Polyion character of globular proteins detected by translational and rotational diffusion

Giuseppe Chirico* and Sabrina Beretta

Istituto Nazionale per la Fisica della Materia, Dipartimento di Fisica, Università di Milano Bicocca, Via Celoria 16, I-20133 Milano, Italy

(Received 12 March 1999; revised manuscript received 26 April 1999)

Photon correlation spectroscopy measurements are performed on a solution of lysozyme, a small, highly charged protein at pH=4, in glycerol-buffer mixtures. From the correlation functions of the polarized and depolarized scattered intensity, we derive an effective protein radius by inverting the Stokes Einstein relation for either the translational or the rotational diffusion, as a function of the protein concentration $0 \leqslant C \leqslant 40$ g/L. The translational radius decreases with the protein concentration and extrapolates at $C \simeq 0$ to a value slightly larger than the mean rotational radius, which is independent of the protein concentration. By employing recent calculations on electrolyte friction effects (eletrolyte-protein interactions) on both the translational and rotational radius and by keeping also into account the protein-protein electrostatic interactions, we are able to account for the observed differences, suggesting also that lysozyme is more highly hydrated in glycerol mixtures than in the pure buffer. These results indicate that depolarized photon correlation spectroscopy measurements can be used as a valuable tool to detect small changes in the overall protein size.
[S1063-651X(99)03908-2]

PACS number(s): 87.15.Vv, 82.70.−y, 87.14.Ee

I. INTRODUCTION

Proteins in ionic solutions far from the isoelectric point have a remarkable polyion character which greatly affect their diffusional properties through the electrostatic protein-protein and electrolyte-protein interactions. In fact, small proteins can bear a number of positive and negative groups resulting in a substantial net charge. As an example, lysozyme, a globular protein $\simeq 14\,300$ Dalton in molecular weight, has $\simeq 10$ positive charges at acidic $p\mathrm{H} \simeq 4.2$ and shows isoelectric conditions at $p\mathrm{H} \simeq 11$.

The change of the translational mutual diffusion coefficient with the protein concentration, C (in g/L), is known [1–4] to depend mostly on the direct and hydrodynamic protein-protein interactions, and can be relevant at low ionic strength and high protein charge. However, the enhancement of the translational mutual diffusion coefficient with finite protein concentration is partially attenuated (dynamic attenuation) [5,6] due to the finite time response of the small ions, which, because of the electrostatic interactions, are dragged together with the protein. Moreover, electrolyte-protein interactions affect the protein diffusional properties also at $C \simeq 0$. In fact, the cloud of electrolytes, whose motion is highly correlated to the protein diffusion, produces an enhancement of the protein friction coefficient (electrolyte friction) [7–9].

Protein-protein interaction effects on the rotational diffusion have been predicted to be remarkably smaller [10,11] than those on the translational diffusion and few experimental studies on small biopolymers have been reported [12]. As to the electrolyte-protein interaction effects on the rotational diffusion, recent theoretical studies [13] do not seem to have been compared yet to experimental results on proteins.

In order to investigate the translational and rotational diffusion properties of proteins, one can use photon correlation spectroscopy of the polarized and depolarized scattered laser light. When a cross-correlation method is used, in which two copies of the light signal scattered at an angle θ are detected by two different photomultipliers, the cross correlation of the outputs of the detectors is equal to the autocorrelogram of the scattered light detected by an ideal photomultiplier with reduced spurious correlations (afterpulsing, electronics reflections, etc.) as originally proposed by Arecchi [14].

We have recently shown [15] that this experimental method allows the measurement of the correlogram of the light depolarized by a lysozyme solution, investigating the possible dependence of the rotational diffusion coefficient with the protein concentration. The aim of this paper, stimulated also by the recent theoretical predictions of electrolyte friction effects for anisotropic molecules [13], is to study experimentally these effects on the translational and rotational diffusional properties.

We use here a home-made setup, described elsewhere [16], capable of detecting tiny light fluctuations ($\simeq 1\%$) with a minimum lag time of 12.5 ns. Both the translational and rotational diffusion coefficients of lysozyme, at $p\mathrm{H} \simeq 4.6$ and at low ionic strength $\simeq 45$ mM, are measured from the relaxation rate of the correlogram of the polarized and depolarized scattered light. In order to bring the rotational relaxation times within the accessible time window (i.e., relaxation times larger than 100 ns), we have slowed the protein diffusion by adding glycerol to the solution [15], while the translational diffusion has been investigated both in buffer solutions and water/glycerol mixtures. By following the behavior of the diffusion coefficients versus the protein concentration and temperature, it is found that the protein-protein interactions, though very effective on the translational diffusion, do not affect appreciably the rotational motions in solution, as expected for such small molecules [15,12].

The novelty of the present communication consists in the comparison of the effects of the electrolyte-protein interactions on the translational and rotational frictional properties,

*Electronic address: giuseppe.chirico@mi.infm.it

 © 1999 The American Physical Society

experimentally determined by means of photon correlation spectroscopy of polarized and depolarized light, to the existing theoretical predictions [13]. It is confirmed also that the electrolyte-protein interactions alter only slightly the rotational diffusion of the protein. The present results indicate that a measure of the protein radius from the inversion of the Stokes relation for the rotational diffusion can be taken directly as a measure of the real protein radius even at finite protein concentration.

II. MATERIALS AND METHODS

The optical source employed for the scattering measurements is an argon laser (Spectra Physics, 2025) tuned at $\lambda = 514$ nm. Typical powers are $P = 600$ mW and $P = 1$ W, for polarized and depolarized light scattering experiments, respectively. The sample cell is a square Hellma 10 mm quartz cell and the true scattering angle has been derived from the angle measured on the goniometer according to Snell's refraction law. The scattering angle in the polarized configuration is $\theta = 113.8°$ (goniometer angle $= 125°$) for the experiments versus temperature, and $\theta = 90°$ for measurements versus protein concentration. For the depolarized light scattering measurements we use $\theta = 2.4°$ (goniometer angle $= 3.4°$) and the polarization state is controlled by two Glan-Thomson polarizers with a procedure previously described [17]. A detailed description of the construction and testing of the cross-correlation apparatus is given elsewhere [16].

Chicken egg white lysozyme (Sigma Co. Lot. 65H7025) was used without further purification and showed a polydispersity less than 0.1 measured by cumulant analysis [18] of the polarized light correlograms. The lysozyme was dissolved in acetate buffer or in 60% w/w (weight fraction) glycerol/acetate buffer mixture. In both cases ionic strength was $\mu = 45$ mM and the solution pH was verified, before and after measurements, to be $4.2 \leq p\mathrm{H} \leq 4.6$. The glycerol (ACS reagent) concentration of the mixture was checked by measuring the refraction index ($n = 1.4127 \pm 0.0005$ at $T = 20\,°\mathrm{C}$). The mixture viscosity at various temperatures and its dielectric permeability have been estimated from an interpolation of published tables [19,20]. The lysozyme concentration has been always measured spectrophotometrically [$\varepsilon(280\,\mathrm{nm}) = 2.64$ L/g/cm].

Possible conformational changes of lysozyme in acetate buffer and 60% w/w glycerol/acetate mixtures was followed at $T = 20\,°\mathrm{C}$ by circular dichroism measurements on a Jasco J500A spectropolarimeter (Japan Spectroscopic Co.). In the near uv region ($250 < \lambda < 350$ nm), spectra were recorded at protein concentration $C \simeq 0.3$ g/L with a square cell with 10 mm path length and the data averaged 40 times. The spectra in the far uv region ($200 < \lambda < 250$ nm) have been recorded at $C \simeq 0.2$ g/L solutions with a barrel quartz cell of 1 mm path length for circular dichroism and the data averaged 90 times. The mean residue molar ellipticities $[\theta]$ in degrees$\times$cm$^2\times$decimole^{-1} have been computed according to the protein concentration assuming for lysozyme a molecular weight of 14300 Dalton and 132 residues.

III. DATA ANALYSIS

Measurements of the normalized intensity correlograms, $g_I(t)$, have been analyzed according to the relation [17,21]

$$g_I(t) = 1 + S_N\left(\frac{I_S^2}{(I_S+I_B)^2}|g(t)|^2 + \frac{2I_BI_S}{(I_S+I_B)^2}\mathrm{Re}\{g(t)h^*(t)\}\right), \tag{1}$$

where S_N indicates the signal-to-noise ratio, I_S is the amount of scattered light due to the solute, I_B is the background scattering, which is assumed to be uncorrelated, $g(t)$ is the normalized field correlation function, and $h(t)$ is the normalized autocorrelation function of the background light due to slow moving large aggregate or ''dust'' in solution, assumed to be constant. In the measurements of the depolarized scattered light one finds $I_S \ll I_B$, and the measured correlation function is approximated by the real part of $g(t)$, i.e., $g_I(t) - 1 \propto \mathrm{Re}\{g(t)\}$, while in the measurements of the polarized scattered light, for which $I_S \gg I_B$, one assumes $g_I(t) - 1 \propto |g(t)|^2$.

The correlograms from polarized light scattering measurements are fitted to a multiple-exponential decay (two or three exponential components). The slowest relaxation (rate $\simeq 500$ Hz) is attributed to residual dust, since the particle size corresponds to the filter pore diameter, and is disregarded in the following discussion. The measurements on glycerol mixtures show a fast (relaxation rate $\simeq 170$ kHz) component which is ascribed to the self-diffusion of glycerol in the mixture [15]. The component with relaxation rates $\Gamma_P \simeq 10-50$ kHz is attributed to the protein mutual diffusion and related to an effective protein radius:

$$R_{\mathrm{eff},T} = \frac{k_BT}{6\pi\eta}\frac{q^2}{\Gamma_P}, \tag{2}$$

where q^2 is the square of the scattering vector. It is important to notice that the above definition, while taking into account the effect of temperature T and of solution viscosity η, does not correspond to the real protein radius, since all the possible interprotein and protein-electrolyte interactions are not explicitly taken into account.

The correlograms of the depolarized light scattering can always be analyzed by a single exponential decay. Since the depolarized scattering intensity was typically very low (depolarization ratio $\simeq 1/200$), measurements required almost 12 h to obtain reasonable photon statistics. A typical protein correlogram and its single exponential fit is shown in Fig. 1 together with the corresponding correlogram from the solvent alone (the 60% w/w glycerol/acetate mixture). The protein correlogram shows an exponential decay while there is no evidence of correlation for the solvent. The relaxation rate $\Gamma_D = \Gamma_P + 6\Theta \simeq 15$ MHz can be approximated as $\Gamma_D \simeq 6\Theta$, where Θ is the rotational tumbling diffusion coefficient, since the contribution Γ_P is only $\simeq 30$ Hz at low q^2, where the depolarized measurements are performed. Similarly to the experiments in polarized scattered light, one can define an effective protein radius as

$$R_{\mathrm{eff},R}^3 = \frac{k_BT}{(8\pi\eta\Theta)} \tag{3}$$

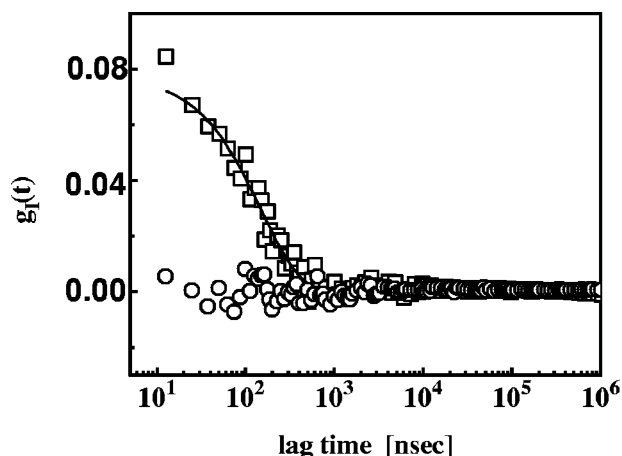

FIG. 1. Correlation function of the depolarized light scattered by a $C=37$ g/L lysozyme solution at $\theta=2.4°$, $T=7.5\,°$C in 60% w/w glycerol/acetate mixture. Squares indicate the lysozyme solution and circles correspond to the glycerol/buffer mixture. The solid line is a single exponential decay best fit to the lysozyme correlogram.

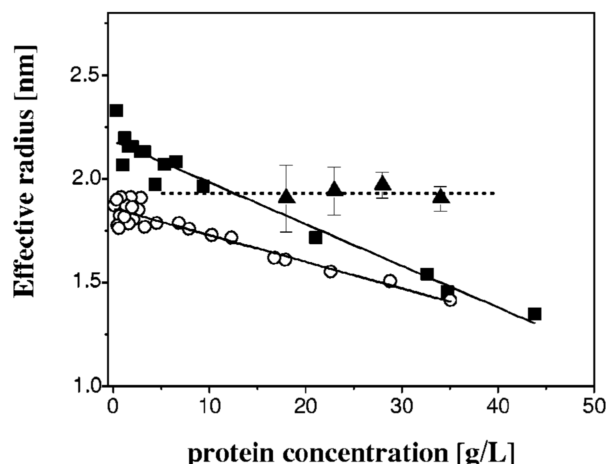

FIG. 3. Translational effective radii versus the lysozyme concentration as measured in 60% glycerol/acetate mixtures (filled squares) and in acetate buffer (open circles) at $T\simeq 20\,°$C. Solid lines are linear fit to the data. The rotational effective radii measured in 60% glycerol/buffer mixtures are also reported (filled triangles) and the horizontal dashed line indicates their average value.

and again the protein size defined above may not be the real protein size since protein-protein and electrolyte-protein interactions are not explicitly accounted for.

IV. RESULTS

The rotational and translational effective radii of lysozyme in 60% glycerol mixtures are reported in Fig. 2 versus the ratio (T/η) of the solution temperature to viscosity in the range $-2\leqslant T\leqslant 12\,°$C for a protein concentration $C\simeq 37$ g/L (volume fraction $\simeq 0.03$). Both the effective radii show an approximately constant behavior indicating that the translational and rotational diffusional properties are scaling with T/η as expected. It is noteworthy that the average sizes obtained by polarized and depolarized scattering are substantially different, $R_{\mathrm{eff},T}=1.40\pm 0.03$ nm and $R_{\mathrm{eff},R}=1.93\pm 0.05$ nm.

The translational diffusion has been followed also versus the protein concentration in the range $0.3<C<40$ g/L at $T\simeq 20\,°$C, both in acetate buffer and the glycerol/acetate mixtures, and the corresponding effective radius, $R_{\mathrm{eff},T}$, is shown in Fig. 3. In both cases one can fit the experimental trend to a linear law

$$R_{\mathrm{eff},T}=R_{\mathrm{eff},T0}(1+S_T C) \tag{4}$$

finding, however, different values for the extrapolation at $C=0$ and for the slope: $R_{\mathrm{eff},T0}=2.18\pm 0.02$ nm and $S_T=-9.2\pm 0.5$ mL/g for the 60% glycerol/buffer mixture and $R_{\mathrm{eff},T0}=1.85\pm 0.01$ nm and $S_T=-6.9\pm 0.5$ mL/g for the acetate buffer solution.

Finally, the rotational diffusion has been studied at different protein concentrations $(18\leqslant C\leqslant 36$ g/L) only in glycerol mixtures and at a low temperature $T\simeq -4.5\,°$C. The corresponding effective radius $R_{\mathrm{eff},R}$ is reported versus C in Fig. 3 (filled triangles) with an average value $R_{\mathrm{eff},R}=1.93\pm 0.03$ nm (dashed line). No appreciable change occurs in the range of concentrations investigated here.

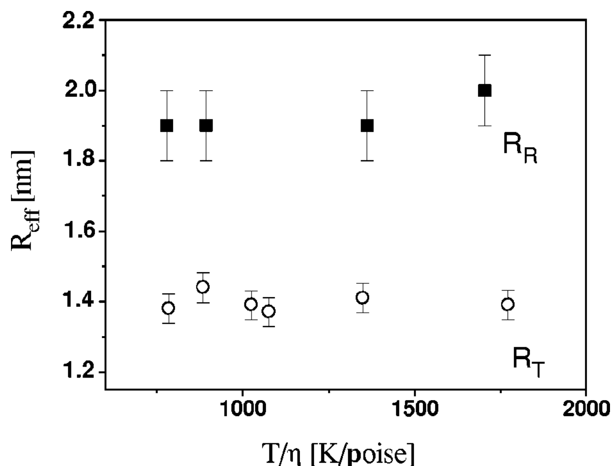

FIG. 2. Rotational (filled squares) and translational (open circles) effective radii versus the ratio of the solution temperature and viscosity as measured in 60% glycerol/acetate mixtures.

V. DISCUSSION

The translational and rotational effective radius, measured at finite protein concentration, does not depend appreciably upon the ratio of the temperature and viscosity (see Fig. 2) in agreement with the prediction of the Stokes-Einstein relations [5]. This result is not surprising, since the temperature dependence of the interaction contribution constant S_T is very small in the narrow temperature range explored here. Interestingly, the remarkable difference observed between the average values of the translational and rotational radii, which is very close to the size reported for lysozyme at infinite dilution [22,23], suggests the presence of substantial protein-protein interactions that affect mainly the translational diffusion.

The presence of interaction effects on the protein diffusion properties is confirmed by the decrease of $R_{\mathrm{eff},T}$ with increasing C (Fig. 3). The slope S_T is dependent on the protein-protein interaction energy [1,24] and can be computed by assuming a DLVO potential [24], which is a com-

bination of a screened Coulomb and a short-range dipolar interaction. Since we are investigating lysozyme far from its isoelectric pH and at relatively low ionic strength ($\simeq 45$ mM), the main contribution is the electrostatic repulsion. As a matter of fact, the steeper dependence of $R_{\mathrm{eff},T}$ in the glycerol/acetate mixtures than in pure acetate buffer correlates with the value of the dielectric permeability ε of the two solvents, $\varepsilon_{\mathrm{water}} \simeq 78.5$ while [19] $\varepsilon_{60\%\ \mathrm{glycerol}} \simeq 62$ at $T = 20°$, which induces more important electrostatic interactions in glycerol mixtures than in a buffer. A more detailed analysis, similar to that reported elsewhere [1,25,15], shows that the slopes measured in the two solutions are compatible with a protein charge of $Q = 9 \pm 2$ for the acetate buffer and $Q = 10 \pm 1.5$ for the 60% w/w glycerol/acetate mixture.

In interpreting the dependence of the hydrodynamic radii with the protein concentration, one should also consider the dynamic attenuation [7,5] due to electrolyte-protein interactions. When taking into account such effects, the dependence of $R_{\mathrm{eff},T}$ on C becomes

$$R_{\mathrm{eff},T} = R_{\mathrm{eff},T0}(1 + S_T C)[1 + f_{\mathrm{BD}}(C,\mu)], \tag{5}$$

where μ is the solution ionic strength and $f_{\mathrm{BD}}(C,\mu)$ is a function given by Belloni and Drifford [26] [Eq. (16) of the cited reference]. However, the correction due to the dynamic attenuation term is not remarkable in our experimental conditions since $f_{\mathrm{BD}}(C,\mu)$ is only $\simeq 0.02$ at the largest protein concentration investigated here while the protein-protein interaction term $S_T C \simeq 0.36$. Therefore, the simple linear relation in Eq. (4) correctly describe the data.

The concentration dependence of the rotational effective radius $R_{\mathrm{eff},R}$ is not appreciable here, in agreement with theoretical predictions verified on large colloids [10,11] and also with experimental results on tRNA [12]. Dynamic attenuation effects on the rotational diffusion are assumed to be negligible.

We discuss now the more interesting finding of the large ($\simeq 15\%$) difference in the extrapolated values of the effective radii $R_{\mathrm{eff},T}$ in the two solutions. It must be noted that this variation is not negligible since our experimental uncertainties are below 1%. The observed discrepancy could be a consequence of a different folding state or hydration of the protein in glycerol [27] with respect to the acetate buffer, as well as an effect of the enhanced protein friction due to interactions with its counterions cloud, a phenomenon known as electrolyte friction [13]. In fact, even in the same glycerol/acetate mixture the extrapolated translational effective radius, $R_{\mathrm{eff},T0}$, is higher than the corresponding rotational radius, $R_{\mathrm{eff},R}$, indicating that translational and rotational frictions are differently affected by (solvent) electrolyte-protein interactions.

However, at least part of the observed difference in the protein radius might be due also to different protein folding in the two solvents. The circular dichroism spectra in the near and far uv region (Fig. 4) for the acetate buffer and the 60% glycerol/acetate mixture solutions have then been measured in order to check the degree of folding. The spectra show that the secondary structure of the protein is not remarkably affected by glycerol and appears similar to those reported in the literature [28]. The minor differences observed in the negative peak of the far uv region (λ

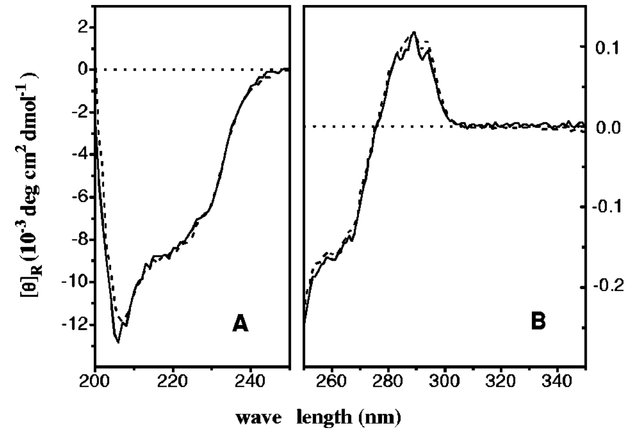

FIG. 4. Circular dichroism spectra in the far- and near-uv region (panels A and B, respectively) for lysozyme in 60% glycerol/acetate mixtures (dashed line) and in acetate buffer (solid line).

$\leqslant 205$ nm) might be either due to changes in the aromatic contribution or to slight changes in the backbone conformation, which are not expected to involve a remarkable increase of the protein radius.

The observed difference between the translational radii in the two solvents should then be ascribed to the interactions between the protein and the surrounding counterions, which may be differently modulated in the two solvents. In general, the friction coefficient of a charged sphere of radius R and total charge Q in an electrolyte solution at ionic strength μ can be expressed as [29,7]

$$\zeta = \zeta_0 + \Delta\zeta_{\mathrm{el}}(\mu,R,Q),$$

where $\zeta_0 = 6\pi\eta R$ is the friction coefficient of the protein in absence of electrolytes, and R can be assumed as a ''bare'' protein radius. The first theoretical studies [29,7] made specific assumptions on the electrolyte size and their hydrodynamic coupling, which have been relaxed by a more recent work by Medina-Noyola *et al.* [8,9] based on the generalized Langevin equation (GLE) approach [30]. Moreover, a treatment of electrolyte friction effects on the protein rotation based on the GLE theory has been recently presented [13].

We compare here Schurr's theoretical prediction [7] and two different approximations of the Medina-Noyola treatment [Eq. (16) and Eq. (18) of Ref. [8]] for the computation of the ''bare'' protein radius. Since the correction term, $\Delta\zeta_{\mathrm{el}}$, depends on the protein radius and charge, the protein radius is recovered by iteratively solving the equation

$$R_T = R_{\mathrm{eff},T0} - \frac{\Delta\zeta_{\mathrm{el}}(\mu,R_T,Q)}{6\pi\eta}. \tag{6}$$

The application of the different models gives a very similar result (see Table I). For the glycerol/acetate mixtures the agreement between the translational radius, $R_T \simeq 1.9$ nm, and the effective rotational radius (see Figs. 2 and 3), $R_{\mathrm{eff},R} \simeq 1.93 \pm 0.03$ nm, is very good. The $\simeq 15\%$ difference observed between the estimates of $R_{\mathrm{eff},T0}$ in the two solvents is therefore to be ascribed almost entirely to electrolyte friction effects. However, even after these corrections, a systematically higher ($\simeq 9\%$) translational radius R_T is found for the data taken in the glycerol/acetate mixtures than in the acetate

TABLE I. Protein radius corrected for the electrolyte friction effect.

Solution	R_T [nm][a]	R_T [nm][b]	R_T [nm][c]	Protein charge[d]
Acetate buffer	1.7	1.7	1.65	9±2
60% glycerol	1.85	1.9	1.85	10±1.5

[a]Computed according to Ref. [7].
[b]Computed according to Ref. [8], Eq. 16.
[c]Computed according to Ref. [8], Eq. 18.
[d]In proton units.

solutions (see Table I), which do not seem to be due to major protein unfolding as checked by the circular dichroism spectra in the two solvents. As a matter of fact, polyols such as glycerol or sorbitol modify the protein-solvent interactions inducing higher protein hydration and stability [31,27]. It is likely that the larger radius observed here in glycerol mixtures is due to a thicker protein hydration layer corresponding to $\simeq 0.2$ nm or almost one water shell.

The good agreement between R_T and $R_{\mathrm{eff},R}$ in the glycerol mixtures suggests that there are no appreciable effects of electrolyte-protein interactions on the rotational diffusion. In order to confirm this, we can consider different types of approximations for the theoretical prediction of the electrolyte friction effect on the rotational motion. [13] However, one gets very similar results when the translational diffusion coefficient of the electrolyte D_{el} is much larger than the protein diffusion coefficient D_{0p}, as is the case here where $D_{\mathrm{el}} \simeq 125\times 10^{-8}\ \mathrm{cm}^2/\mathrm{s}$ (computed assuming an ion radius $\simeq 0.15$ nm) and $D_{0p} \simeq 10\times 10^{-8}\ \mathrm{cm}^2/\mathrm{s}$ (computed assuming a protein radius $\simeq 2$ nm) in glycerol mixtures. An estimate of the electrolyte friction contribution to protein rotational friction, $\Delta\zeta_R$, is given by [13]

$$\Delta\zeta_R = \frac{Q^2\sqrt{a^2-b^2}}{12\varepsilon(D_{\mathrm{el}}+D_{0p})} G_{\mathrm{rot}}(\kappa b, a/b), \qquad (7)$$

where a and b indicate the long and short axis of the protein assumed as a prolate ellipsoid and κ^{-1} is the Debye screening length [5]. The function G_{rot} must be computed numerically as an integral of Bessel functions and its expression is given in Eq. (56) of Ref. [13]. The predicted behavior for the present case, with $\varepsilon \simeq 62$ (for 60% glycerol/acetate mixtures) [19] and protein axial ratio [22] $a/b \simeq 2$, is reported in Fig. 5 and the abscissa value corresponding to $\mu \simeq 45$ mM is indicated by a vertical line.

By assuming the values of $a = 2.75$ nm and $b = 1.65$ nm reported for lysozyme [22], one can estimate a relative

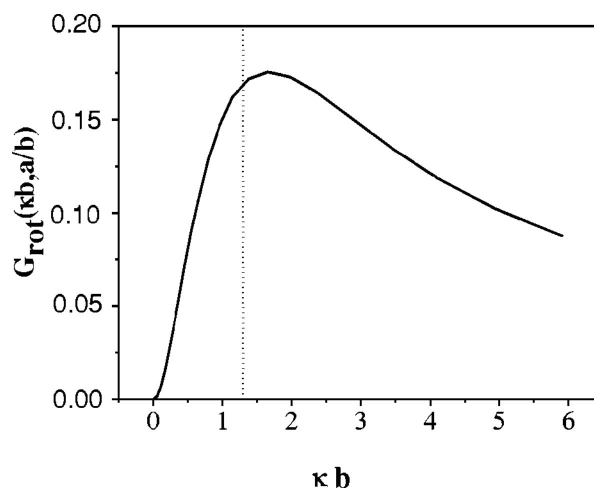

FIG. 5. Correction factor for the electrolyte friction on the rotational diffusion, G_{rot}, versus the product κb of the Debye screening constant and the smaller protein axis. The computation is performed for axial ratio $a/b = 2$ according to Eq. (56) of Ref. [13].

change in the effective protein radius $\Delta R_R/R_R \simeq \Delta\zeta_R/(3\zeta_R) \simeq 0.01$, which amounts to $\simeq 0.02$ nm, a negligible correction compared to our experimental uncertainty on $R_R = 1.9 \pm 0.1$ nm.

VI. CONCLUSIONS

We have shown that the hydrodynamic radius obtained from the relaxation rate of the correlograms of the polarized light is fully compatible with the estimate made from the depolarized light scattering measurements once the effects of protein-protein and electrolyte-protein interactions are carefully taken into account. Moreover, the depolarized scattering is shown to give a direct measurement of the hydrodynamic radius almost free of contributions from electrolyte friction or protein-protein interactions at protein concentration $C \leqslant 40$ g/L. Finally, the slightly larger protein radius detected in glycerol mixtures is compatible with an enhanced protein hydration in this solvent and supports the use of depolarized photon correlation spectroscopy for the measurements of fine changes in protein size.

ACKNOWLEDGMENT

This work has been partially supported by a grant of the Istituto Nazionale per la Fisica della Materia, advanced research project ''PROCRY.''

[1] M. Corti and V. Degiorgio, J. Phys. Chem. **85**, 711 (1981).
[2] G. D. J. Phillies, J. Phys. Chem. **99**, 4265 (1995).
[3] B. U. Felderhof, J. Phys. A **11**, 929 (1978).
[4] J. K. Batchelor, J. Fluid Mech. **74**, 1 (1976).
[5] K. S. Schmitz, *An Introduction to Dynamic Light Scattering by Macromolecules* (Academic Press, Boston, 1990).
[6] J. M. Schurr, Chem. Phys. **111**, 55 (1987).
[7] J. M. Schurr, Chem. Phys. **45**, 119 (1980).
[8] G. Cruz de Leon, M. Medina-Noyola, and O. Alarcon-Waess, Chem. Phys. Lett. **207**, 294 (1993).
[9] A. Vizcarra-Rendon, M. Medina-Noyola, and R. Klein, Chem. Phys. Lett. **173**, 397 (1990).
[10] R. Piazza, V. Degiorgio, M. Corti, and J. Stavans, Phys. Rev. B **42**, 4885 (1990).

[11] V. Degiorgio, R. Piazza, and R. B. Jones, Phys. Rev. E **52**, 2707 (1995).
[12] J. C. Thomas, J. M. Schurr, and D. R. Hare, Biochemistry **23**, 5407 (1984).
[13] M. Hernandez, O. Alarcon-Waess, and M. Medina-Noyola, J. Chem. Phys. **106**, 2492 (1997).
[14] F. T. Arecchi, M. Corti, V. Degiorgio, and S. Donati, Opt. Commun. **3**, 284 (1971).
[15] G. Chirico, S. Beretta, and G. Baldini, J. Chem. Phys. **110**, 2297 (1999).
[16] G. Chirico and M. Gardella, Appl. Opt. (to be published).
[17] G. Chirico and G. Baldini, J. Chem. Phys. **104**, 6020 (1996).
[18] D. E. Koppel, J. Chem. Phys. **57**, 4814 (1972).
[19] J. W. Lawrie, *Glycerol and the Glycols: Production, Properties and Analyses*, ACS Monograph Series (Chemical Catalog Co., New York, 1928).
[20] C. S. Miner and N. N. Dalton, *Glycerol*, ACS Monograph Series No. 117 (Reinhold, New York, 1954).
[21] B. J. Berne and R. Pecora, *Dynamic Light Scattering* (Wiley, New York, 1976).
[22] S. B. Dubin, N. A. Clark, and G. B. Benedek, J. Chem. Phys. **54**, 5158 (1973).
[23] D. E. Kuehner, C. Heyer, C. Rämsch, U. M. Fornefeld, H. W. Blanch, and J. M. Prausnitz, Biophys. J. **73**, 3211 (1997).
[24] J. K. G. Dhont, in *Studies in Interface Science*, edited by D. Möbius and R. Miller (Elsevier, Amsterdam, 1996).
[25] S. Beretta, G. Chirico, D. Arosio, and G. Baldini, J. Chem. Phys. **106**, 8427 (1997).
[26] L. Belloni and M. Drifford, J. Phys. (France) Lett. **46**, L-1183 (1985).
[27] S. N. Timasheff, Annu. Rev. Biophys. Biomol. Struct. **22**, 66 (1993).
[28] F. Tanaka, L. S. Forster, K. P. Pranab, and J. A. Rupley, J. Biol. Chem. **250**, 6977 (1975).
[29] F. Booth, J. Chem. Phys. **22**, 1956 (1954).
[30] M. Medina-Noyola and A. Vizcarra-Rendon, Phys. Rev. A **32**, 3596 (1985).
[31] K. Gekko and I. Hiroharu, J. Biochem. Biophys. Methods **107**, 572 (1990).