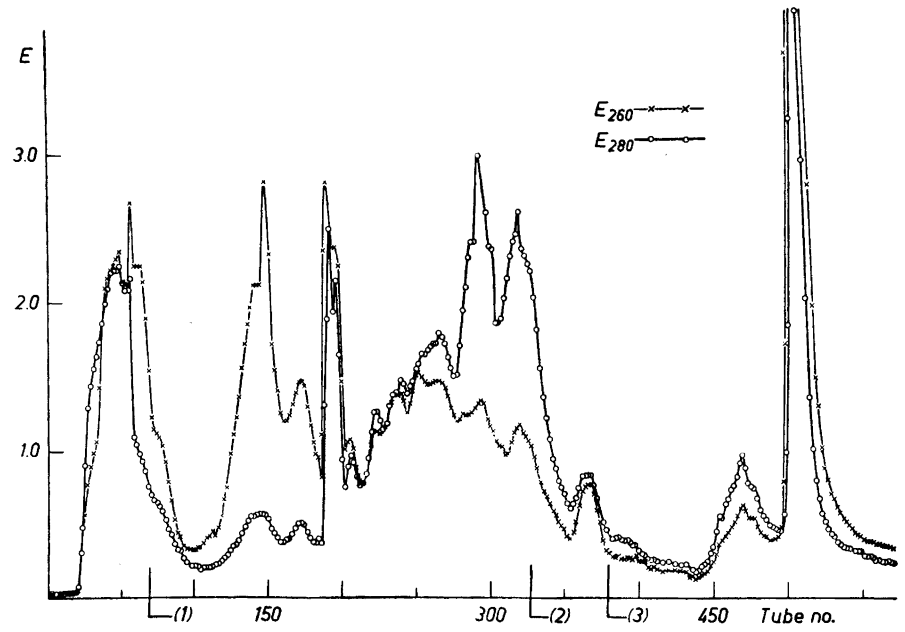

Fig. 1. Chromatogram of whole rat liver supernatant on TEAE cellulose. 4 livers used. The column contained 35 g TEAE cellulose. 1 000 ml vessels used to obtain the gradient. The first marker on the abscissa indicates the beginning of the first step of the gradient. From the second to the third marker, 0.2 N NaCl in 0.01 N Tris-HCl, pH 7.4 was filtered through the column. The third marker indicates the beginning of the second step of the gradient.

It will be seen that a satisfactory fractionation has been obtained, comparable with that obtained earlier [10] using a formic acid gradient system on Dowex 1. It was of primary interest for subsequent experiments (to be published) to determine the positions of inorganic phosphate, ADP, and ATP on the chromatogram. These are peaks IV, VIII, and IX, respectivly, and are the only radioactive peaks obtained under the conditions of the experiment. The successful use of TEAE cellulose for nucleotide chromatography would appear to be of value as a complement to those ion exchange methods already available, especially with compounds containing labile groups which might otherwise be destroyed by the lower pHs generally used in conventional nucleotide chromatography.

The third type of experiment was performed with acetone-precipitated liver supernatant protein. The precipitation was performed as quickly as possible to reduce the risk of denaturation. The combined precipitates were dissolved in buffer and chromatographed in the same way as in Fig. 1 without, however, having attempted to remove the small amount of residual acetone present. This explains the absorption properties of the first peak in Fig. 3, a typical chromatogram from this type of experiment.

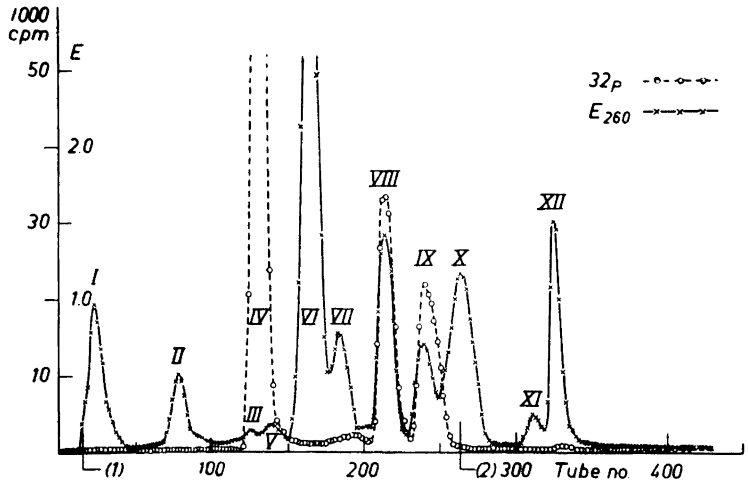

Fig. 2. Chromatogram of radioactive TCA extract of rat liver mitochondria on TEAE cellulose. Mitochondria from 4 rat livers used. 15 g of TEAE cellulose. 500 ml gradient vessels. The first and second markers on the abscissa indicate the beginning of steps 1 and 2 of the gradient, respectively.

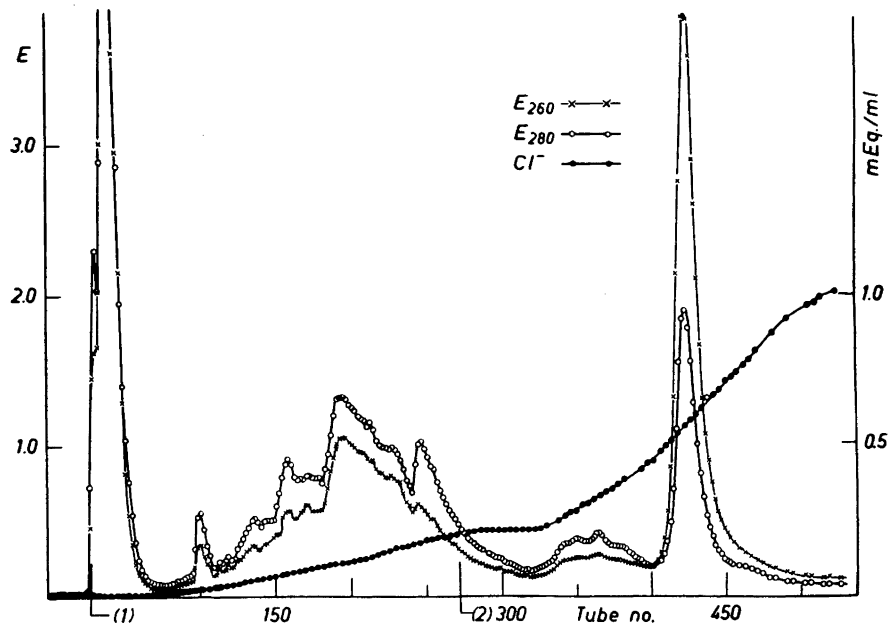

Fig. 3. Chromatogram of acetone-precipitated protein of rat liver supernatant on TEAE cellulose. 4 livers. 35 g of TEAE cellulose. 1 000 ml gradient vessels. The first and second markers on the abscissa indicate the beginning of steps 1 and 2 of the gradient, respectively. The chloride concentration in the effluent in mequiv. per ml may be determined from the ordinate at the right.

In this experiment the chloride concentration of the effluent was followed in order to indicate the nature of the gradient obtained. It will be seen that the nucleotide material present in the experiment reproduced in Fig. 1 has been removed and that the only two peaks with a high relative absorption at 260 mμ are the first and last, respectively. The first owes its absorption properties to the acetone present, while the last peak, as in Fig. 1, contains RNA. Although the chromatogram shown in Fig. 3 is of interest in that it gives a picture of the normal pattern of soluble rat liver proteins (and as such may profitably be compared with the electropherograms of soluble liver protein fractions obtained previously [8]) the identity of the remaining peaks has not been investigated further at this time. The object of the present experiments has been to study the chromatographic behaviour of nucleotide extracts and the soluble proteins of rat liver on gradient elution from columns of TEAE cellulose. The use of this method for the separation of the phosphoproteins of rat liver supernatant will be described in a subsequent paper.

Acknowledgments. This investigation was supported by a grant from the *Medical Faculty of the University of Uppsala.* The author is indebted to Dr. J. Porath for a generous gift of DEAE cellulose. He is also grateful to Dr. W. Kirsten for making available the chloride determination method.

REFERENCES

1. Sober, H. A., Gutter, F. J., Wyckoff, M. M. and Peterson, E. A. *J. Am. Chem. Soc.* **78** (1956) 756.
2. Boman, H. G. and Kaletta, U. *Biochim. et Biophys. Acta* **24** (1957) 619.
3. Sober, H. A. and Peterson, E. A. *J. Am. Chem. Soc.* **76** (1954) 1711.
4. Peterson, E. A. and Sober, H. A. *J. Am. Chem. Soc.* **78** (1956) 751.
5. Porath, J. *Arkiv kemi* **11** (1957) 97.
6. Boman, H. G. and Westlund, L. E. *Arch. Biochem. Biophys.* **70** (1957) 572.
7. Porath, J. *Arkiv kemi* **11** (1957) 259.
8. Glomset, J. *Acta Chem. Scand.* **11** (1957) 512.
9. Schneider, W. C. and Hogeboom, C. H. *J. Biol. Chem.* **183** (1950) 123.
10. Beyer, R. E., Glomset, J. and Beyer, T. *Biochim. et Biophys. Acta* **18** (1955).
11. Boman, H. G. *Biochim. et Biophys. Acta* **16** (1955) 245.
12. Bergmann, J. G. and Sanik, J. *Anal. Chem.* **29** (1957) 241.

Received February 13, 1958.