ELSEVIER

Journal of Chromatography B, 753 (2001) 67–77

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Specific adsorption of phosphate ions on proteins evidenced by capillary electrophoresis and reversed-phase high-performance liquid chromatography

Murielle Rabiller-Baudry*, Bernard Chaufer

Laboratoire des Procédés de Séparation, UC 991 INRA-Université Rennes 1, Campus de Beaulieu, Batiment 10A, 263 Avenue du Général Leclerc, 35042 Rennes Cedex, France

Abstract

Specific adsorption of phosphate ions at pH=7.0 was studied on different proteins, either counter-ions of phosphate (lysozyme, lactoferrin) or co-ion of phosphate (α-lactalbumin). The theoretical electrophoretic mobility of globular proteins lysozyme and α-lactalbumin (apo and holo (+1 calcium per molecule) forms) was compared with those measured by capillary electrophoresis in phosphate at pH 7.0, versus the ionic strength (I) in the range 0–0.775 mol L^{-1}. The specific adsorption of phosphate ions was evidenced by difference. From the experimental charge number (Z^{eff}) of protein in phosphate medium, a phosphate content per protein molecule was determined at pH=7.0.

- For lactoferrin (pI=8–9), the electrophoretic mobility (μ) was constant and negative, highlighting a charge reversal due to phosphate adsorption.
- For α-lactalbumin (holo form) experimental μ was roughly constant and more negative than predicted. Z^{eff} increased continuously from −4 to −11 in the ionic strength range from 0.005 to 0.775 mol l^{-1}, respectively. Accordingly, one to six phosphates were bound per molecule, respectively.
- For lysozyme, experimental electrophoretic mobility was positive but lower than predicted. Z^{eff} was only discrete values +5 for I in the range 0.001–0.020 mol l^{-1} and about +3 in the range 0.050–0.500 mol l^{-1}, whereas the theoretical Z value was +7 at pH=7.0. Lysozyme bounds one phosphate at low ionic strength and about two — three at higher ionic strength.

Reversed-phase HPLC confirms that adsorption of phosphate is different for the three proteins.

Keywords: Phosphate ions; Proteins

1. Introduction

The capillary electrophoresis (CE) of protein is often performed for analytical purposes, and few

*Corresponding author.

E-mail address: murielle.rabiller-baudry@univ-rennes1.fr (M. Rabiller-Baudry).

0378-4347/01/$ – see front matter
PII: S0378-4347(00)00431-X

studies deal with their characterisation. Generally, the electrophoretic mobility (μ), which is roughly the ratio of the charge to the radius, was expressed as a function of the (Ze/M^n) ratio, where Ze represents the charge and M the molecular mass. The reported n values were mostly 1/3, 1/2, 2/3 ([1,2 and Refs. cited herein 3]).

In fact, the ratio of the charge (corresponding to infinite dilution) to the radius ought to be corrected by a term depending on the ionic strength of the electrolyte solution in which the protein is dissolved [4,5]. The dependence of the electrophoretic mobility to the ionic strength is important but often neglected. Moreover, two different kinds of electrolyte have to be considered according to the classification used in colloid science [4]:

(i) Indifferent electrolyte interacts with the protein through electrostatic attractive interactions. Counter-ions (opposite charge) surround the protein and consequently decrease its effective net charge. In other words the electrophoretic mobility decreases with the increase of ionic strength (double electric layer compression).

This property is commonly used in ion-exchange chromatography to cancel interactions between a protein and the stationary phase in order to elute the protein.

(ii) Specifically adsorbed electrolyte interacts with proteins through electrostatic and non-electrostatic interactions. Consequently, when the electrolyte concentration increases, the protein charge is screened. In some cases the charge reversal of the protein occurs. The isoelectric point is then quite different from the theoretical one (pI) based on the amino acid content.

The specific adsorption of electrolyte is studied by following the electrophoretic mobility measured at constant pH versus the ionic strength of different background electrolytes (BGE) [6–8]. Different behaviours were evidenced:

(i) The charge reversal is shown, as for lysozyme (isoelectric point pI = 10.7) in phosphate at pH 9.0. Lysozyme is positively charged at low ionic strength, neutral for I close to 0.010 mol l^{-1}, then negatively charged. The isoelectric point shifts to 9.0 in phosphate for I = 0.010 mol l^{-1} at pH = 9.0 [6].

(ii) The mobility remains constant with the ionic strength, as for lactoferrin (pI = 8–9) in phosphate at pH = 9.0, for which the electrophoretic mobility is constant and negative for I between 0.001 and 0.500 mol l^{-1} [7].

(iii) Abnormal low electrophoretic mobility is shown [8]:

- Lysozyme in phosphate at pH = 7.0 for I lower than 0.020 mol l^{-1}, but the protein remains positively charged; whereas theoretical mobility matches well with experimental mobility in an indifferent electrolyte medium as (NaCl).
- α-lactalbumin either at pH = 7.0 in phosphate or at pH = 8.0 in borate for I between 0.020 and 0.200 mol l^{-1}. It can be underlined that phosphate and borate are both oxyanions.

Moreover, the specific adsorption of phosphate on lysozyme was indirectly checked by reversed-phase HPLC (RP-HPLC): the phosphate ions bound to the protein decrease its hydrophobic character, at pH = 9.0 [6] and pH = 7.0 [8].

In this work, the objective was focused on the specific adsorption of phosphate on three different proteins, that act either as counter-ion of phosphate as lysozyme and lactoferrin or as co-ion of phosphate as α-lactalbumin at pH = 7.0. The electrophoretic mobility was measured on a large ionic strength range (I from 0.001 to 0.775 mol l^{-1}) and then related to the apparent net charge of the proteins. The specific adsorption of phosphate on the three proteins was also studied by RP-HPLC.

2. Theory

2.1. Liquid chromatography

In reversed-phase HPLC, the protein is eluted according to its hydrophobicity. The k' capacity factor (dimensionless) defined according to Eq. (1) allows an easy comparison between component of different hydrophobicity, under isocratic elution conditions: the smaller k' is, the less hydrophobic the protein is:

$$k' = (Ve - Vm)/Vm \quad (1)$$

where Ve = volume of elution of the protein and Vm = volume of the mobile phase.

HPLC determination of molecular interactions is generally carried out by addition of the interacting molecule, at various concentrations, to the mobile phase. We have previously proposed another procedure to highlight strong non-electrostatic interactions, due to a pre-treatment of the protein and not to chromatographic conditions. The molecule of interest was dissolved in solutions containing various concentrations of the interacting molecule at fixed pH, and eluted with the same eluent in isocratic mode. We have shown that the pH, the ionic strength and the chemical nature of the electrolyte in the sample solution can have a role on k' when strong interactions occurred, even if the eluent has a pH of roughly 2. According to this procedure, we have shown that k' remained unchanged when lysozyme was dissolved either in water or in potassium chloride solution of various ionic strengths in the range 0–1 mol l^{-1}, at pH = 7.0. Whereas k' varied according to the pH (4 and 9) and the ionic strength of potassium phosphate solution [6]. At pH = 4.0 and pH = 9.0, the specific adsorption of phosphate ions provokes the decrease of lysozyme hydrophobicity, in good agreement with the variation of the electrophoretic mobility.

2.2. Electrophoretic mobility

Electrophoretic mobility (μ) occurs when a charged species is placed in an electric field E. According to the Debye-Hückel theory, charged solutes are surrounded by ions of the electrolyte. The thickness of the resulting sphere (or double electric layer) is the Debye length κ^{-1}, which depends on the ionic strength I of the medium [4,5,9]. The effect of ionic strength is taken into account by including a corrective term $X(\kappa R)$. For κR in the range 0.1–300, μ can be expressed as the following equation (in SI units) [8,9]:

$$\mu = [Ze/6\pi\eta R].[X(\kappa R)/(1 + \kappa R)]$$
$$= \mu_0 [X(\kappa R)/(1 + \kappa R)] \quad (2)$$

where Ze = the net charge of the protein (C), Z = the charge number of the protein, μ_0 = the mobility in an insulating medium ($I = 0$), η = the electrolyte viscosity (Pa s), κ = the reciprocal Debye length (m^{-1}), R = the protein radius (m), $X(\kappa R)$ = Henry's corrective term, which expression when $\log(\kappa R) < 1$ is given in [5,8].

The term $[X(\kappa R)/(1 + \kappa R)]$ take into account the role of the ionic strength, on the electrophoretic mobility, and depends only on the ionic strength (via κ) and on the protein (R) without any consideration of the electrolyte chemical nature. It varies from 1 to 0 when the ionic strength (I) increases from 0 to infinity.

To calculate the theoretical mobility using Eq. (2), the following hypotheses have been made:

- the protein radius is the Stokes radius.
- the theoretical net charge number (Z) of proteins was calculated, from the amino acid composition of protein, and pK_a values of the side chain amino-acids (the pK_a values used were those of free amino acids) [10]. Such hypotheses have been formerly validated for lysozyme in an indifferent electrolyte where the theoretical electrophoretic mobility matches well with the experimental one [8].

No theoretical calculation was made for lactoferrin, because the amino acid sequence is not completely solved (Asx and Glx). Fig. 1 shows the theoretical mobility (μ) of lysozyme and Fig. 2 shows those of α-lactalbumin of both the holo form and apo form calculated at pH = 7.0 in relation to the ionic strength. The absolute value of mobility decreases significantly when the ionic strength increases from 0 to 0.775 mol l^{-1}, as expected.

In the following, the effective charge number (Z^{eff}) of the protein is obtained by substituting the experimental mobility (μ^{exp}) to μ in Eq. (2).

3. Experimental

3.1. Samples and reagents

All reagents were of an analytical grade. Potassium dihydrogenophosphate, NaOH and HCl were from Merck (Darmstadt, Germany).

Acetonitrile (Carlo Erba) and trifluoroacetic acid

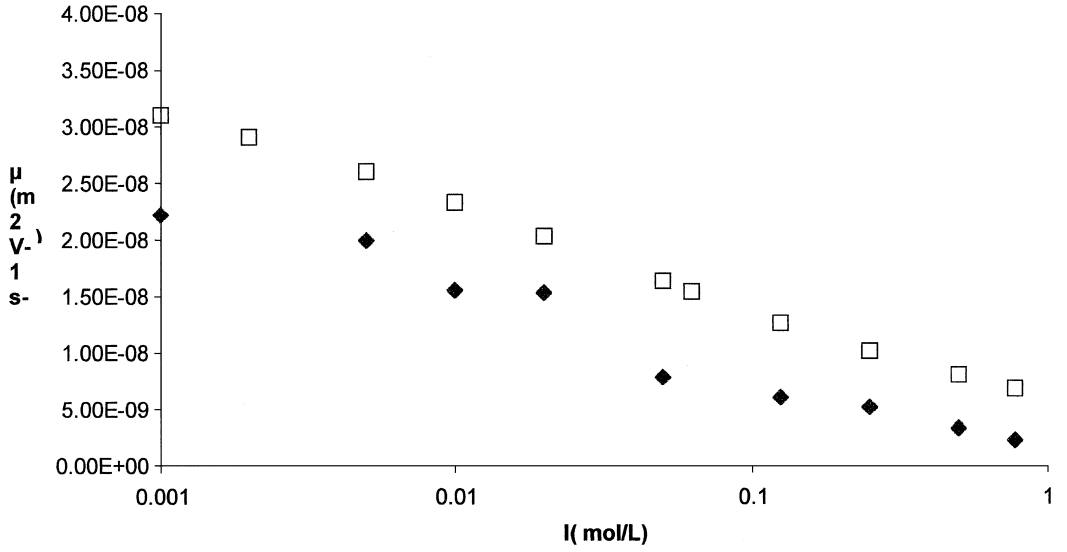

Fig. 1. Theoretical electrophoretic mobility (□, Eq. (2)) and experimental electrophoretic mobility (♦) of lysozyme versus the ionic strength (I) of potassium phosphate at pH = 7.0.

(TFA, Pierce Chemicals) of a spectroscopic grade were used for HPLC experiments.

The proteins used are from the white egg (lysozyme, pI = 11 [11], Stokes radius Rs = 1.83 nm [12]) and from cow milk (α-lactalbumin, pI = 4.2–4.5 [13] and Rs = 1.95 nm [14] and lactoferrin, pI = 8–9 [15]

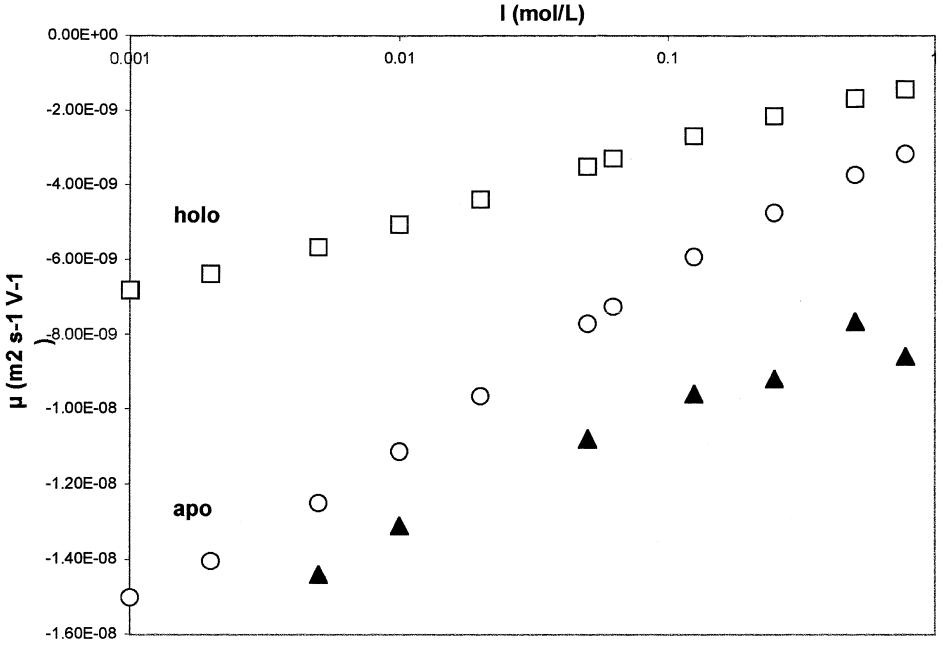

Fig. 2. Theoretical electrophoretic mobility (□: holo form, ○: apo-form, Eq. (2)) and experimental electrophoretic mobility (▲) in potassium phosphate at pH = 7.0 of α-lactalbumin versus the ionic strength (I).

and $Rs = 2.2$ nm for $I < 0.200$ mol l^{-1} and $Rs = 4.4$ nm for $I > 0.200$ mol l^{-1} [7]). α-Lactalbumin is a metallo-protein and two forms are known to exist, the apo form, which is stable at acid pH, and the holo form (one protein chelates about one calcium) stable at upper pH. Holo α-lactalbumin was used in this study (Sigma, Type III). Lysozyme in hydrochloride form was from Ovonor (Tregueux, France), and lactoferrin, which is also a protein that bounds ion (iron) from Armor Proteines (Saint-Brice en Coglès, France).

For CE, single protein was dissolved in deionised water and 1 μm filtered (Aquadem-Elga, Vern sur Seiche, France).

3.2. Liquid chromatography (RP-HPLC)

The HPLC system included a pump unit (Beckman 126), an automatic sample injector (Gilson 231–401) equipped with a valve (Rheodyne 7125) and a diode-array UV detector (Beckman 168) at 280 nm and 220 nm. The flow-rate was 1 ml min^{-1} and 50 μl samples were injected. The data were processed with Gold 8 software (Beckman).

Reversed-phase HPLC in isocratic elution mode was performed for the hydrophobic characterisation of protein. The stationary phase was made of polystyrene divinylbenzene, 300 A, 8 μm, 150×4.6 mm, PLRP-S from Polymer Laboratories. The eluents were 0.1% (v/v) trifluoro acetic acid (TFA) in water (eluent A) and 0.1% (v/v) TFA in water–acetonitrile (20:80, v/v) (eluent B). Elution pH was roughly 2.

Prior to injection, the protein (1 g l^{-1}) was dissolved in potassium phosphate solution or in potassium chloride solution, at pH 7.0 for various ionic strengths from 0 to 0.775 mol l^{-1}. Then it was eluted in isocratic mode with an acetonitrile percentage depending on the protein. The relative hydrophobicity of the three proteins is as follows: lysozyme < α-lactalbumin < lactoferrin. For a given protein, in order to evidence apparition, or not, of less hydrophobic contribution than the native protein, we have chosen the smaller percentage of eluent B allowing the elution of the protein. Consequently, lysozyme was eluted with 41% of eluent B (32.8% acetonitrile), α-lactalbumin with 46% (36.8% of acetonitrile) and lactoferrin with 47% (37.6% of acetonitrile), respectively.

3.3. Capillary electrophoresis

Two capillaries were used, an unmodified silica capillary (Beckman with an inner diameter of 75 μm) and a modified silica capillary. The capillary total length was $L_t = 27$ cm, and only $L_d = 20$ cm from the inlet to the detector. The modification was performed in our laboratory by grafting the silica surface with isopropyl tri (*N*-ethylene diamino) ethyl titanate. The chemical modification reduced the electroosmotic flow (EOF) to 57% of its initial value, when measured in potassium phosphate at pH = 7.0 and $I = 0.020$ mol l^{-1}.

The electrophoretic mobility of a protein was the same with the two capillaries in good agreement with previous works [8,16]. Consequently no attention will be paid in the following to the chemical nature of the capillary used.

Capillary electrophoresis was performed on a P/ACE 2100 instrument, operated under gold system 6.01 for control, data acquisition and software analysis (Beckman). The temperature was controlled at 25°C. The samples were injected by applying pressure (0.5 p.s.i., 3435 Pa). All separations were carried out applying a constant voltage between 3 kV and 10 kV. UV detection at 214 nm was set at the cathodic end of the capillaries. An electroosmotic flow marker (benzylic alcohol 0.01 vol% in water) was co-injected (0.5 p.s.i., 1 s) with the protein. Consequently, the migration order was as follows: cations, neutral species (EOF), then anions migrating slower than the EOF.

The pH of the phosphate-based background electrolyte was adjusted to 7.0. At this pH, phosphate is both monovalent and divalent (50:50) and the ionic strength (I) are roughly two times its concentration. One can consider that phosphate charge is -1.5.

Apparent electrophoretic mobility (μ^{app}) was related to the experimental one (μ^{exp}) and the electroosmotic flow EOF (μ^{EOF}) according to:

$$\vec{\mu}^{app} = \vec{\mu}^{exp} + \vec{\mu}_{eo}^{EOF} \quad (3)$$

μ^{EOF} is the EOF contribution to electrophoretic

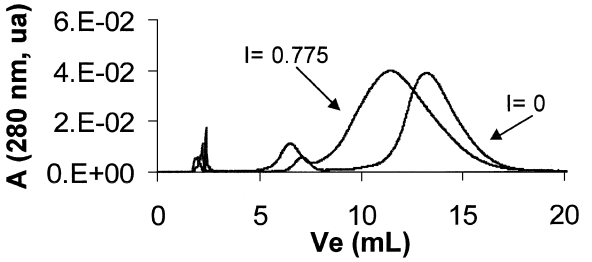

Fig. 3. Chromatograms of lysozyme dissolved in water and in potassium phosphate solution at $I=0.775$ mol l^{-1} at pH 7.0 prior to injection obtained in RP-HPLC. Isocratic elution mode: 32.8% acetonitrile, 0.1% TFA (v/v).

mobility determined on the basis of the migration time of the neutral marker.

For each background electrolyte (BGE) composition, the mobility of protein is the average of 10 measurements. The relative standard deviation (RSD) was about 1.5% for high ionic strengths ($I>0.020$ mol l^{-1}) and less than 7% at lower ionic strengths.

4. Results

4.1. Hydrophobic character by reversed-phase chromatography

Fig. 3 shows the chromatogram of lysozyme dissolved in water. The pH was adjusted to 7.0 by NaOH. A main peak of protein was eluted at an eluent volume *Ve* close to 14 ml; a minor (less than 10% in surface area) and not well-identified contribution of less hydrophobic lysozyme was eluted at *Ve* close to 6 ml. In the following, only the major peak will be considered as the ''native'' protein. When lysozyme is dissolved in potassium chloride solutions of increasing ionic strength from 0.001 to 0.775 mol l^{-1}, the chromatogram remained unchanged; the k' values were constant and close to 5 (Fig. 4). When lysozyme is dissolved in potassium phosphate solutions of increasing ionic strengths, the peak of the native protein disappeared and was replaced by a less hydrophobic peak (Fig. 3) leading to k' values decreasing with the ionic strength increase (Fig. 4).

The same procedure was followed with α-lactal-

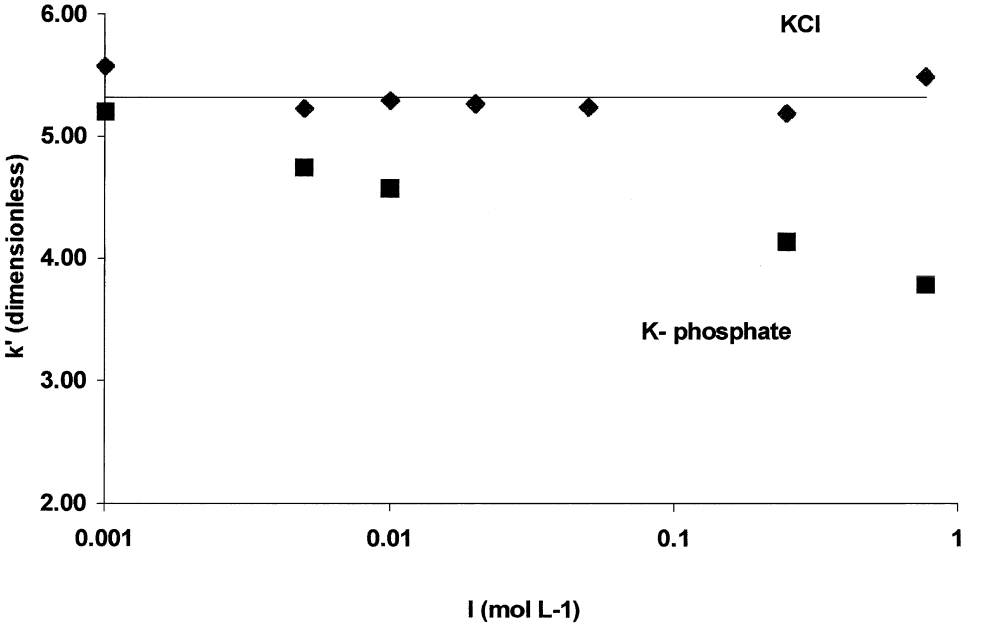

Fig. 4. $k'=(Ve-Vm)/Vm$ of lysozyme dissolved in KCl (♦) and potassium phosphate (■) at pH 7.0 prior injection, versus the ionic strength (I) determined from RP-HPLC.

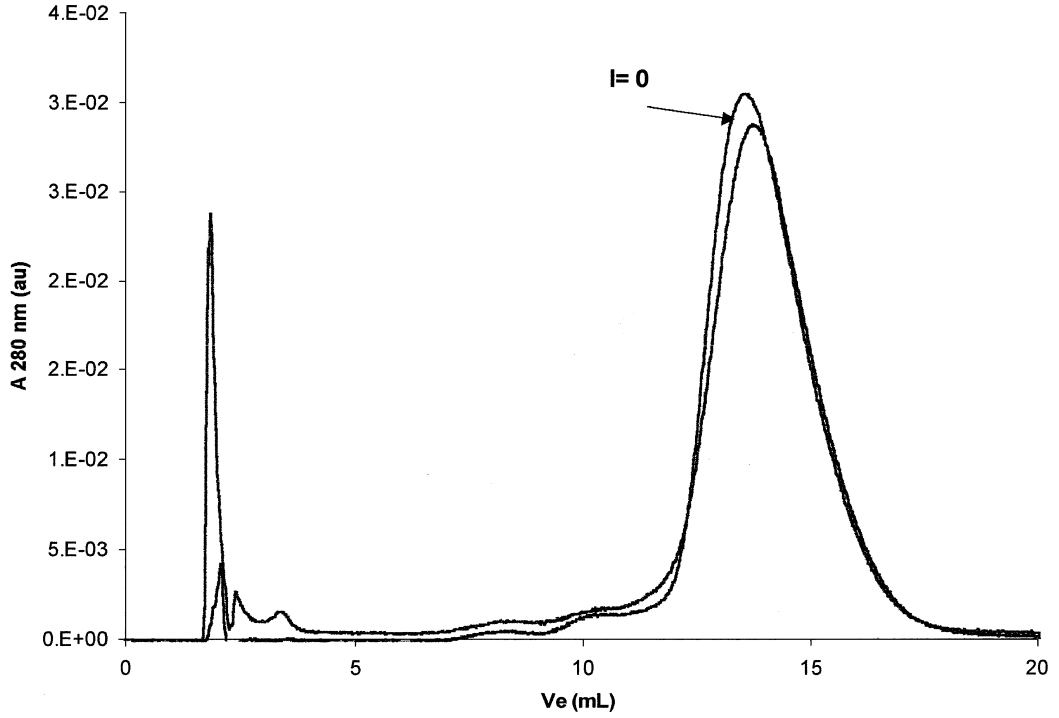

Fig. 5. Chromatograms of α-lactalbumin dissolved in water and in potassium phosphate solution at $I=0.775$ mol l^{-1} at pH 7.0 prior to injection obtained in RP-HPLC. isocratic elution mode: 36.8% acetonitrile, 0.1% TFA (v/v).

bumin (Fig. 5) and lactoferrin (not shown). In both cases, the chromatograms remains unchanged when the samples were dissolved either in water or in potassium phosphate solution of varying ionic strength ($I=0$–0.775 mol l^{-1}) at pH = 7.0.

By phosphate adsorption, the hydrophobic character of lysozyme is decreased as evidenced in RP-HPLC, whereas no change of the hydrophobic behaviour of α-lactalbumin and lactoferrin was observed whereas they also bind phosphate ions. Table 1 shows the ratio of the molar absorptivity (ϵ) at 280 nm to that at 220 nm for the three proteins in various electrolyte solutions. This ratio varies for lysozyme in phosphate at $I=0.775$ mol l^{-1} when compared with lysozyme in water, meaning that the nature of the chromophore varies, i.e. that phosphate is bound to the protein. As for k', the $\epsilon^{280}/\epsilon^{220}$ ratio is constant for lactoferrin and α-lactalbumin.

4.2. Electrophoretic mobility by CE

4.2.1. Lactoferrin

Lactoferrin has a basic isoelectric point pI = 8–9 and is expected to be positively charged at pH 7.0.

Table 1
$\epsilon^{280}/\epsilon^{220}$ from RP-HPLC (isocratic elution mode, see text for details) for different proteins versus the ionic strength of KCl or phosphate at pH = 7.0 in the protein samples

Protein	Lysozyme	Lysozyme	Lysozyme	α-Lactalbumin	Lactoferrin
"electroyte"	water	KCl 0.775 mol l^{-1}	K phosphate 0.775 mol l^{-1}	K phosphate $I=0$ to 0.775 mol l^{-1}	K phosphate $I=0$ to 0.250 mol l^{-1}
$\epsilon^{280}/\epsilon^{220}$	0.136	0.135	0.157	0.122	0.096

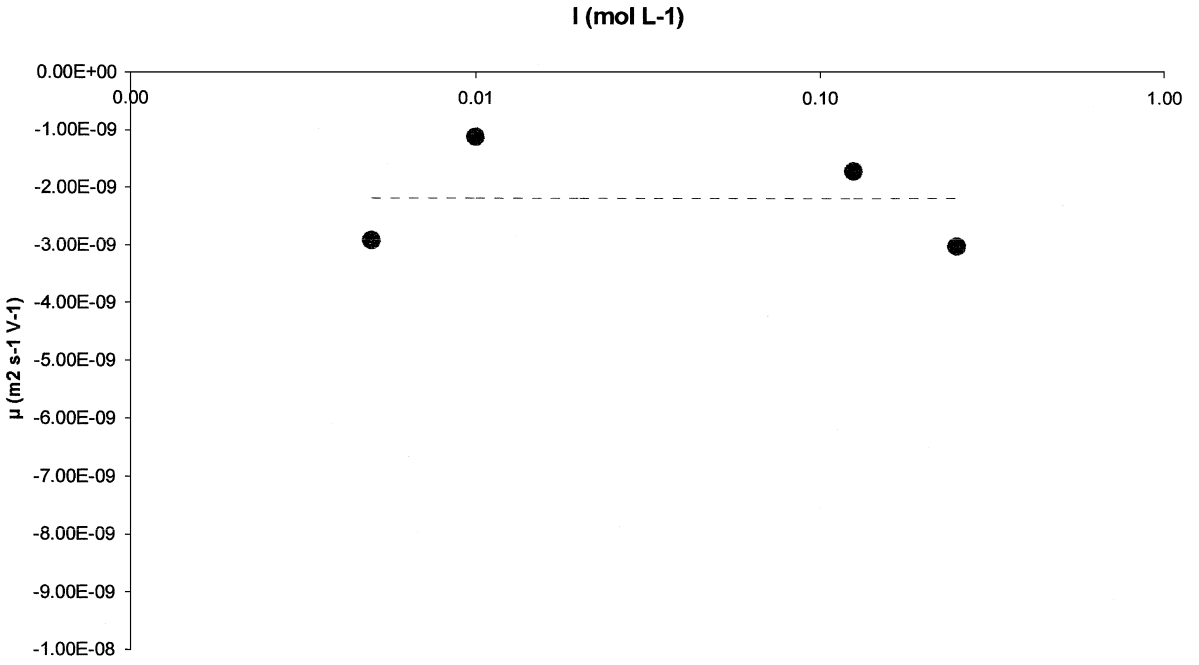

Fig. 6. Experimental electrophoretic mobility (●) of lactoferrin versus the ionic strength (I) of potassium phosphate at pH = 7.0.

Fig. 6 shows the experimental electrophoretic mobility in BGE made of phosphate at pH 7.0, in the ionic strength range 0.005–0.250 mol l^{-1}. The mobility was slightly negative and roughly constant. This unexpected sign of mobility evidenced the reversal of the lactoferrin charge and the specific adsorption of phosphate ions on the positively charged lactoferrin.

4.2.2. α-Lactalbumin

α-Lactalbumin has an isoelectric point close to 4 and is expected to be negatively charged at pH 7.0. Fig. 2 shows the electrophoretic mobility in the ionic strength range 0.005–0.775 mol l^{-1}, in phosphate at pH 7.0: it was always lower than the theoretical mobility of the holo form (with one calcium bound per molecule) used in this experiments. Moreover, experimental data were lower than that predicted for the apo form (without calcium). The absolute value of the experimental mobility decreases slightly with the increase of ionic strength in the range beyond 0.1 mol l^{-1}.

Fig. 7 compares the effective charge number Z^{eff} and the theoretical values of both holo and apo forms. The absolute value of Z^{eff} increased from about −4 at $I = 0.005$ mol l^{-1} to about −11 at $I = 0.775$ mol l^{-1}, whereas the theoretical Z value for the holo-form used is −1.7. This behaviour is typical of phosphate adsorption on the protein, which is a co-ion in this case, and increases continuously with the ionic strength. Considering that phosphate charge is −1.5, the Z^{eff} values of α-lactalbumin suggest that about one phosphate by α-lactalbumin is adsorbed at $I = 0.005$ mol l^{-1} and about six phosphates are adsorbed at $I = 0.775$ mol l^{-1}.

4.2.3. Lysozyme

Lysozyme has a basic isoelectric point pI = 10.7 and is expected to be positively charged at pH 7.0. Fig. 1 shows the electrophoretic mobility in the ionic strength range 0.001–0.775 mol l^{-1}, in phosphate at pH 7.0: it was always lower than the theoretical mobility that matches well with experimental data in an indifferent electrolyte, as previously shown [8]. The absolute value of the experimental mobility decreases 10 times when the ionic strength increases from 0.001 to 0.775 mol l^{-1}.

Fig. 8 compares the effective charge numbers Z^{eff}

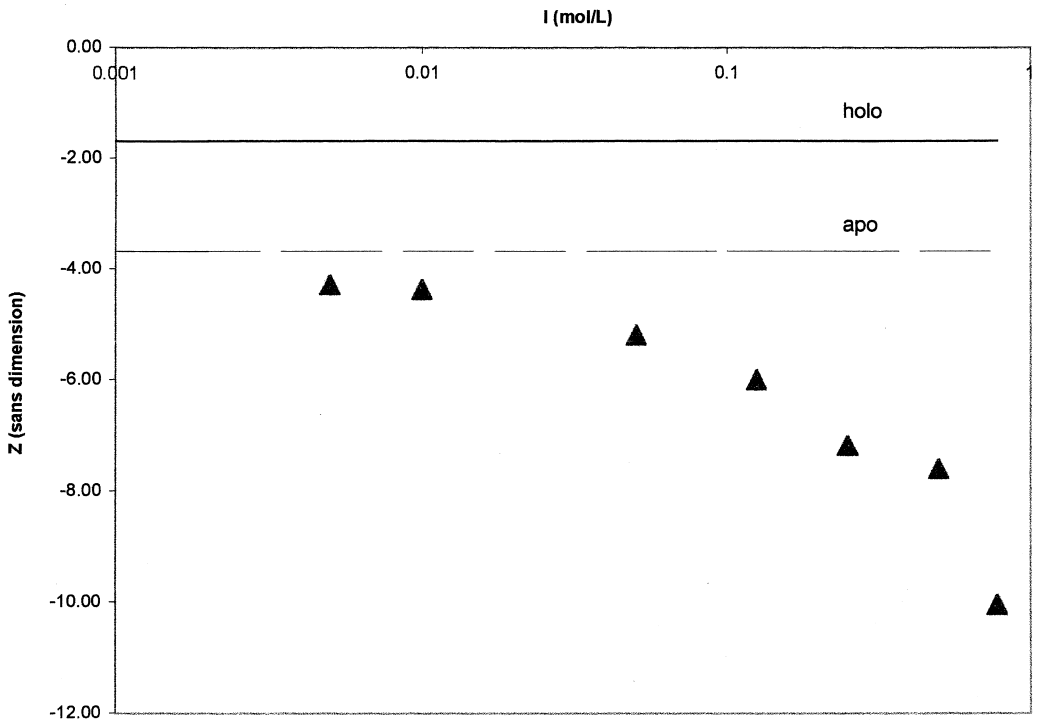

Fig. 7. Theoretical charge number (Z) (holo form and apo-form, Eq. (2)) and experimental effective charge number (Z^{eff}) (▲) of α-lactalbumin versus the ionic strength (I) of potassium phosphate at pH = 7.0.

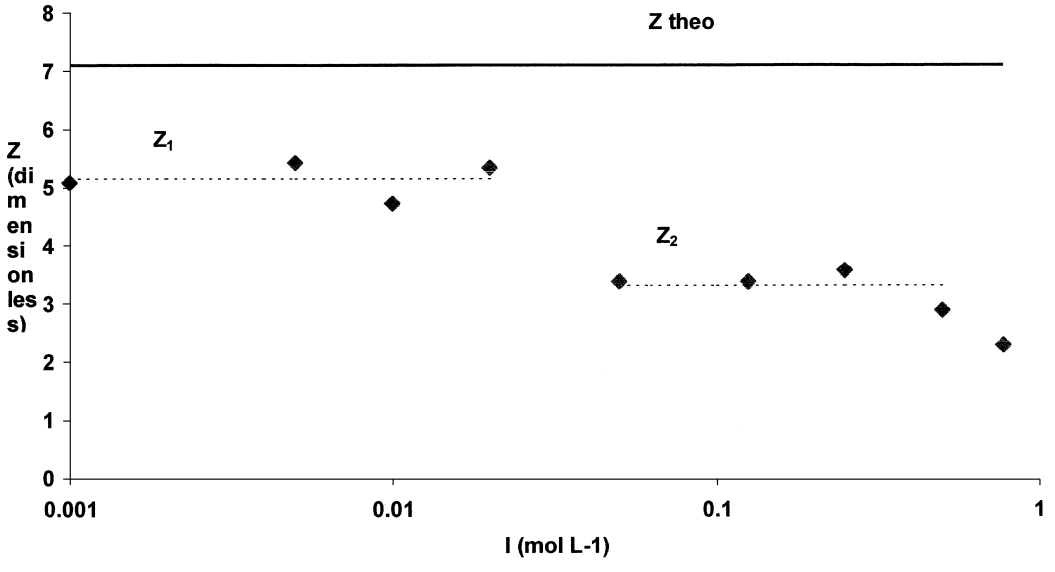

Fig. 8. Theoretical charge number (Z theo, Eq. (2)) and experimental effective charge number (Z^{eff}) (♦) of lysozyme versus the ionic strength (I) of potassium phosphate at pH = 7.0.

and the theoretical value (+7.1). The value of Z^{eff} increased in a discrete way with the ionic strength: for I in the range 0.001–0.020 mol l^{-1}, Z^{eff} is close to 5, and decreases until 3 in the ionic strength range 0.050–0.500 mol l^{-1}. This behaviour is typical of phosphate adsorption occurring on the protein, which is here a counter-ion. Considering that phosphate charge is −1.5, the Z^{eff} value of lysozyme suggests that about one phosphate is adsorbed in the ionic strength range 0.001–0.020 mol l^{-1} and between two and three phosphates are adsorbed in the range 0.050–0.500 mol l^{-1} per protein molecule. The capability to adsorb one phosphate per lysozyme at low ionic strength at pH 8.0 has been previously reported [17].

5. Discussion

Lysozyme and α-lactalbumin are globular proteins of close compositions: 129 amino acids (32 of hydrophobic character) and 123 (39 of hydrophobic character) respectively. But according to Arai and Norde [18], they are relevant of two protein types:

(i) ''Hard'' proteins have high structure stability and adsorb on hydrophobic interfaces under all conditions of charge interaction and on hydrophilic surfaces only if electrostatically attracted. Lysozyme behaviour is typical of ''hard'' protein.

(ii) ''Soft'' proteins have low structure stability and consequently structure rearrangements occur in the molecule in order to allow its adsorption on hydrophobic surface and on hydrophilic surface under attractive and repulsive electrostatic conditions. α-Lactalbumin behaviour is typical of ''soft'' protein.

In other words, lysozyme conformation remains rigid, whereas α-lactalbumin conformation can change according to its environment.

From the effective charge number (Z^{eff}) determined, two different type of specific adsorption behaviour seems occur:

(i) A ''not well defined'' adsorption, as for α-lactalbumin and lactoferrin for which the phosphate content varies continuously with the ionic strength, evidenced by a slight variation of the mobility or a roughly constant one with the ionic strength, respectively.

(ii) A ''step by step'' adsorption, as for lysozyme, for which the phosphate contents varies in a discrete way. In this case, the final composition looks like a ''complex'' of well defined stoechiometry, highlighting likely the existence of higher affinity sites between lysozyme and phosphate ions than with α-lactalbumin and lactoferrin.

One can suppose that deformation of α-lactalbumin occurring in the RP-HPLC eluent, whatever the phosphate content and that phosphate are in the inner hydrophilic part of the molecule during elution, whereas no deformation of lysozyme occurred and that hydrophobic parts are partly screened by phosphate ions changing its hydrophobicity.

Investigations by other methods as ES/MS or FTIR are needed in order to elucidate the physical meaning of the variation of the hydrophobic character induced by the specific adsorption of oxyanions on proteins.

6. Conclusion

In this work, specific adsorption of phosphate ions on different proteins, either counter-ions of phosphate (lysozyme, lactoferrin) or co-ion of phosphate (α-lactalbumin) was studied. Measurement of the protein electrophoretic mobility (μ) by capillary electrophoresis (CE) was achieved at constant pH (7.0) versus the ionic strength of potassium phosphate in the range 0–0.775 mol l^{-1}. The specific adsorption of phosphate was evidenced when experimental mobility was significantly different from the theoretical one. The experimental net charge number (Z^{eff}) of proteins was then calculated allowing the determination of a phosphate content per molecule and highlighting either a step by step adsorption (lysozyme) or a not well defined one (α-lactalbumin). Reversed-phase HPLC confirms that adsorption of phosphate is different for lysozyme and α-lactalbumin.

Acknowledgements

The authors thank Marie-Christine Hautbois from IUT Rennes for technical assistance.

References

[1] E.C. Rickard, M.M. Strohl, R.G. Nielsen, Anal. Biochem. 197 (1991) 197.
[2] S.K. Basak, M.R. Ladisch, Anal. Biochem. 226 (1995) 51.
[3] M. Wronsky, J. Chromatogr. A 657 (1993) 165.
[4] J. Lyklema, Fundamentals of Interface and Colloid Science, Academic Press, London, 1995.
[5] P.C. Hiemenz, R. Rajagopalan, in: Principles of Colloid and Surface Chemistry, 3rd ed, Marcel Dekker, New York, 1997, p. 548.
[6] D. Lucas, M. Rabiller-Baudry, F. Michel, B. Chaufer, Colloids Surfaces A: Physicochem, Eng. Aspects 136 (1998) 109–122.
[7] B. Chaufer, M. Rabiller-Baudry, D. Lucas, F. Michel, M. Timmer, Lait 80 (2000) 197–203.
[8] M. Rabiller-Baudry, A. Bouguen, D. Lucas, B. Chaufer, J. Chromatogr. B 706 (1998) 23–32.
[9] C. Tanford, in: Physical Chemistry of Macromolecules, Wiley, New York, 1961, p. 416.
[10] R.M.C. Dawson, D.C. Elliott, W.H. Elliott, K.M. Jones, Data for Biochemical Research, 3rd ed., Oxford Science, 1987.
[11] R.E. Dickerson, I. Geis, in: J. Belmont (French edition), Structure et Action des Proteines, Ediscience, Paris, 1972.
[12] C. Loret, B. Chaufer, B. Sebille, M. Hanselin, Y. Blain, A. Le Hir, Int. J. Biol. Macromol. 10 (1988) 366.
[13] W.N. Eigel, J.E. Buttler, C.A. Ernestrom, H.M. Farrell Jr., V.R. Harwalkar, R. Jenness, R.Mc.L. Whithney, J. Dairy Sci. 67 (1984) 1631.
[14] H.E. Swaisgood, in: P.F. Fox (Ed.), Developments in Dairy Chemistry, Applied Science Publication, London, 1982, Chap. 1.
[15] S. Lentsch, Ultrafiltration et électrofiltration: mécanismes impliqués dans le fractionnement de mélanges albumine-lactoferrine et albumine-poly(éthylèneglycol) Thèse d'Université, Toulouse, France, 1993.
[16] W. Nashabeh, Z. El Rassi, J. Chromatogr. 559 (1991) 367.
[17] S. Moreau, Etude des interactions entre le lysozyme du blanc d'oeuf de poule et les minéraux par ultrafiltration et ES-MS, DEA, Univ. Nantes, France, 1993.
[18] T. Arai, W. Norde, Colloids and Surfaces 51 (1990) 1–15.