A DIELECTRIC STUDY OF TWO ACID POLYELECTROLYTES, PHOSVITIN AND A POLYMETAPHOSPHATE

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SUMMARY

The phosphoprotein phosvitin (as partial sodium salt) and a polymetaphosphate (potassium salt) were studied dielectrically in aqueous solution at low concentrations. Comparatively high dielectric increments were obtained. For phosvitin $\Delta\varepsilon/g$ was of the order of 10 at the lowest concentration studied (0.24 g/l). The increments for both substances showed a non-linear concentration dependence. $\Delta\varepsilon/g$ increases markedly with decreasing concentration. The phosvitin solutions showed dielectric dispersions in the frequency range used. The critical wavelength is concentration-dependent, being displaced toward longer wavelengths with decreasing concentration.

The dielectric properties correspond to those of other polyelectrolytes studied.

INTRODUCTION

Dielectric studies[1–5] of a number of linear polyelectrolytes in aqueous systems have revealed high dielectric increments for such substances in comparison with, for example, less asymmetric proteins. This difference may be ascribed partly to differences in shape and thus in "surface charge" but seems to be explained mainly by different nature and *number* of the charged groups. It was then thought of interest to include in the basic dielectric studies of polyelectrolytes a complex protein with a high content of orthophosphate groups, such as phosvitin, although this protein is probably not homogeneous. This would allow a comparison with the results obtained with polynucleotides and nucleoproteins. Phosvitin has a phosphorus content of about 10%, probably mainly as serine phosphate[6] (threonine phosphate and amide-phosphate linkages may also occur[7]). The minimum molecular weight has been determined in sedimentation studies to be 12,000 and from osmotic pressure measurements 21,000[6]. Nucleoproteins[8] give dielectric increments of the same order as for linear polyelectrolytes but in that case the dielectric properties seem to be mainly explained by the nucleic acid moiety. A phosphoprotein such as ovalbumin gives dielectric increments of the same order as for simple proteins[9]. In that case the phosphorus content, *ca.* 0.1%, is probably too low to influence the charge and the dielectric properties. A further group of biologically interesting polymers with high phosphorus content, namely the polymetaphosphates, does not seem to have been studied dielectrically.

Some dielectric results with a synthetic potassium polymetaphosphate are included here for comparison.

EXPERIMENTAL

Phosvitin was prepared from hens' eggs according to MECHAM AND OLCOTT[6,10]. It was dialysed to distilled water and lyophilized. The moisture content was 11.9% (determined by drying over night at 80°, 0.05 mm Hg over phosphorus pentoxide). The phosphorus content of the dry protein was 9.43% (determined according to a modification of Fiske and Subbarow's method), and the nitrogen content 14.0% (modified titrimetric Conway technique after combustion with sulfuric acid–hydrogen peroxide). The sodium content of the dry protein was 4.45%, potassium content $\leqq$ 0.03% (flame photometry with internal lithium standard). Spectrographic analysis (Dr. S. LANDERGREN, Geological Survey of Sweden) showed 0.1–1% iron, 0.1–1% magnesium and 0.01–0.1% calcium.

The phosphoric acid groups were thus mainly in the first ionization step, and the pH of the dilute solutions studied dielectrically was about 6.2.

Paper electrophoresis was carried out in the apparatus described by KÖIW *et al.*[11] using Whatman No. 1 filter paper and pH 8.6 barbital buffer (ionic strength 0.1). The position of the phosvitin fraction was obtained by cutting the papers in 1 cm wide strips and analysing for nitrogen (nesslerization) and phosphorus (as given above) after combustion with sulfuric acid–perchloric acid. Phosvitin may also be detected by spraying with ferric chloride solution followed by sulfosalicylic acid solution[12], or by using the bromophenol blue staining, which gives much weaker spots, however. No staining was obtained with naphthalene blue-black (Amidoschwarz).

At pH 8.6 phosvitin moved about 8 cm towards the anode in 8 h and about 18 cm in 16 h (150 V, 5–6 mA). Only one fraction was obtained and the position of the nitrogen and phosphorus peaks coincided.

Synthetic potassium polymetaphosphate was prepared according to MALMGREN[13] by dialysing commercial sodium "hexametaphosphate" in potassium chloride solution in the cold, followed by dialysis with distilled water. MALMGREN has determined the molecular weight of such preparations to about 8,000. The dialysed solutions were used directly for dielectric measurements. Concentrations were calculated from the phosphorus content analysed after acid hydrolysis. The pH of the solutions studied dielectrically was about 5.5. The molar ratio of the content of sodium, potassium and phosphorus in the solutions studied was Na:K:P = 1:25.6:29.4 (flame photometric determination of sodium and potassium).

The dielectric measurements were carried out with a modification of the "ellipsoid method" as described previously[1,8]. All measurements were performed at 20°. As the solutions of phosvitin did not show any marked "time effects" (*cf.*[1,8]) the preparation of the solutions was not critical, but in general they were investigated in the time interval 2–8 h after dissolution.

RESULTS AND DISCUSSION

The static dielectric constant, ε_1, of phosvitin is shown in Fig. 1 as a function of concentration. There is a non-linear relationship as has also been obtained with other polyelectrolytes studied[1–5,8]. In contrast to this finding, simple proteins show a linear relationship[9].

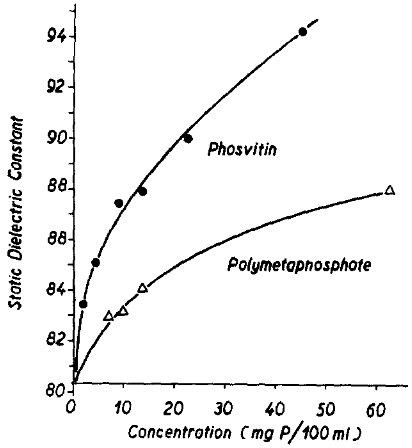

Fig. 1. Static dielectric constant of phosvitin and polymetaphosphate in aqueous solution as a function of concentration.

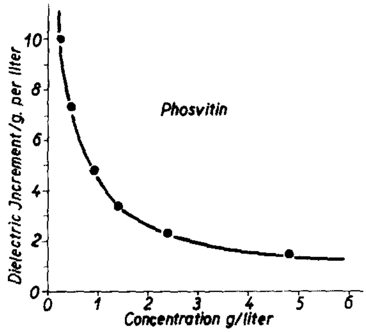

Fig. 2. Dielectric increment/g/l as a function of concentration of phosvitin in aqueous solution.

TABLE I

DIELECTRIC INCREMENTS AND CRITICAL WAVELENGTHS OF PHOSVITIN IN AQUEOUS SOLUTION AT VARIOUS CONCENTRATIONS

Concentration		ε_l**	ε_h**	*Increment* $(\varepsilon_l - \varepsilon_h)$	*Reduced increment*	*Critical wavelength (meters)***
*g/l**	*mg P/100 ml*				$\frac{\varepsilon_l - \varepsilon_h}{g/l}$	
4.8	45	94.2	(87.0)	(7.2)	(1.5)	(16)
2.4	23	90.0	(84.5)	(5.5)	(2.3)	(25)
1.4	14	87.9	83.1	4.8	3.4	32
0.95	9.0	87.4	82.8	4.6	4.8	35
0.48	4.5	85.1	81.6	3.5	7.3	50
0.24	2.3	83.4	80.9	2.5	10	63
0.48 in $5 \cdot 10^{-4}$ *N* NaCl	4.5	84.5	82.3	2.2	4.6	48

Values in parentheses are uncertain owing to high electric conductivity of solutions and incomplete accessibility of lower dispersion region.

* Calculated from phosphorus analysis of solution and "moisture-free" protein.

** Values obtained graphically from dispersion curve.

The reduced increment/g/l, $\Delta\varepsilon/g$, in accordance with this finding increases sharply with decreasing concentration (Fig. 2). At the lowest concentrations studied it is about 10 (Table I). This is thus considerably higher than with simple proteins, for which $\Delta\varepsilon/g$ does not exceed 2. With polymetaphosphate a similar ε_l *vs.* concentration curve is obtained as with phosvitin (Fig. 1). $\Delta\varepsilon/g$ is about 8 at the lowest concentrations studied.

The solutions of phosvitin and of polymetaphosphate showed comparatively high electric conductivity at the highest concentrations studied, evident in the form of disturbing heat convections during measurements. The high frequency dielectric constant, ε_h, of phosvitin solutions (Table I, Fig. 3) is considerably higher than the dielectric constant of the solvent ($\varepsilon_{\text{water}} = 80.3$) and increases with concentration. This is probably an artefact due to the high conductivity of the solutions. To eliminate this error the dielectric increment, $\Delta\varepsilon$, is calculated as ($\varepsilon_l - \varepsilon_h$).

The sharp rise of the reduced dielectric increment, $\Delta\varepsilon/g$, with decreasing concentration (Fig. 2) may be tentatively interpreted as an increase of the net charge of the polyions due to a decreasing polyion-counter ion association[4]. An increase in counter ion concentration would then give a screening effect (increasing polyion–counter ion association) with a decrease of $\Delta\varepsilon/g$. This is in fact obtained when the dielectric increment of phosvitin is measured in dilute sodium chloride solution (Table I). These dielectric properties seem to be characteristic of polyelectrolytes; they have been obtained with sodium carboxymethylcellulose[3], dextran ester sulfate[2], alginate[4], and others. With other physico-chemical properties of polyelectrolytes such as equivalent conductance, reduced osmotic pressure, and reduced viscosity, similar findings have been obtained previously[14–16]. The interpretation given above has been derived from the results of such studies.

Dielectric dispersions were obtained within the available frequency range with phosvitin solutions at low concentration. At high concentrations only part of the dispersion was available with the present method (Fig. 3). The dispersions largely proceed according to the relation

$$\varepsilon_\lambda = \varepsilon_h + \frac{\varepsilon_l - \varepsilon_h}{1 + \frac{\lambda_c^2}{\lambda^2}} \qquad (\text{DEBYE}^{17})$$

where ε_λ is the dielectric constant at the wavelength λ and λ_c is the critical wavelength.

With solutions of polymetaphosphate no dispersions were obtained, although there was a tendency for the dielectric constant to decrease at the shortest wavelengths. No estimate of the critical wavelengths could be made but they must be less than 10 meters.

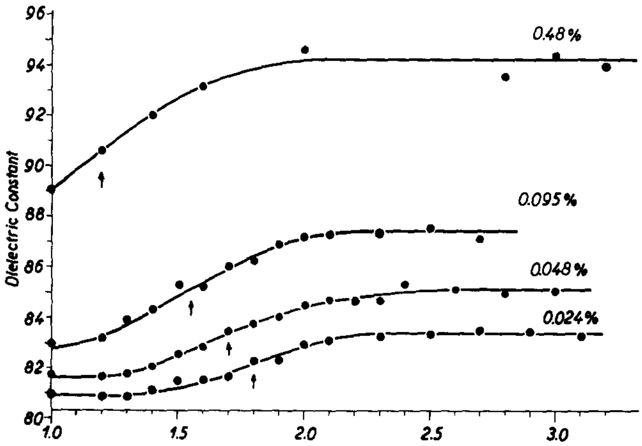

Fig 3. Dielectric dispersion curves for phosvitin in aqueous solution at various concentrations. The arrows indicate the critical wavelengths. Fulldrawn curves are theoretical curves according to the Debye relation, assuming a single relaxation time in each case.

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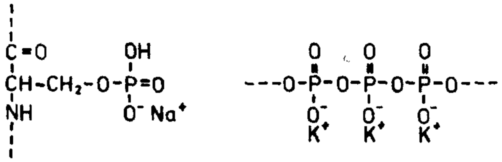
The critical wavelength of phosvitin solutions was displaced towards longer wavelengths with decreasing concentration (Fig. 3, Table I). This is contrary to the case with simple proteins but has been obtained with other polyelectrolytes studied[1-5,8]. An orientation polarization according to Debye cannot explain this effect, but it may be explained by a Maxwell-Wagner effect or an ion relaxation effect according to Debye-Falkenhagen (*cf.* [5]). In conclusion it may be stated that phosvitin (and polymetaphosphate) show dielectric properties which correspond to those obtained with other polyelectrolytes rather than those of simple proteins. This seems to be a difference in degree rather than in quality and must be ascribed to the high content of ionizable, organically linked phosphate groups in phosvitin (Fig. 4).



$$\underset{\underset{\vdots}{NH}}{\overset{\overset{\vdots}{C{=}O}}{CH}}{-}CH_2{-}O{-}\underset{O^-\,Na^+}{\overset{OH}{P}}{=}O \qquad ---O{-}\underset{\underset{K^+}{O^-}}{\overset{O}{\overset{\|}{P}}}{-}O{-}\underset{\underset{K^+}{O^-}}{\overset{O}{\overset{\|}{P}}}{-}O{-}\underset{\underset{K^+}{O^-}}{\overset{O}{\overset{\|}{P}}}{-}O---$$

Fig. 4. Formulae of serine phosphate in polypeptide chain (left) and of polymetaphosphate (right).

MECHAM AND OLCOTT[6] suggested that the function of phosvitin in egg yolk may be that of a polyvalent metal storer. It is then interesting to note that a selective uptake of cations corresponding to that of an ion-exchange resin has been shown for some polyelectrolytes in solution[3,18], and that there is an unexpectedly high content of iron and magnesium in our phosvitin specimen in spite of the previous treatment with strong ammonium sulphate and sodium chloride solutions.

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