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Protein Does Not Interfere with the Ion-Selective Electrode Determination of Calcium, Sodium or Potassium Ions

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Summary: The protein interference with the determination of ionised Ca^{2+}, Na^+ and K^+ reported in several studies could be due to effects on liquid/liquid junction potentials or on the ion-selective electrodes, but could also be due to *Donnan* effects during sample preparation, e.g. ultrafiltration or dialysis. The possible interference of protein was studied by subjecting a bovine serum albumin solution, 100 g/l, to gel-filtration in Sephadex G-25 columns equilibrated with 150 mmol/l NaCl, 5 mmol/l KCl, 20 mmol/l MOPS, pH 7.4 and varying concentrations of Ca^{2+}; 0.75 and 1.25 mmol/l. The albumin was dissolved in the basic electrolyte solution and pH adjusted before the gel-filtration. Samples were taken for measurements before, during and after the elution of the protein peak. In this way it was ensured that the activities were not changed by the presence of albumin. Also the temperature, the ionic strength of the electrolyte and the bridge solution were varied. Ca^{2+}, Na^+ and K^+ activities were measured with the aid of a Kone Microlyte instrument and Ca^{2+} in addition with the Radiometer ICA-1 instrument or a measuring system consisting of a Philips IS 561-Ca electrode, a Beckman KCl reference electrode connected to the sample chamber via a 2% agarose bridge containing either 0.15 or 2 mol/l KCl, a pH/voltmeter and a voltage-bucking device. No interference by protein was found.

Introduction

Concern has been expressed (1) that protein interferes with the measurement of calcium ion concentration (calcium activity) in serum when Ca-selective electrodes are used. The interference has been claimed to be large enough to compromise the clinical interpretation of data. In hypoproteinaemia, this positive interference may lead to spuriously low values for ionised calcium, and to an overestimation of ionised calcium at high protein concentrations (1, 2). Indeed, there are several reports of a positive correlation between serum protein or albumin concentration and ionised calcium in patients (3–5). In these studies samples were subjected to dialysis or ultrafiltration. *Freaney* et al. (6) on the other hand found the interference to be so small – 0.02 mmol/l per 10 g/l albumin – that it would rarely be significant.

The claimed interference can be accounted for by formation of *Donnan* equilibria in the distribution of ions across semipermeable membranes during ultrafiltration or dialysis (7–9). In a recent paper *Thode* et al. (9) found that after dialysis of solutions with varying albumin concentrations, the distribution not only of Ca^{2+}, but also of Na^+, K^+ and Cl^- between dialysate and the albumin-containing solution was in full agreement with the *Donnan* theory. However, *Payne & Jones* (10) noted that the interference was influenced by the composition of the liquid junction solution of the reference electrode and believed that protein might influence the liquid-liquid junction potential, possibly by forming precipitates when hypertonic liquid junction solutions were employed. On the other hand, *Buckley* et al. (11), used electrode assemblies with or without liquid-liquid junctions, in which

either a conventional reference electrode or a Na^+ or K^+-sensitive electrode was used as the reference electrode. They found the same Ca^{2+} activities with both kinds of reference electrode, though the analytical values varied with the albumin concentration. They concluded that it was unlikely that protein would affect liquid-liquid potentials.

In the studies quoted above it was difficult to prepare albumin-electrolyte solutions without perturbing the ion activities, since commercial albumin preparations contain some cations, and albumin will bind cations, particularly Ca^{2+} ions, present in the electrolyte solution. In the present study this difficulty has been circumvented by subjecting the albumin to gel-filtration in columns equilibrated with defined electrolyte solutions. During passage through the column the protein binds cations present in the electrolyte solution with which the Sephadex column has been equilibrated until binding equilibrium is reached; or, if excess Ca^{2+} has been added to the sample before application to the column, the excess is left behind during the passage of the protein through the column. If the albumin were to bind more Ca^{2+} during gel filtration, a solution with reduced Ca^{2+} content would be eluted some time after the albumin. If equilibrium has been reached, the cation activities of the eluate emerging before the protein peak would be the same as immediately after the peak. If protein causes an interference with the assays the sensed signals would be changed during the elution of the protein peak. In this way it was possible to ensure that the albumin or protein had reached equilibrium with the cations in the solution without changing their activities.

Materials and Methods

Bovine serum albumin fraction V, fatty acid free, was obtained from Boehringer Mannheim (Federal Republic of Germany) and dissolved to give a concentration of 100 g/l in solution 1, which contained 150 mmol/l NaCl, 5 mmol/l KCl, 1.25 mmol/l $CaCl_2$, 20 mmol/l 3-(N-morpholino)propanesulphonic acid (MOPS), pH 7.4 at room temperature. Solution 2 contained KCl and the buffer as in solution 1 but Ca^{2+} was lower (0.75 mmol/l) and the concentration of NaCl was 151 mmol/l.

Sephadex G-25 (Pharmacia, Uppsala, Sweden) columns, 2 cm diameter, 30 cm high, were equilibrated with solutions 1 and 2, respectively; 3.0 ml of albumin solution was applied, and the column was eluted with the respective solution. With solution 2 enough Ca^{2+} was added to the albumin prior to the run, and the pH was read-justed, to ensure that equilibrium between free and bound Ca^{2+} was reached during the gel-filtration. The eluate was passed through a Pharmacia Single Path Monitor UV-1 and the absorbance at 280 nm recorded. Based on the absorbance there was a slight dilution of albumin during gel filtration. The eluate was collected in 1.0 ml fractions. Ca^{2+}, Na^+ and K^+ activities were measured at room temperature (22–23 °C) with the aid of a Microlyte instrument (Kone Instrument Division, Espoo, Finland) or a Radiometer (Copenhagen, Denmark) ICA-1 instrument with a junction of 6 mol/l sodium formate and calibrated according to the instructions of the manufacturers. In addition, Ca^{2+} activity was meaured in a cell consisting of a Philips IS 561-Ca^{2+} electrode and a Beckman KCl reference electrode connected to the sample chamber *via* a 2% agarose bridge containing either 150 mmol/l or 2 mol/l KCl. The chamber contents were stirred with the aid of a magnetic stirrer and a circular follower. The output from the electrode was relayed from a pH/voltmeter, IM-555 (Instrumentarium, Helsinki, Finland) to a strip chart recorder *via* a voltage-bucking device providing additional sensitivity and zero-point adjustments. For these experiments the system was calibrated with the solutions 1 and 2 and a 1:1 mixture of them.

Results and Discussion

The experimental design allowed continuous monitoring of the protein concentration of the eluate from the gel-filtration column while cation activities were assayed from aliquots of the eluate. The volume of protein solution applied to the column was large enough to give on elution a protein peak with a plateau (see figures), where the concentration of protein was 61.7–67.9 g/l (mean 64.2, n = 4) based on the absorbance at 280 nm.

Figure 1 shows that the Ca^{2+} activities sensed both by the Kone Microlyte and by the cell assembled in the laboratory with a Philips electrode as sensor did not change noticeably when the albumin peak appeared, irrespective of whether the free Ca^{2+} concentration was 1.25 mmol/l (fig. 1 a), or 0.75 mmol/l (fig. 1 b). It is also seen that binding of Ca^{2+} to albumin had reached equilibrium, since the plateau before the protein peak was the same as after the peak. In figure 1 b it is also shown that it did not make any difference whether the agarose bridge to the reference electrode contained isotonic (150 mmol/l) KCl (open squares), or hypertonic KCl solution. The Microlyte instrument employs a hypertonic junction solution as does every ion-selective electrode instrument. The higher values for Ca^{2+} reported by the Microlyte instrument are due to the composition of the calibration solution (12).

Figure 2 shows the corresponding data for measurements of Na^+ and K^+ activities with the Microlyte instrument. A protein effect on the liquid-liquid junction potential should influence these measurements as well, but the presence of albumin did not have any effect on the results.

It could be argued that the protein interference is not due to albumin (which, as a highly negatively-charged protein, has been regarded as the most important component), but to some other proteins in serum that may be precipitated at the liquid-liquid junction. However, no interference could be seen in the measurement of calcium activity when a human serum

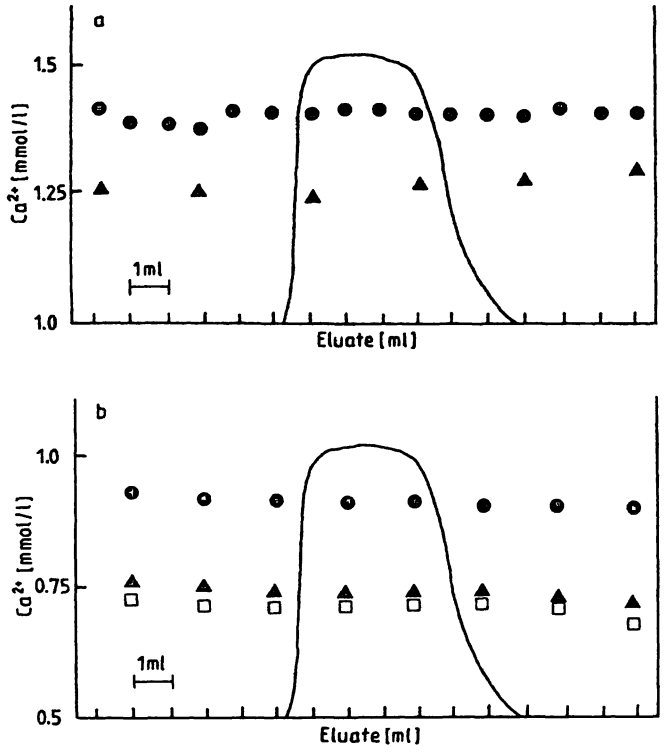

Fig. 1. Calcium activities in the presence and absence of albumin.
a) at higher Ca^{2+} concentration; solution 1,
b) at lower Ca^{2+} concentration, solution 2.
For details see Materials and Methods.
Continuous trace, protein peak in gel-filtration; the plateau represents 100 g/l;
Filled circles, mmol/l Ca recorded by the Microlyte instrument;
Triangles, Ca recorded by the Ca-electrode assembly using 150 mmol/l KCl junction solution;
Squares, Ca recorded by the Ca-electrode assembly using 2 mol/l KCl junction solution.

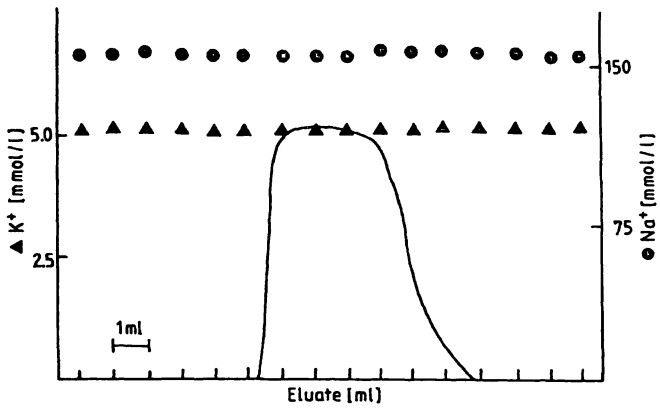

Fig. 2. Na^+ and K^+ concentrations in the presence and absence of albumin. Gel filtration was carried out in the presence of solution 1. Continuous trace, protein peak;
Filled circles, mmol/l Na^+ recorded by the Microlyte instrument;
Triangles, recorded K^+.

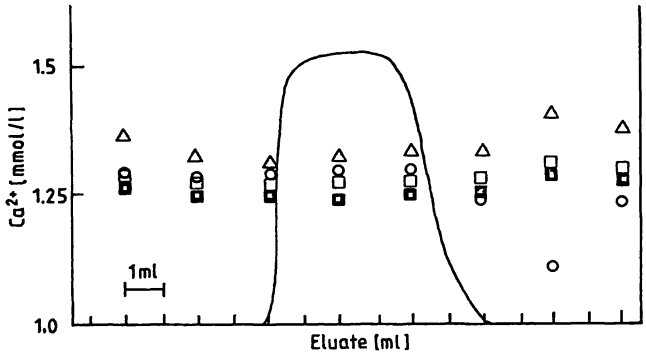

Fig. 3. The effect of temperature and ionic strength on ionised Ca^{2+} in the presence of albumin.
Triangles, Ca^{2+} recorded by the Radiometer ICA-1 instruments;
Open squares, Ca^{2+} recorded by the Ca-electrode assembly at room temperature with 2 mol/l KCl bridge solution;
Filled squares, the same at 37 °C;
Circles, experimental conditions as for open squares but only 20 mmol/l NaCl was present and the electrode was calibrated with Ca^{2+} added to this solution.

sample was used instead of bovine albumin (not shown). It could also be argued that the interference is not seen at room temperature but at 37 °C where the tendency of protein to form precipitates would be enhanced. Figure 3 shows that no interference was seen even at 37 °C using either our own assembly (filled squares) or the Radiometer instrument (triangles), which is operated at 37 °C.

The low peak after the albumin peak is due to excess Ca^{2+} added to the samples before the gel filtration. It is concluded that protein does not appreciably affect results with measuring systems employing open, flowing, liquid junctions. A possible effect on the liquid-liquid junction potentials may be compensated by opposite effects on the level of the ion-selective electrode, or by a decrease in Cl^- activities in protein-containing fractions, which may change liquid-liquid potentials. Since different cation-selective electrodes were used, a compensating effect of proteins on these seems highly unlikely. A Cl^- effect on the liquid junction was not studied directly. A significant effect of the Cl^- ion should be detectable by varying the concentration of NaCl in the electrolyte solution. Figure 3 shows that changing the concentration of NaCl from 150 (open squares) to 20 mmol/l (circles) failed to reveal any interference. Ca^{2+}-binding by albumin was increased in the low ionic-strength solution, as could be expected, since the Ca^{2+} added was in excess in the presence of 150 mmol/l NaCl, while additional Ca^{2+} was bound by the albumin during gel-filtration in the presence of 20 mmol/l NaCl, resulting in lowered Ca^{2+} values after the protein peak.

It is concluded that protein *per se* does not cause interference with measuring systems employing open liquid junctions. It appears likely that the interference reported may be due to perturbation of cation equilibria on addition of protein or to *Donnan* effects during sample preparation or when semipermeable membranes are part of the measuring system. In the experimental design used, the ion activities of the bulk solution should have remained intact even in the presence of albumin. The negative charges of the macromolecules should have been largely masked by the presence of countercations already in equilibrium with the surrounding electrolyte solution. It should be borne in mind, however, that there might be mechanisms *in vivo* by which changes in protein contents of body fluids may change their calcium activities, e. g. during venous stasis (13) and changes in posture (14).

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