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CHROMATOGRAPHY OF $Na^{125}I$ ON SEPHADEX GELS

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SUMMARY

Elution volumes of NaI and $Na^{125}I$ appear to differ significantly when chromatographed on Sephadex G-15 or G-10. Using distilled water as the eluent, radioiodide was eluted in the void volume of the column while NaI was found eluted after NaCl. However, in most other eluent systems studied, radioiodide was retarded in comparison with NaI. This retardation was greatest with organic acid eluents and was more pronounced on Sephadex G-10 than on G-15. The difference in the elution volumes between NaI and $Na^{125}I$ could be decreased by elution with 0.1 *M* NaOH or by addition of NaI or KI to the loading sample. Sephadex G-25 did not appear to discriminate between NaI and $Na^{125}I$ under the conditions studied.

INTRODUCTION

Separation of halides has been described in the literature using paper chromatography[1], anion-exchange chromatography[2], column and thin-layer chromatography on microcrystalline cellulose[3], on Sephadex G-10 and G-25[4] and, more recently, on Sephadex G-15[5]. In comparison with other halides, iodide was found retarded on Sephadex gels by several groups of workers[4–6]. The present study resulted from a search for a purification system for radioiodinated neurohypophyseal peptides. Since NaI and $Na^{125}I$ are used in our iodination reaction[7], and our experience has been that native neurohypophyseal hormones are not easily desalted by conventional Sephadex chromatography[8], we included several inorganic salts in defining the elution parameters of our Sephadex G-15 column. In the course of this work it became apparent that the elution volume of radioiodide differed from that of NaI and KI, and that the elution volume of $Na^{125}I$ was sensitive to (a) the composition of the eluent buffer and (b) the concentration of carrier iodide in the loading sample.

MATERIALS AND METHODS

Reagents of analytical grade were used. Sephadex gels were purchased from Pharmacia (Piscataway, N.J., U.S.A.). Radioactive iodide was obtained from New England Nuclear (Boston, Mass., U.S.A.; Cat. No. NEZ-033 for $Na^{125}I$ and NEZ-

035A for $Na^{131}I$) and from Amersham/Searle (Don Mills, Canada; Cat. No. IMS-30). $Na^{125}I$ (New England Nuclear) was used for all the experiments, except those done for purposes of isotope comparison, as indicated in the text.

The same glass chromatographic column (Pharmacia; K 15/30, 15 × 300 mm) was used for all experiments reported here. Water was used for bed volume calibration[9] and appropriate temperature corrections were made.

Fractions were collected by volume (1.2 ml). The flow-rate ranged from 38 to 56 ml/h; within this range of flow-rate no significant alterations in the elution profiles could be observed. The loading sample was made up fresh in the eluent buffer to a volume of 0.4 ml. Unless otherwise indicated, approx. 2 μCi of radioactive iodide was used for chromatography.

Eluent buffers are listed in Table I. The gels were fined, and when re-packed in each new buffer, equilibrated with three column volumes of the eluent buffer prior to use. After chromatography in buffers F and G (Table I), the gels were discarded.

Radioactivity was monitored using a Model 4227 automatic gamma counter (efficiency, 67% for $Na^{125}I$) from Nuclear Chicago (Des Plaines, Ill., U.S.A.). The elution of non-radioactive salts was monitored by conductance measurements on a Model CDM-2e Radiometer (Copenhagen, Denmark) conductivity meter.

TABLE I

ELUENT BUFFERS

Letter code	*Composition*	*pH*	*Specific conductivity ($m\Omega^{-1}$)*
A	0.05 *M* Tris–HCl	8.0	2.6
B	0.5 *M* NaCl in buffer A	8.1	40.4
C	7 *M* urea in buffer A	8.3	1.3
D	0.2 *M* acetic acid	2.6	1.1
E	0.1 *M* formic acid	1.9	5.1
F	0.1 *M* NaOH	12.0	20.7
G	ethanol, 0.1% in HCl–water (60:40)	2.0	2.5
H	distilled water	6.3	0.005–0.009
I	7 *M* urea in buffer H	8.5	0.026
J	0.15 *M* sodium acetate	5.0	3.3
K	0.2 *M* ammonium acetate	5.0	11.1

In the initial experiments the conductance measurements were checked qualitatively by spotting of the fractions on filter paper and exposing the paper to iodine vapor[10]. Elution of HCl was monitored both by conductance and pH measurements.

Elution volume corresponding to the maximum of an effluent peak was used for V_0 (void volume; ml) and V_e (elution volume; ml) measurements. In most experiments Blue Dextran 2000 (Pharmacia) dissolved in eluent buffer was used for V_0 estimations. In the Sephadex G-10 series and in the Sephadex G-15 series which were run at low pH, cytochrome *c* or myoglobin (Mann Res. Labs., New York, U.S.A.) was substituted for Blue Dextran.

Calculations of K_{av} were done as described in the literature[11], using the following relationship:

$$K_{av} = \frac{V_e - V_0}{V_b - V_0}$$

where V_b is the bed volume of the gel (ml) obtained from the calibration of the column as described above.

Iodination control experiments consisted in subjecting $Na^{125}I$ solution to the routine steps of peptide iodination procedure with buffer blank in place of the hormone. The iodination procedures used were the method of Greenwood *et al.*[12], and the method of Chard *et al.*[13], both methods preceded by treatment of the radioiodide with sulfurous acid[14], and thallium chloride iodination[7].

RESULTS

Chromatography on Sephadex G-15

The elution volume of radioiodide in Tris–HCl buffer (A, Table I) was approximately two times greater than that of NaCl. Elution volumes of $CaCl_2$, HCl and $NaIO_3$ were the same as that of NaCl. Iodides (NaI and KI) were found retarded in comparison with NaCl, but not to the same extent as $Na^{125}I$ or $Na^{131}I$ (Fig. 1). Three different lot numbers of the gel tested were found to behave identically with respect to these ions (Table II).

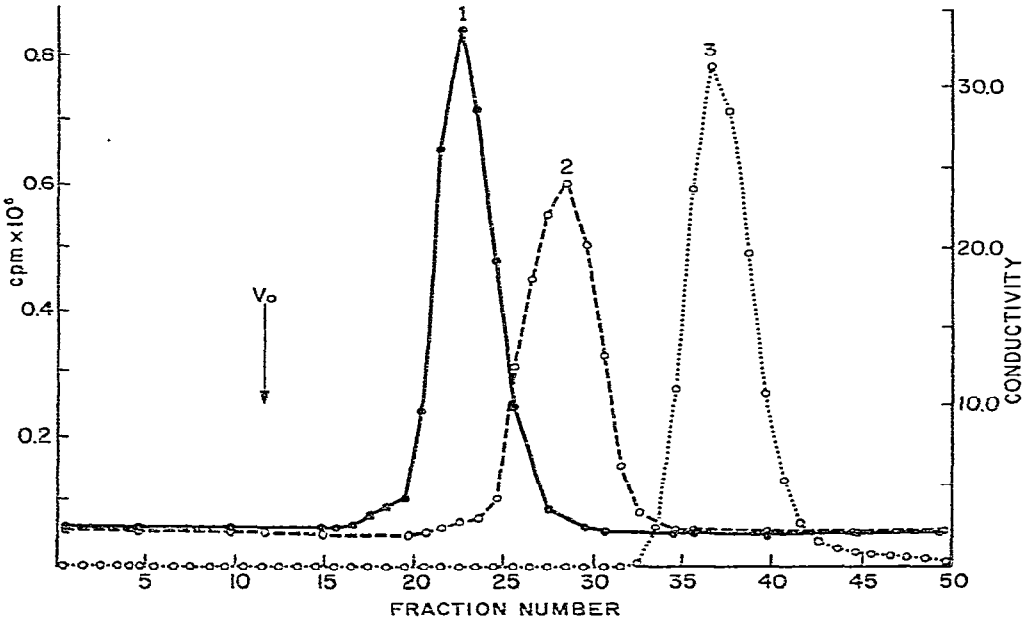

Fig. 1. Elution of NaCl (1), NaI (2) and $Na^{125}I$ (3) from Sephadex G-15 (Lot No. 268). Eluent, buffer A; temperature, 2°; fraction volume, 1.2 ml; flow-rate, 42 ml/h; column, 227 × 15 mm (V_b = 38.8 ml).

Changes in the composition of the eluent buffer had relatively little effect on the elution of the non-radioactive salts tested. The elution maximum of radioiodide on the contrary was found to depend on the eluent. In distilled water radioiodide was found eluted in the void volume of the column, while acetate-containing buffers tended to accentuate the retardation observed in Tris–HCl (Table II). The use of aqueous

TABLE II

K_{av} VALUES FOR GEL FILTRATION OF SALTS ON SEPHADEX G-15

Buffer	*Lot No.*	V_b *(ml)*	*NaCl*	*NaI*	$Na^{125}I$*	$Na^{125}I$**	*KI*	$NaIO_3$	*HCl*	$CaCl_2$	$Na^{131}I$*
A	268	38.8	0.548 ± 0.012◊	0.853 ± 0.033◊	1.250 ± 0.021◊	1.24	0.96	0.54	—	0.59	—
A	2014	38.6	0.60	0.99	1.43	—	1.07	0.55	0.66	—	—
A	5490	39.4	0.59	0.93	1.33	1.32	1.03	0.55	0.59	—	1.32
B	268	38.8	—	1.24	1.65	—	—	—	—	—	—
B	5490	38.7	0.63	1.25	1.63	—	1.01	—	0.73	—	1.61
C***	5490	40.6	0.71	0.98	1.09	—	1.04	0.73	0.73	—	—
D	268	38.8	0.54	0.90	1.91	—	—	—	—	—	—
D	2014	40.6	0.62	1.09	1.92	—	0.93	0.57	0.62	—	—
D	5490	40.0	0.61	0.96	3.65	—	1.11	—	0.61	—	—
E	268	38.8	0.48	0.85	1.65	—	—	—	—	—	—
H	2014	39.6	0.55	0.83	0.06	—	1.13	0.49	0.56	—	—
H***	5490	39.9	0.52	0.86	0.08	—	0.85	—	0.61	—	—
I***	2014	40.9	0.66	0.92	0.18	—	—	—	—	—	—
J	268	47.0	0.48	0.85	1.72	—	—	—	—	—	—
K	2014	39.9	0.61	1.22	2.23	—	—	—	—	—	—

* From New England Nuclear.

** From Amersham/Searle.

*** Experiments at room temperature; all others at 2°.

◊ Standard error of the mean (n = 8).

ethanol as the eluent has been reported to eliminate certain types of adsorptions on Sephadex[15]. In our experience, the use of buffer G (Table I) increased the retardation of iodides: K_{av} of NaI was 2.34 and that of $Na^{125}I$ was 12.86. Moreover, the recovery of radioiodide from such a column was only 10–20%.

Two sets of conditions were found to change the elution volume of radioiodide to that of NaI from Sephadex G-15: (1) elution with 0.1 *M* NaOH, and (2) addition of carrier iodide to the loading solution. The latter procedure showed that the elution volume of the radioiodide peak was inversely proportional to the concentration of carrier iodide used (Table III; Fig. 2). Addition of KI to the loading sample had the same effect as that of NaI, while the addition of $NaIO_3$ had no effect. Addition of carrier NaI to the loading sample of $Na^{125}I$ when distilled water was the eluent also resulted in the migration of the radioactive peak from the V_0 region to V_e of NaI. This migration was found to be independent of the order of mixing of the two isotopes.

TABLE III

CARRIER-DEPENDENT ELUTION OF RADIOACTIVE PEAK

Conditions: Sephadex G-15 (lot No. 268); eluent buffer, A; V_b = 38.8 ml; temperature, 2°.

Composition of loading sample (400 µl)		K_{av}
$Na^{125}I$ (µCi)	*NaI (mg)*	
0	235–390	0.853 ± 0.033*
2	390.0	0.813
2	230.0	0.820
2	34.0	0.867
2	10.0	0.961
4	10.0	0.961
40	10.0	0.961
2	5.0	1.008
40	5.0	0.984
2	2.5	1.055
2	1.5	1.102
2	0.25	1.172
2–100	0	1.250 ± 0.021*

* Standard error of the mean (n = 8).

Chromatography on Sephadex G-10 and G-25

Sephadex G-25 was found not to discriminate appreciably between the ions tested (Table IV). However, the retardation of iodides, and radioiodide in particular, was even more pronounced on the Sephadex G-10 than on Sephadex G-15 (Table IV).

To determine whether our separation systems were applicable to actual iodination reaction mixtures, several iodination control experiments were performed, using $Na^{125}I$ purchased from both commercial sources as outlined in the methods. We were unable to demonstrate any difference in the elution from Sephadex G-15 and G-10 of the $Na^{125}I$ subjected to the iodination control experiments and that of $Na^{125}I$ itself. Similarly, elution of $Na^{131}I$ was found to correspond to that of $Na^{125}I$ (Table II).

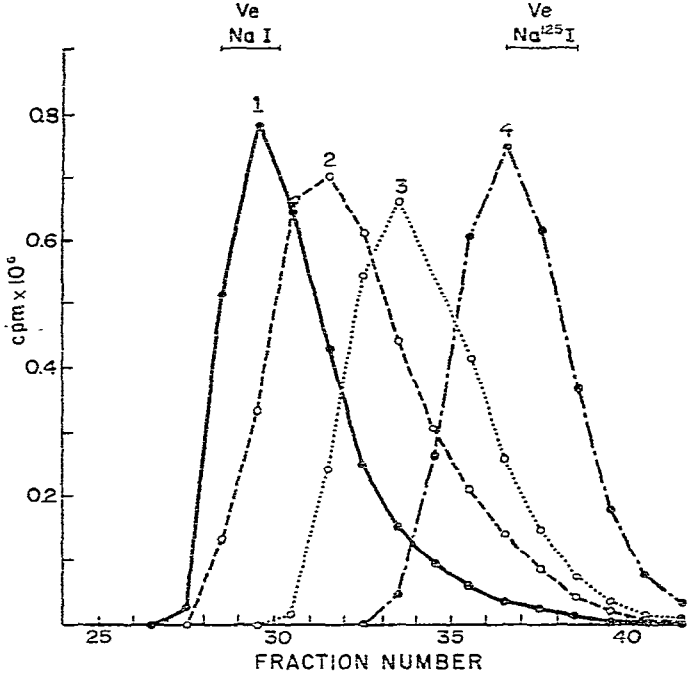

Fig. 2. Carrier-dependent elution of $Na^{125}I$ on Sephadex G-15 (Lot No. 268). Eluent, buffer A; temperature, 2°; fraction volume, 1.2 ml; flow-rate, 45 ml/h; column, 227 × 15 mm (V_b = 38.8 ml). Loading samples, 2 μCi $Na^{125}I$ + x mg NaI: (1), x = 230 mg; (2), x = 10 mg; (3), x = 2.5 mg; (4), x = 0.25 mg.

TABLE IV

K_{av} VALUES FOR GEL FILTRATION OF SALTS ON SEPHADEX G-10 AND G-25

V_0 for Sephadex G-10 was measured with cytochrome *c*, in buffer A and D; with myoglobin in buffer F. Temperature, 2° (unless otherwise indicated).

Column	*Buffer*	*Lot No.*	V_b *(ml)*	K_{av}				
				NaCl	*NaI*	*$Na^{125}I$*	*KI*	*HCl*
Sephadex G-10	A	1032	39.7	0.46	1.12	1.75	1.37	0.55
	D	1032	39.4	0.52	1.14	3.96	1.18	—
	F	1032	39.4	0.44	1.08	1.38	—	—
Sephadex G-25	A	6453	39.9	0.70	0.86	0.94	0.88	0.66
	A*	9833	39.9	0.77	0.94	0.97	—	—
	D	6453	39.9	0.75	1.01	1.04	1.04	—

* At room temperature.

DISCUSSION

It can be seen from our results on the elution volumes of NaCl and HCl, and from the corresponding volumes for iodides that, according to the definition of Eaker and Porath[16], iodides did not participate in the process of gel filtration, but were subject to a secondary type of interaction.

Secondary interactions of substances with the Sephadex gel have been ascribed to adsorption[5,6,17,18] and to ion-exchange effects[16,17]. Solvent systems designed to minimize some adsorption effects have been reported[15,19]. In our experience, one of these systems[15] enhanced the adsorption of NaI and of $Na^{125}I$. The degree of cross-linking of Sephadex has been reported to influence retardation of iodide[4] and our studies confirm this observation.

The elution volume of chlorides from Sephadex G-10 in water was reported to increase proportionally with the concentration of the loading sample: at low sample concentrations the elution maximum approached the void volume[20]. The concentration of the radioactive sample in our experiments with water as the eluent was < 2.5 nM of iodide, and radioiodide eluted in the void volume. A small number of fixed anionic exchange groups on the Sephadex backbone are not neutralized in water and may contribute to an ion-exclusion effect[21,22]. Some reports indicate that carrier-free radioiodide solutions may have a higher proportion of non-iodide contaminants[23–26]. It was reported that the unidentified radioactive material can be minimized by reducing substances, NaOH, and iodide concentrations in excess of 1 μM (ref. 25).

Our experiments do not exclude the possibility that the differences in the elution between the two isotopes simply reflect the difference in the sensitivities of the detection techniques (conductivity, iodine vapor on one hand, and gamma radiation on the other). In such a case the difference between the two elution maxima may reflect the upper and the lower range of iodide adsorption.

Regardless of the underlying causes for the differences in the elution of radioiodide on one hand, and NaI and KI on the other, the phenomenon can find practical application in purification of low-molecular-weight radioiodinated compounds from unreacted radioiodide. Our experiments demonstrate that the concentration of carrier will affect the elution volume of radioiodide, and therefore the columns can be standardized with that in mind. The iodination procedure itself may govern the choice of purification, since carrier iodide is added at the end of iodination reaction in high concentration by some workers[12], in lower concentration by others[7,27], and not at all by some[13]. Since it has been shown in this report that iodide retardation takes place in acid as well as in slightly basic eluent buffers, elution conditions can be chosen that are suitable to the preservation of the integrity of iodinated molecules.

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