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THE BINDING OF SODIUM AND POTASSIUM IONS BY HEPARIN

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SUMMARY

1. The effect of heparin on the relative activities of sodium and potassium ions has been calculated from the distribution of small ions between a solution of heparin and its ultrafiltrate.

2. It was found that the activities of sodium and potassium ions are markedly decreased by heparin, and that the potassium ion is bound by heparin in preference to the sodium ion.

3. It is suggested that the excess of sulfate groups over carboxyl groups in the heparin is responsible for this selectivity of heparin for alkali-metal ions.

4. It is proposed that heparin-like cation exchangers participate in the selective distribution of sodium and potassium ions in the living cell in connection with certain metabolic non-equilibrium processes.

INTRODUCTION

Alkali metal ions do not readily form stable compounds with other ions or molecules. However, intensive studies with polyelectrolytes, such as polyacrylate, have shown that the thermodynamic activities of alkali metal ions can be lowered by binding these cations electrostatically, owing to the high density of charge of the closely spaced anionic groups along the chain of the polyion[1–3].

Heparin is a natural polymer of D-glucuronic acid and D-glucosamine, apparently containing five sulphate groups and two carboxyl groups per tetrasaccharide unit[4]. In accordance with the high density of negative charge of this structure of heparin, it was found in our laboratory[5] that heparin lowers the activity coefficients of alkali metal ions, and some experiments suggested a greater affinity of heparin for K^+ than for Na^+. Independently, ASCOLI *et al.*[6] found that heparin binds Na^+. Some other anionic polysaccharides, such as arabic acid[7] and chondroitin sulphate[8], have also been found to bind alkali metal ions. No marked differences in behaviour were observed between Na^+ and K^+ in these experiments.

In relation to the different behaviour of Na^+ and K^+ in biological systems, the selective interactions of these cations with natural macromolecules are of interest. With the aid of the ultrafiltration technique, we have studied the states of Na^+ and K^+ in a heparin solution, and special attention has been paid to the selective affinities of heparin for Na^+ and K^+.

METHODS

Experimental methods

Dilute aqueous solutions of heparin were prepared from a concentrated solution of a sodium salt of heparin ("Heparin", supplied by Orion OY, Helsinki). The original heparin solution contained no K^+, and therefore a suitable amount of KCl was added to the solutions. The pH was adjusted with HCl or NaOH and KOH.

The ultrafiltrate was obtained by passing the heparin solution through a dialyzing membrane under hydrostatic pressure as described previously[5]. The experiments were performed in duplicate or triplicate. The membrane used was a cellulose dialyzing membrane (No. 4465-A2, Arthur H. Thomas, Philadelphia). By filtration of aqueous solutions of NaCl and KCl it was shown that this membrane was permeable to the ions of these salts. As tested with toluidine blue the ultrafiltrate contained no heparin, indicating that the membrane was impermeable to heparin.

The concentrations of Na^+ and K^+ in the heparin solutions and their ultrafiltrates were determined with a flame photometer; that of Cl^- was determined titrimetrically with $AgNO_3$.

Attempts were made to measure the Donnan membrane potential between the heparin solution and its ultrafiltrate. The potential difference between two calomel electrodes was measured through saturated KCl bridges in the heparin solution and ultrafiltrate with a compensation potentiometer. This potential difference was found to be 30–40 mV, the heparin solution being negatively-charged in relation to its ultrafiltrate. These values are of the same order as the liquid-junction potentials between a heparin solution and its diffusate fluid without the intervention of a semipermeable membrane[9]. In fact, the semipermeable membrane is not essential for determining these physicochemical properties of a polyelectrolyte solution[10].

The pH of the solutions was measured with a glass electrode, a calomel electrode being used as reference. The difference in pH between the heparin solution and its ultrafiltrate was 0.3–0.5 of a pH unit, the ultrafiltrate having a higher pH value than the heparin solution. However, it should be noted that these measurements of the pH as well as those of the Donnan membrane potential suffer from the uncertainty of the liquid-junction potential of the salt bridge.

Calculations of the ability of heparin to bind sodium and potassium ions

The activity coefficients of Na^+ and K^+ in the heparin solution in relation to those of Na^+ and K^+ in the corresponding ultrafiltrates were calculated by means of Donnan's theory. We have assumed in these calculations, in accordance with previous investigators[11–12], that the activity coefficient of the small ion having the same sign of charge as the polyion is not noticeably affected by the latter. Thus the ratio of the activities of Cl^- in the heparin solution and in its ultrafiltrate is considered to be equal to the ratio of the concentrations of Cl^- in these fluids.

$(Na)_h$ is the sodium ion concentration on the heparin side, and γ_{Na_h} its activity coefficient. $(Na)_{uf}$ is the sodium ion concentration on the ultrafiltrate side, and $\gamma_{Na_{uf}}$ its activity coefficient. Analogous expressions are used for K^+ and Cl^-. Then, according to Donnan's theory:

$$\frac{\gamma_{Na_{uf}} \cdot (Na)_{uf}}{\gamma_{Na_h} \cdot (Na)_h} = \frac{\gamma_{K_{uf}} \cdot (K)_{uf}}{\gamma_{K_h} \cdot (K)_h} = \frac{(Cl)_h}{(Cl)_{uf}} .$$

The ratios of the activity coefficients in the heparin solution and the ultrafiltrate are then for Na^+ and K^+, respectively:

$$\frac{\gamma Na_h}{\gamma Na_{uf}} = \frac{(Cl)_{uf} \cdot (Na)_{uf}}{(Cl)_h \cdot (Na)_h}$$

$$\frac{\gamma K_h}{\gamma K_{uf}} = \frac{(Cl)_{uf} \cdot (K)_{uf}}{(Cl)_h \cdot (K)_h}$$

The concentrations of Na^+ and K^+ bound by heparin are calculated as follows:

$$\text{bound } Na^+ = (Na)_h - \frac{\gamma Na_h}{\gamma Na_{uf}} \cdot (Na)_h$$

$$\text{bound } K^+ = (K)_h - \frac{\gamma K_h}{\gamma K_{uf}} \cdot (K)_h$$

The selectivity coefficient of heparin for Na^+ and K^+, $K\frac{K}{Na}$, is calculated as follows:

$$K\frac{K}{Na} = \frac{(K)_h \cdot (Na)_{uf}}{(K)_{uf} \cdot (Na)_h}.$$

Thus all parameters for the calculation of the selectivity coefficient can be determined experimentally.

RESULTS

The results in Table I show that heparin has a marked effect on the activity of alkali metal ions. In Expt. 5, in which the concentration of alkali metal chloride in relation to that of heparin is low, the activity coefficients of Na^+ and K^+ in the heparin solution are 0.54 and 0.38, respectively, of those in the ultrafiltrate. When the concentration of NaCl (Expt. 6) or of KCl (Expt. 4) or of HCl (Expt. 3) in the heparin solution is increased, the activity coefficients of Na^+ and K^+ increase, suggesting competitive binding of Na^+, K^+, and H^+ with heparin. Dilution of the heparin solution with water has little effect on the relative binding of Na^+ and K^+ with heparin (Expts. 1 and 4).

At pH 12.0 the binding of Na^+ and K^+ increases, but the selectivity coefficient of heparin for K^+ in preference to Na^+ does not increase when compared with the

TABLE I

BINDING OF Na^+ AND K^+ BY HEPARIN

Expt. No.	Heparin (g/l)	pH_h	$(Cl)_h$	$(Cl)_{uf}$	$(Na)_h$	$(Na)_{uf}$	Bound Na	$\frac{\gamma Na_h}{\gamma Na_{uf}}$	$(K)_h$	$(K)_{uf}$	Bound K	$\frac{\gamma K_h}{\gamma K_{uf}}$	$K\frac{K}{Na}$
			mmoles/l						mmoles/l				
1	2.5	7.1	12.5	13.4	13.2	8.9	3.6	0.72	9.6	4.5	4.8	0.50	1.44
2	2.5	12.0	8.0	9.0	13.9	8.0	4.9	0.65	8.4	3.5	4.5	0.47	1.38
3	2.5	2.2	20.0	22.0	13.3	12.0	0.1	0.99	10.5	8.1	1.6	0.85	1.16
4	25.0	7.1	117.0	128.0	132.0	88.0	36.0	0.73	85.0	40.0	41.0	0.52	1.40
5	25.0	7.3	30.0	45.0	124.0	44.0	56.0	0.54	3.9	1.0	2.4	0.38	1.42
6	25.0	7.3	165.0	178.0	250.0	175.0	60.0	0.74	5.7	2.8	2.7	0.53	1.39

values obtained at neutral pH (Expts. 1 and 2). At pH 2.2 there is barely any binding of Na^+ with heparin, the binding of K^+ is also low and the selectivity coefficient is almost unity (Expt. 3).

DISCUSSION

The cation-binding properties of heparin found in the present work are similar to those previously found with other negatively-charged polyelectrolytes, in that the activity coefficients of small cations increase with increasing concentrations of simple electrolytes, such as NaCl, KCl, or HCl, in the polyelectrolyte solution, while dilution of the polyelectrolyte solution with water has little effect on the activity coefficients of the small cations. In these respects the polyelectrolyte solutions differ from simple electrolyte solutions[1,13].

Quantitatively, the Na^+-binding ability of heparin found here is in agreement with that found by ASCOLI *et al.* using quite a different method, *i.e.*, a charged collodion membrane electrode[6].

It was found in the present work that heparin binds K^+ in preference to Na^+. The studies of TEUNISSEN AND BUNGENBERG DE JONG[14] are of interest in regard to the mechanism of selectivity of macromolecules for small cations. These workers determined the concentration of a salt, such as NaCl or KCl, at which the charge of the polyelectrolyte is reversed. They found that the ability of a cation to change the charge of a polyelectrolyte depends on both the naked, and the hydrated, size of the cation and the polarizability of the negatively-charged groups of polyelectrolyte in relation to water. According to them, when the anionic groups of a polyion, such as PO_4^{3-} or COO^-, are more polarizable than water, the electrostatic immobilization of a cation is determined by its naked size, and hence small (naked) Na^+ is bound in preference to K^+. On the other hand, when the polarizability of the anionic group of a polyion, such as SO_4^{2-}, is less than water, the K^+, having a smaller hydrated diameter than Na^+, is bound preferentially. These hypotheses are supported by BREGMAN's studies with cation-exchange resins[15] The latter worker found, for example, that at pH 2.6 the phosphonic acid resin binds K^+ in preference to Na^+, the selectivity coefficient being 1.2. On the other hand, at pH 10, at which the second dissociation of the phosphonic acid increases, the selectivity was changed and the Na^+ was bound in preference to K^+.

It is in accordance with the above concept that heparin, containing sulphate groups in excess of carboxyl groups, binds K^+ in preference to Na^+. The greatest selectivity coefficient was about 1.4.

It is of interest that chondroitin sulphate, containing equal numbers of both sulphate and carboxyl groups, has a barely detectable preference for K^+ over Na^+ (see ref. 8).

Unfortunately, the selectivity coefficients of the natural cation-exchangers, including heparin, found so far in equilibrium experiments are not quantitatively great enough to explain the high selectivity of distribution of Na^+ and K^+ between the extracellular fluid and the protoplasm of the living cell. However, natural cation-exchangers may participate in the selective distribution of Na^+ and K^+ in connection with certain metabolic non-equilibrium processes, such as the flow of water[16] and of hydrogen ion[9], which have been found to produce a selective distribution of Na^+ and K^+ in model experiments.

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