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Physico-chemical characterization of proteins by capillary electrophoresis

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Abstract

The electrophoretic mobility of proteins was successfully determined by means of capillary electrophoresis (CE) with various background electrolytes (BGEs). The objective was focused on the variation in BGE physico-chemical composition and the consequential impact on the observed protein charge. Experimental and calculated mobilities, according to Henry's equation, versus ionic strength have been compared. For positively-charged lysozyme, a good agreement between observed and calculated mobilities was observed using triethanolamine chloride at pH 7.0 as the BGE. Mobility close to zero was shown using borate (pH 8.0) and phosphate (pH 7.0) at a low ionic strength of about 20 mmol l^{-1}, and as a consequence, specific adsorption of oxyanions was evidenced. Lysozyme retention in the case of reversed-phase high-performance liquid chromatography (RP-HPLC) was decreased by the presence of phosphate ions. CE and HPLC are complementary tools for characterizing the behaviour of lysozyme. On the other hand, the mobility of the negatively-charged α-lactalbumin remained constant as regards phosphate at pH 7.0 in the 20–200 mmol l^{-1} range, contrary to the decrease that had been expected with the increasing ionic strength. β-Lactoglobulin exhibited increasingly lower mobilities than those expected of boric acid/borate at pH 7.0 and 8.0 (I=20 mmol l^{-1}). © 1998 Elsevier Science B.V.

Keywords: Proteins; α-Lactalbumin; β-Lactoglobulin; Lysozyme

1. Introduction

The capillary electrophoresis (CE) of proteins is often performed for analytical purposes. The optimum conditions for separation generally require the use of additives in the background electrolyte (BGE), and denaturing conditions are often used [1–5].

The mobility (μ) from CE, which is roughly the charge-to-size ratio, was compared with traditional techniques. For proteins, μ was expressed as a function of the (Ze/M^n) ratio, where Ze represents the charge and M the molecular mass. Pioneering work was established with paper electrophoresis by Offord [6]. The reported n values were mostly 1/3, 1/2, 2/3 for proteins and peptides ([7,8] and Refs. cited herein [9]).

Whitesides and coworkers [10–12] have described an original method for the determination of the effective charge (Z) of an unknown protein, based on the ''charge ladder concept''. A protein charge ladder is a family of derivatives of the protein obtained after modification of the charge of the protein by means of chemical bonding (typically by acetylation). The observed Z values tallied with the net charge calculated from the amino acid sequence for a set of twelve proteins. Nevertheless, for α-lactalbumin, a protein relevant to this paper, a

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difference of two charges was shown, when 25 mmol l^{-1} Tris HCl/200 mmol l^{-1} glycine pH 8.3 was used as the BGE. In order to take into account the impact of ionic strength on mobility, a mean constant coefficient (Cr) (for the Henry equation) was experimentally determined. On the other hand, denaturation of proteins can occur during chemical modification set at pH 12.

Solute mobility depends on electrolyte ions: pure electrostatic interactions occurred in indifferent electrolytes, whereas electrostatic and non-electrostatic interactions between the protein and the electrolyte ions occurred in specifically adsorbed electrolytes. For specifically adsorbed ions, it is possible to reverse the net charge of the protein according to the electrolyte concentration [13].

In this work, attention was focused on the electrophoretic mobility of proteins resulting from CE in relation to its physico-chemical environment. Both the ionic strength and the chemical nature of the background electrolyte were considered. Different cases were considered: when the protein and the BGE ions under consideration were counter-ions or co-ions. We will show that the study of mobility versus ionic strength is a useful way to show the existence of interactions between a protein and a BGE.

The charge variation of lysozyme in phosphate was indirectly checked by reversed-phase high-performance liquid chromatography (RP-HPLC).

2. Theory: electrophoretic mobility

Electrophoretic mobility (μ) occurs when a charged species is placed in an electric field E. According to the Debye–Hückel theory, in a conducting medium (electrolyte), charged solutes are surrounded by ions of the electrolyte. The thickness of the resulting solvation sphere (or double electric layer) is the Debye length κ^{-1}, which depends on the ionic strength I of the medium [13–15]. The effect of ionic strength was taken into account by including a corrective term $X(\kappa R)$, and μ can be expressed as the following Henry's equation (in SI units) [14]:

$$\mu = [2\varepsilon\zeta/3\eta]X(\kappa R) \quad (1)$$

where ζ is the zeta potential of the particule (V), depending on ionic strength, η the medium viscosity (Pa s), ε the dielectric constant (C/V m), κ the reciprocal Debye length (m^{-1}), R the radius (m) and $X(\kappa R)$ Henry's corrective term.

For theoretical calculation [$\log(\kappa R) < 1$], the following expression was used for $X(\kappa R)$ (Eq. (2)) with correction of typing errors from [15]. It was checked that $X(\kappa R)$ tallied with the figure obtained by Tanford [14].

$$X(\kappa R) = 1 + \frac{1}{16}(\kappa R)^2 - \frac{5}{48}(\kappa R)^3 - \frac{1}{96}(\kappa R)^4 + \frac{1}{96}(\kappa R)^5 - \left[\frac{1}{8}(\kappa R)^4 - \frac{1}{96}(\kappa R)^6\right]\exp(\kappa R)\int_{\infty}^{\kappa R} e^{-t}\frac{dt}{t} \quad (2)$$

Mathematically, Henry's equation varies from 1.0, when $\kappa R < 0.1$, to 1.5, when $\kappa R > 100$–300.

The term $\int_{\infty}^{\kappa R} e^{-t}\frac{dt}{t}$ was determined by means of the Romberg method [16].

For the proteins and the ionic strength considered in the following, most of Henry equation values were between 1.01 and 1.06 (except for α-lactalbumin at $I = 200$ mmol l^{-1} 1.09).

For κR in the range 0.1–300, the zeta potential can be expressed as a function of the protein size with the hypothesis of a spherical shape. Thus, Eq. (1) can be expressed as (in SI units):

$$\mu = [Ze/6\pi\eta R] \cdot [X(\kappa R)/(1 + \kappa R)] \quad (3)$$

where Ze is the net charge of the particule (C).

The term $[X(\kappa R)/(1+\kappa R)]$ varies from 1 to 0 when I increases from 0 to infinity. When ionic strength approaches zero (infinite dilution) κ^{-1} is large with a small charge density and the surface charge of the solute is maximum. At high ionic strength, κ^{-1} is small but with a large charge density which more efficiently balances the solute surface charge, the solute net charge remaining minimal.

In an insulating medium ($I = 0$), Eq. (2) reduces to the well-known Stokes' law:

$$\mu = [Ze/6\pi\eta R] \quad (4)$$

3. Experimental

3.1. Samples and reagents

All reagents were of an analytical grade. Boric acid and sodium tetraborate were obtained from Janssen (Noisy le Grand, France). Sodium and potassium chloride, potassium dihydrogenophosphate, triethanolamine (TEA) were from Merck (Darmstadt, Germany). Acetonitrile and trifluoroacetic acid (TFA, Pierce Chemicals) of a spectroscopic grade were used for HPLC experiments.

Lysozyme in hydrochloride form came from Ovonor (Tregueux, France). α-Lactalbumin (purity >90% higher than Type III from Sigma), as well as β-lactoglobulin (purity 90% by RP-HPLC), were kindly donated by the INRA research agency (LRTL, Rennes, France). β-Lactoglobulin (L2506) and α-lactalbumin (Type III) from Sigma were also used. The same results were obtained with the different available proteins. For CE, single proteins were dissolved in water, and stock solutions were frozen.

The water was deionised and 1 μm filtered (Aquadem-Elga, Vern sur Seiche, France).

Polyethyleneimine PEI 40,000 (Polymin P, Basf) and diglycidyl ether of bisphenol A DGEBA (Epikote 828, Shell) were kindly provided by the manufacturers and used as received.

3.2. HPLC apparatus and procedure

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 detection system (Beckman 168) at 220 nm. The flow-rate was 1.0 ml/min and 50 μl samples were injected. The data were processed with Gold 8 software.

RP-HPLC in isocratic elution mode was performed for the hydrophobic characterization of lysozyme in solution (PLRP-S polystyrene divinylbenzene, 300 A, 8 μm, 150×4.6 mm I.D., Polymer Laboratories). The eluents were 0.1% (v/v) 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. Before injection, lysozyme (1 g l^{-1}) was dissolved either in water (natural pH ca. 4–5) or in phosphate solutions at pH 7.0.

3.3. CE apparatus and procedure

CE was performed on a P/ACE 2100 instrument, operated under Gold system 6.01 for control, data acquisition and software analysis (Beckman, Gagny, France). Capillaries were mounted in a cartridge the temperature of which 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. UV absorbance detection at 214 nm was set at the cathodic end of the capillaries.

3.4. BGE used in CE

The pH of the phosphate-based BGE was adjusted to 7.0 by means of hydrochloric acid. At this pH, phosphate is monovalent and divalent (50:50) and ionic strength is two-times its concentration.

Boric acid-based BGE was prepared by mixing boric acid and sodium tetraborate in order to get a pH 8.0 solution. As the pK_a of boric acid is about 9.2, the ionic strength proves to be roughly 1/10 times boric acid concentration (a 200 mmol l^{-1} boric acid solution is only 20 mmol l^{-1} sodium borate, $I=20$ mmol l^{-1}).

The boric acid solution was adjusted to pH 7.0 by adding HCl (which is, in fact, a titration of the remaining borate form in the pH 8.0 solution: 200 mmol l^{-1} boric acid and 20 mmol l^{-1} NaCl, $I=20$ mmol l^{-1}).

3.5. CE capillaries

Many capillaries were used, four unmodified silica capillaries (Beckman with an inner diameter of 75 μm) and six modified silica capillaries, with polyethyleneimine (PEI) as first proposed by Towns and Regnier [17]; they were then crosslinked with various DGEBA concentrations leading to more or less hydrophobic coatings. The chemical modification generally reduced the electroosmotic flow (EOF) to 53–57% of its initial value.

3.6. Electrophoretic mobility determination

Apparent electrophoretic mobility (μ_{app}) was related to μ and the EOF according to Eq. (5):

$$\vec{\mu}_{app} = \vec{\mu} + \vec{\mu}_{eo} \quad (5)$$

where μ_{eo} was the EOF contribution to electrophoretic mobility determined on the basis of the migration time of a neutral marker (benzyl alcohol, Prolabo, Paris, France).

4. Results and discussion

4.1. Reversed-phase chromatography of lysozyme

Lysozyme was dissolved in different electrolytes, prior to injection, and its behaviour was investigated with a polymer-based phase (RP-HPLC) characterized by constant acetonitrile content and pH 2.

The chromatogram of lysozyme dissolved in water is shown in Fig. 1. The main peak at ca. 12 min (Lys a, 92%) was assumed to be the native protein. A minor peak (Lys b, 7%) was eluted at ca. 7 min (less hydrophobic species). No chromatogram change was observed when lysozyme was dissolved in KCl or TEA Cl solutions of increasing ionic strengths of up to 100 mmol l^{-1}.

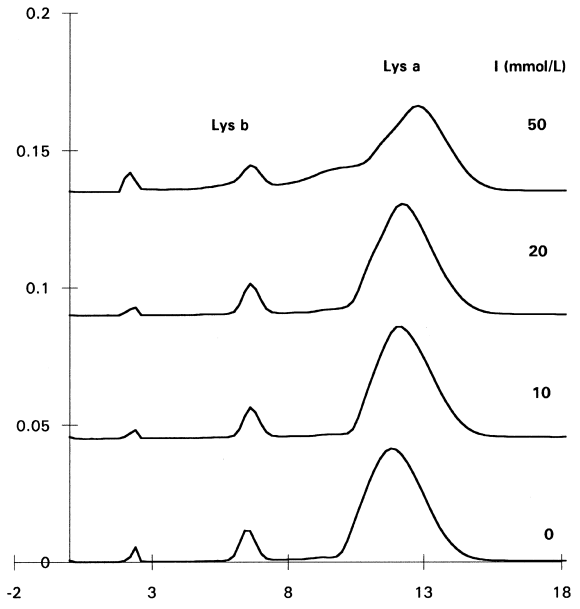

Fig. 1. Chromatograms of lysozyme dissolved in various phosphate concentrations at pH 7 prior to injection obtained in RP-HPLC isocratic elution mode: 33.6% acetonitrile, 0.1% TFA (v/v). Lys a: native protein, Lys b: minor species (see Section 4.1).

The lysozyme peak was enlarged with a shoulder (less hydrophobic) when dissolved in phosphate solutions (I above 20 mmol l^{-1}) (Fig. 1). When I increased up to 50–150 mmol l^{-1} the remaining native protein was only 57%: phosphate ions were adsorbed on lysozyme.

RP-HPLC shows that lysozyme dissolved in phosphate solution yields a partly less hydrophobic form than the native form, presumably due to phosphate adsorption.

4.2. CE: protein mobility and chemical nature of the capillary

The adsorption of proteins in the case of silica is so strong that in some cases no detection of proteins has been reported [18–20]. For analytical purposes, in order to avoid this adsorption phenomenon, additives were added to the BGE, such as rare-earth ions [21] and PEG [5], or the capillary was modified [17,19,22–24].

Generally speaking, do interactions between proteins and capillary surface modify the electrophoretic mobility measured? Nashabeh and El Rassi [23] noticed that the electrophoretic mobility of lysozyme and ribonuclease A was independent of the nature of the coatings with various kinds of PEG modified capillaries.

To answer this question, the comparison of mobility between PEI-modified and unmodified silica capillaries was made. The BGE based on 200 mmol l^{-1} boric acid (20 mmol l^{-1} NaCl) pH 7.0 was used with single standard protein solutions.

In this study, before any electropherograms were actually available, we observed that the capillary walls must be saturated by proteins. This stage of protein adsorption was evidenced by the EOF reduction. In most cases, it required several injections before electrophoretic mobilities could be measured.

α-Lactalbumin and β-lactoglobulin (A+B) were injected in four different capillaries (two modified and two unmodified, between ten and twenty-five injections for each capillary). Protein mobility was the same, within a precision of 15%, regardless of the capillary. Therefore mobility determination was

found to be independent of the chemical nature of the capillary surface, contrary to quantitative determination.

Thus, in the following study, only the BGE composition will be described and no attention will be paid to the chemical nature of the capillary used (however, measurements were made on different capillaries for each BGE composition).

4.3. Theoretical mobility versus ionic strength

The actual size of proteins in solutions is often discussed in literature. The use of the gyration radius for describing the diffusive behaviour of proteins in solutions was proved to be satisfactory [25] while other workers have used the Stokes radius with more or less satisfaction (Ref. [7] and Refs. cited herein). Loret et al. [26] previously observed that the Stokes radius produces a unique calibration curve for globular proteins, dextrans and gelatin in size-exclusion chromatography. According to this study, because lysozyme and whey proteins are globular, the Stokes radius (Rs) was used in the following (Table 1).

The net charge (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) (Table 1). Mobility was calculated according to Stokes' law ($I=0$) (Eq. (4)) (Table 1).

Lysozyme mobilities at pH 7.0 and 8.0 (Fig. 2) and holo and apo α-lactalbumin at pH 7.0 (Fig. 3) were calculated in relation to ionic strength. The mobility decrease turned out to be significant when the ionic strength increased from 0 to 20 mmol l^{-1}.

4.4. A basic protein: lysozyme

Lysozyme has a basic isoelectric point (i.e.p.) approaching 11 (Table 1).

The electrophoretic mobility of lysozyme was studied in different BGEs: triethanolamine (TEA Cl) pH 7.0, phosphate pH 7.0 and borate pH 8.0, versus ionic strength, with three different capillaries (one silica and two modified capillaries).

4.4.1. TEA Cl pH 7.0: indifferent electrolyte

In a 5 mmol l^{-1} TEA Cl BGE, the lysozyme electropherogram was characterized by a main peak (Lys 1), the mobility of which was $+2.8\pm0.2\cdot10^{-8}$ $m^2\ s^{-1}\ V^{-1}$. This peak obviously corresponds to Lys a of the RP-HPLC chromatograms.

An increase in ionic strength was brought about through the addition of NaCl in the 5 mmol l^{-1} TEA

Table 1
Calculated charges (Z) from amino acid composition with pK_a values of the side chain of free amino acid from [33], and calculated μ (10^{-8} $m^2\ s^{-1}\ V^{-1}$) according to Eq. (4) at 25°C ($\eta=0.8937$ Pa s, for water) (see Section 4.4 for details) and Stokes radius Rs (10^{-10} m) at pH 7.0 and 8.0 for different proteins

Proteins Reference (AA composition)	i.e.p.	Rs	Z pH 7.0	Z pH 8.0	μ pH 7.0	μ pH 8.0
αLactalbumin [28]	4.2–4.5	19.5				
apo		[31]	−3.7	−4.0	−1.80	−1.96
holo ($+Ca^{2+}$)			−1.7	−2.0	−0.82	−0.99
β-Lactoglobulin[b] [28]		mono[a]	dimer	mono	dimer	mono
A	5.13	20.1	−17.6	−9.0	−6.49	−4.28
B	5.13	dimer 25.7 [31]	−15.6	−8.0	−5.75	−3.80
Lysozyme [32]	11	18.3 [26]	+7.1	+7.0	+3.68	+3.62

[a] Mono: monomer.
[b] A, B: genetic variants.

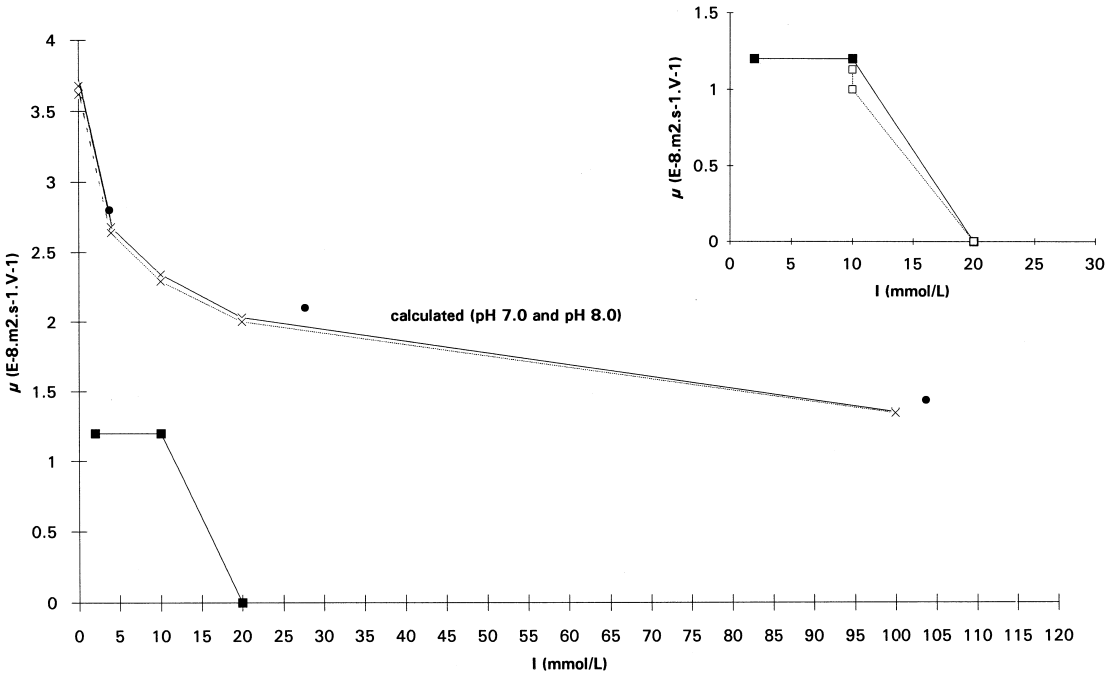

Fig. 2. Theoretical (according to Eq. (3)) and experimental μ at 25°C pH 7 of ''native'' lysozyme versus ionic strength I (dashed line: pH 8.0). ●: TEA Cl BGE pH 7.0, ■: phosphate BGE pH 7.0, □: borate pH 8.0 (insert figure). Each point represents the mean of minimum seven, maximum thirteen measurements effected on one capillary (four different capillaries used).

Cl BGE. The mobility of native lysozyme decreased to $+1.43\pm0.07\cdot10^{-8}$ m^2 s^{-1} V^{-1} with $I=104$ mmol l^{-1}. Fig. 2 shows a good agreement between observed values and calculated ones (Eq. (3)). As a result TEA Cl and NaCl may be assumed to be electrolytes that are indifferent to lysozyme.

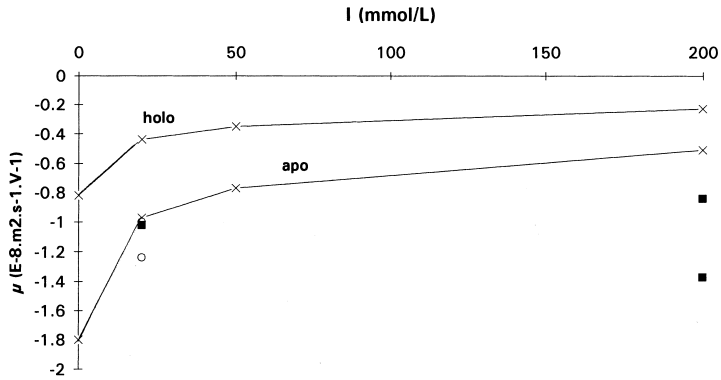

Fig. 3. Theoretical (according to Eq. (3)) and experimental μ at 25°C pH 7.0 of α-lactalbumin versus ionic strength I. ■: Phosphate BGE, ○: borate BGE. Each point represents the mean of minimum seven, maximum twenty-five measurements effected on one capillary (three different capillaries used).

There was strong agreement with the unchanged peak of the native protein obtained by RP-HPLC in single TEA Cl or KCl (range: 0–100 mmol l^{-1}).

4.4.2. Na, K phosphate pH 7.0: specifically adsorbed electrolyte

The electrophoretic mobility of lysozyme was studied in 2.5, 5 and 10 mmol l^{-1} phosphate concentrations at pH 7.0.

With 5 mmol l^{-1} phosphate ($I=10$ mmol l^{-1}), two different peaks appeared, the main one (Lys 1′), assumed to be the native protein ($\mu=1.2\pm0.2\cdot10^{-8}$ m^2 s^{-1} V^{-1}, $n=11$), represents about 91% in the corrected area (in good agreement with RP-HPLC), whereas a small peak ($\mu=0.17\pm0.03\cdot10^{-8}$ m^2 s^{-1} V^{-1}, $n=11$; 9% in corrected area) was located just before the EOF (Fig. 4). This second peak (Lys 2) corresponded to Lys b of the RP-HPLC chromatograms.

A single peak, located in the EOF area was detected when lysozyme was injected, with 10 mmol l^{-1} phosphate ($I=20$ mmol l^{-1}) BGE.

''Native'' lysozyme mobility, according to phosphate concentration is shown in Fig. 2. Observed mobility was lower than in TEA Cl BGE. Therefore, the i.e.p. of lysozyme becomes about 7.0 in the phosphate BGE of I close to 20 mmol l^{-1}.

Mobility variation reported in the literature showed different behaviour than expected. Lysozyme mobility was constant when increasing the ionic strength of phosphate pH 3.8, (a calculation based on the migration time given in [19]), which probably indicates that interactions occurred between lysozyme and phosphate.

As shown by RP-HPLC, lysozyme was only slightly phosphated when $I\geq20$ mmol l^{-1}. In CE, only one peak was detected at $I=20$ mmol l^{-1}, perhaps because lysozyme phosphatation occurred dynamically in the capillary and not prior to injection in reference to the sample injected in the RP-HPLC system.

4.4.3. Boric acid pH 8.0

The electrophoretic mobility of main lysozyme determined at pH 8.0 was about $1\cdot10^{-8}$ m^2 s^{-1} V^{-1} in a 100 mmol l^{-1} boric acid BGE ($I=10$ mmol l^{-1})

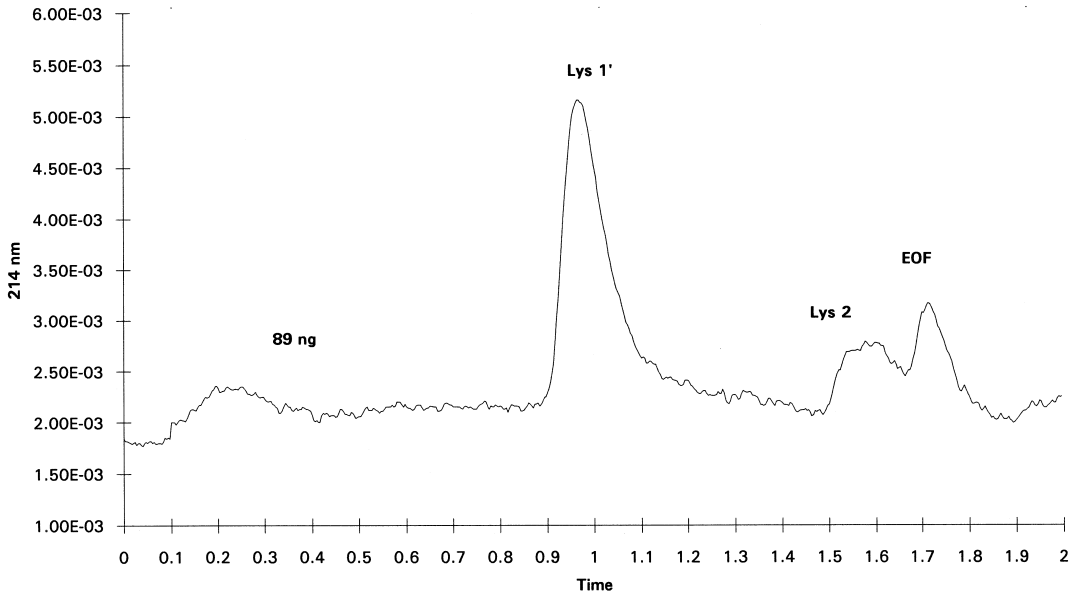

Fig. 4. Electropherogram of (89 ng injected) lysozyme dissolved in water when 5 mmol l^{-1} phosphate pH 7.0 was used as BGE. Lys 1′: native lysozyme; Lys 2: minor species.

and close to zero in 200 mmol l^{-1} boric acid BGE ($I=20$ mmol l^{-1}). Fig. 2 showed again that mobility was lower than expected. The i.e.p. of lysozyme is close to 8.0 in 200 mmol l^{-1} boric acid electrolyte.

Accordingly, monovalent borate anions or boric acid, as phosphate multivalent anions, are assumed to be specifically adsorbed on lysozyme.

4.5. Acid proteins: α-lactalbumin, β-lactoglobulin

α-Lactalbumin and β-lactoglobulin have i.e.p. values close to 5 (Table 1). 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 higher pH. Holo α-lactalbumin was used in this study. β-Lactoglobulin is a subunit protein, in dimer form at pH 7.0. At pH approaching 8.0, the denaturation of disulfide bonds may occur and the monomer form is predominant [27,2].

4.5.1. α-Lactalbumin with borate and phosphate pH 7.0

The electrophoretic mobility of α-lactalbumin at 25°C and pH 7.0 was studied with boric acid and phosphate BGE. A typical electropherogram of single α-lactalbumin was characterized by two peaks, and the main one was assumed to be the native protein, whereas the minor one was unidentified. Minor unidentified species present in α-lactalbumin sample were detected in gel electrophoresis [28].

Mobility [$\mu=(-1.1\pm0.1)\cdot10^{-8}$ m^2 s^{-1} V^{-1}] was the same in phosphate and boric acid at $I=20$ mmol l^{-1}, surprisingly close to that of the apo α-lactalbumin form (Fig. 3).

Moreover, in phosphate BGE pH 7.0, and $I=200$ mmol l^{-1}, μ remained unchanged and far from calculated values with regard to both the apo form and the holo form.

Interaction between phosphate at pH 7.0 and holo α-lactalbumin occurs, possibly through a calcium bridge, as mobility is independent of ionic strength in the 20–200 mmol l^{-1} range.

Further investigations performed with apo α-lactalbumin could evidence the role of divalent calcium.

4.5.2. Electrophoretic mobility of β-lactoglobulin with boric acid at pH 7.0 and pH 8.0

Electrophoretic mobility of β-lactoglobulin in 200 mmol l^{-1} boric acid BGE at 25°C, was studied both at pH 7.0 and 8.0, where the protein is in dimer and monomer form, respectively.

The two genetic variants A and B were well-resolved, as expected according to their charge difference. At pH 7.0, the mobilities of β-lactoglobulin A and B were close together but far from their calculated values. The assumption that dimer could be dissociated by the electric field no longer holds true, as the mobility observed was not intermediate between those of monomer and dimer (Table 2). Similar abnormal mobilities were observed at pH 8.0, close to those measured at pH 7.0.

As for lysozyme in phosphate, abnormal observed mobility could indicate that interactions occurred between the protein and the electrolyte.

We still suspected that phosphate probably interacted with negatively charged α-lactalbumin. Our results suggest that interactions occurred between borate or boric acid and β-lactoglobulin at basic pH.

Separation of the two A and B variants of β-lactoglobulin were shown at acidic pH in phosphate (pH 2–2.5, perhaps denatured protein) in different studies [20,29], whereas no charge difference was predicted according to the amino acid composition. According to lysozyme behaviour, an explanation could be that differential interactions between

Table 2
Calculated mobilities, according to Eq. (3), and experimental values (μ 10^{-8} m^2 s^{-1} V^{-1}) obtained in single solutions when 200 mmol l^{-1} boric acid and 20 mmol l^{-1} NaCl pH 7.0 was used as BGE ($I=20$ mmol l^{-1}) at 25°C, E between 270 and 811 V cm^{-1}.

Protein	μ_{theor}		μ_{exp} single
α-Lactalbumin			−1.1
apo	−0.97		
holo	−0.4		
β-Lactoglobulin	dimer	monomer	
A	−3.06	−2.20	−1.6
B	−2.71	−1.94	−1.5

μ average of between 29 to 43 measurements for each protein measured with three different capillaries. Standard deviation 0.2.

positively-charged β-lactoglobulin and negatively-charged phosphate occurred.

The β-lactoglobulin behaviour in CE remains unclear.

5. Conclusions

In this study, the experimental mobility of proteins versus the ionic strength of various background electrolytes, was compared with theoretical mobility according to the Henry's equation.

The physicochemical behaviour of proteins was investigated.

First of all, in spite of the adsorption stage which occurs in the case of proteins on unmodified and PEI modified capillaries, the mobility of a protein in a given BGE was the same, with a precision of 15%, regardless of the capillary.

The theoretical mobility of lysozyme matches well with that obtained when using an indifferent BGE, as such as chloride (TEA, Na).

As the mobility of lysozyme versus phosphate, and borate, concentration was close to zero, the isoelectric point becomes close to 7.0 in phosphate and close to 8.0 in borate, in a medium of 20 mmol l^{-1} ionic strength in both cases. The phosphate multivalent anion as well as borate monovalent anion, both oxyanions, are specifically adsorbed on lysozyme.

RP-HPLC shows that the native lysozyme peak area decreases with increasing phosphate concentration, with the appearance of less hydrophobic species, presumably phosphated lysozyme.

It was shown elsewhere [30] that with divalent phosphate at pH 9 the lysozyme charge can be reversed into negative charge over $I=20$ mmol l^{-1}.

Thus, CE and RP-HPLC are complementary tools for characterizing the charge and/or the hydrophobic behaviour of protein in indifferent electrolytes as well as with specifically adsorbed ions.

As the mobility of α-lactalbumin in phosphate remained constant when increasing I from 20 to 200 mmol l^{-1}, at pH 7.0, interactions between the protein and phosphate were assumed. Presumed interactions between the protein and boric acid and/or borate also occurred at pH 8.0 and $I=20$ mmol l^{-1}.

β-Lactoglobulin consistently exhibited lower mobilities than those expected in boric acid at pH 7.0 and 8.0, ($I=20$ mmol l^{-1}), when calculated for monomer form as well as for dimer form. These results suggested that interactions occurred between the protein and boric acid and/or borate at basic pH.

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