Characterization of lactate–guanidinium and lactate–lactate interactions in aqueous solution by spectropolarimetry

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Spectropolarimetry has been used to quantify the lactate–guanidinium and lactate–lactate equilibrium interactions in aqueous solution. Association constants for the lactate–guanidinium and lactate–lactate formations are 6.11 dm^3 mol^{-1} and 1.12 dm^3 mol^{-1}, respectively, in aqueous solution; 6.11 dm^3 mol^{-1} is a high value for an electrostatic interaction in water. This stability, however, cannot account for the extremely strong lactate–protein binding, observed earlier by NMR spectroscopy. The molar rotation coefficients for both the heteroassociation and homoassociation complexes are also calculated.

The homoassociative lactate–lactate binding is the first such interaction, whose constant has been determined by spectropolarimetry in aqueous solution.

Lactic acid, a vitally important biomolecule, has been recognized as an indicator of pathophysiological processes.[1–8] In fact, the lactic acid or lactate serum concentration can be used in the detection of several diseases,[1] and lactate interactions are likely to be important.

The guanidinium ion has also recently been the focus of related studies. Guanidinium was shown to be the least acidic protonated nitrogen-base in microequilibria,[9] its ion-pair formation with intramolecular carboxylates has been investigated in peptides[10] and its intermolecular binding selectivity has been described in chemical sensors.[11] Endocyclic guanidinium compounds have been synthesized for enantiodifferentiation of abiotic aliphatic[12] and aromatic[13] carboxylates. The strength of aqueous binding, however, has not been quantified in any of the above cases.

Here we report quantitative studies on lactate–guanidinium interactions in aqueous solution. To obtain an unbiased lactate–guanidinium constant, we also quantified the homoassociative lactate–lactate weak interaction. Carboxylic acid dimerization data appear in the literature,[14] but not for an aqueous solution.

In order to monitor the lactate–guanidinium and lactate–lactate associations, we used spectropolarimetry where rotation signals were detected as a function of concentration. Spectropolarimetry has not been used before to characterize weak interactions in aqueous solution.

The most important advantage of polarimetry and other chiroptical methods[15] is the inherent selectivity. Spectral effects of association are manifested on the chiral component only, and there is no interference from the guanidinium ion to increase the noise level of the measurement. In addition, the UV–VIS based evaluation methods for weak interactions can be readily applied here.

Polarimetry is certainly not among the most sensitive analytical methods. Weak interactions, however, necessitate the use of relatively high component concentrations which can be comfortably measured by polarimetry.

The amino acid arginine would have been a more obvious choice for a simple compound to use as a model for the lactate binding guanidinium residue of a protein. Nevertheless, we used the simple guanidinium ion instead of arginine, for the following reasons. First, the carboxylate and the ammonium sites may make the binding properties of arginine significantly different from that of an arginyl guanidinium residue in a protein. Also arginine, as a second chiral agent in solution, would almost certainly have caused composite chiroptical signals and concomitant difficulties in evaluating interactions.

The lactate–guanidinium association and the related equilibrium constant were defined as shown in eqns. (1) and (2),

$$L + G \rightleftharpoons LG \quad (1)$$

$$K_{1:1} = [LG]([L][G])^{-1} \quad (2)$$

where [L], [G] and [LG] are the equilibrium concentrations of lactate, guanidinium and the lactate–guanidinium complex, respectively. $K_{1:1}$ stands for the equilibrium constant (dm^3 mol^{-1}) of the lactate–guanidinium complex. The homoassociative dimerization process and its equilibrium constant are described in eqns. (3) and (4), where $[L_D]$ is the lactate dimer concentration and K_D is the dimerization constant.

$$L + L \rightleftharpoons L_D \quad (3)$$

$$K_D = [L_D]([L]^2)^{-1} \quad (4)$$

The lactate mass-balance equation in terms of L_T lactate total (analytical) concentration, [L] lactate equilibrium concentration and K_D dimerization constant is as shown in eqns. (5) and (6).

$$L_T = [L] + 2[L_D] \quad (5)$$

$$L_T = [L] + 2K_D[L]^2 \quad (6)$$

The observed rotation ($\alpha^{\lambda i}_{obs}$) at wavelength λ_i is given in eqn. (7); where $[\varphi_{1:0}]$ and $[\varphi_{2:0}]$ are the molar rotation (10^{-1} deg cm^2 mol^{-1}) of the lactate monomer and dimer, respectively, at wavelength λ_i.

$$\alpha^{\lambda i}_{obs} = [L][\varphi_{1:0}]^{\lambda i} + K_D[L]^2[\varphi_{2:0}]^{\lambda i} \quad (7)$$

The observed rotation in a lactate–guanidinium solution is as shown in eqn. (8), where $[\varphi_{1:1}]$ is the molar rotation of the lactate–guanidinium complex.

$$\alpha^{\lambda i}_{obs} = [L][\varphi_{1:0}]^{\lambda i} + K_D[L]^2[\varphi_{2:0}]^{\lambda i} + K_{1:1}[L][G][\varphi_{1:1}]^{\lambda i} \quad (8)$$

The homoassociation species are certainly the minor species in most solutions. Minor species may, however, be the active species in some biochemical processes,[6] such as receptor binding, membrane penetration, *etc.*

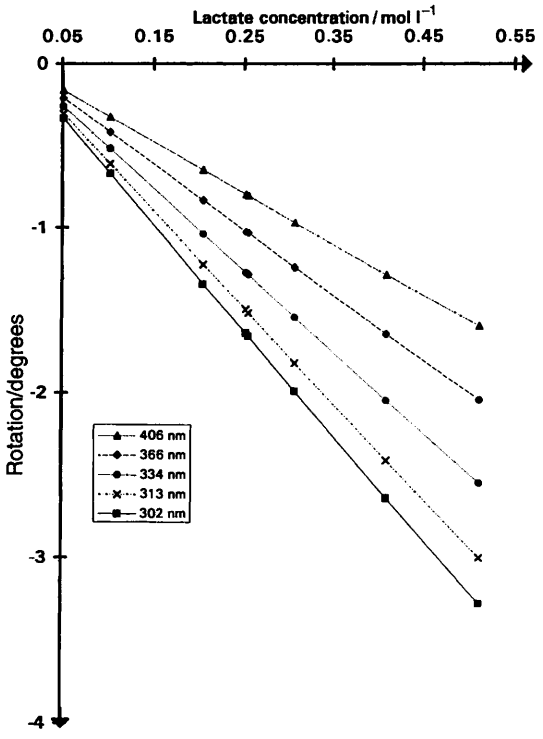

Fig. 1 Plot of the rotation of lactate ion *vs.* lactate concentration at pH 9

Experimental

Polarimetric measurements were carried out on a Perkin-Elmer 241 MC spectropolarimeter at λ 302, 313, 334, 336 and 406 nm at 25.0 ± 0.2 °C and at 1 dm pathlength. A Radelkis OP-208/1 pH meter and a Radelkis OP-0808P combination electrode were used for pH measurements. The pH calibration was made by using Merck Tirisol buffer solutions at pH 2.0, 4.0, 6.0, 7.0, 9.0, 11.0 and 12.0. Equilibrium constants were calculated by using Barcza's computer program.[17]

Guanidinium hydrochloride (Merck) and L-(+)-lactic acid (Aldrich) fine chemicals of analytical grade were used without further purification. The total (lactoyl- and lactide-) lactic acid content was determined by back-titration after hydrolysis with an excess of KOH.[18] During the preparation of the lactate stock solution (0.6 M), special care was taken to complete the lactoyl- and lactide-lactic acid hydrolyses, always keeping the pH below 11, to avoid lactate-racemization.[19]

After completion of the hydrolyses, the pH was set to 9. In order to avoid the presence of foreign ions, which may cause perturbations, a constant ionic strength was not used. The concomitant pH-error hardly influences the protonation state at all. A pH of 9 was also set for the guanidinium stock solutions. The stock solution was diluted to make a concentration series in the range of 0.05 to 0.5 M. The lactate solution was set to have a constant sum of lactate plus guanidinium concentration (JOB method).[20] This principle of continuous variation also allowed a qualitative assessment of the stoichiometry of the composite species also.

Results and discussion

The lactate–lactate dimer formation causes only slight nonlinearity on the rotation *vs.* lactate-concentration function. The graphical plot (Fig. 1) is apparently linear and even the correlation coefficient of a linear regression analysis is near unity (r = 0.9999). The intercepts at different wavelengths, however, are dispersed between −0.012 and −0.026. Values of residual analysis also indicated that the hypothesis of linearity had to be rejected. Differences between experimental points

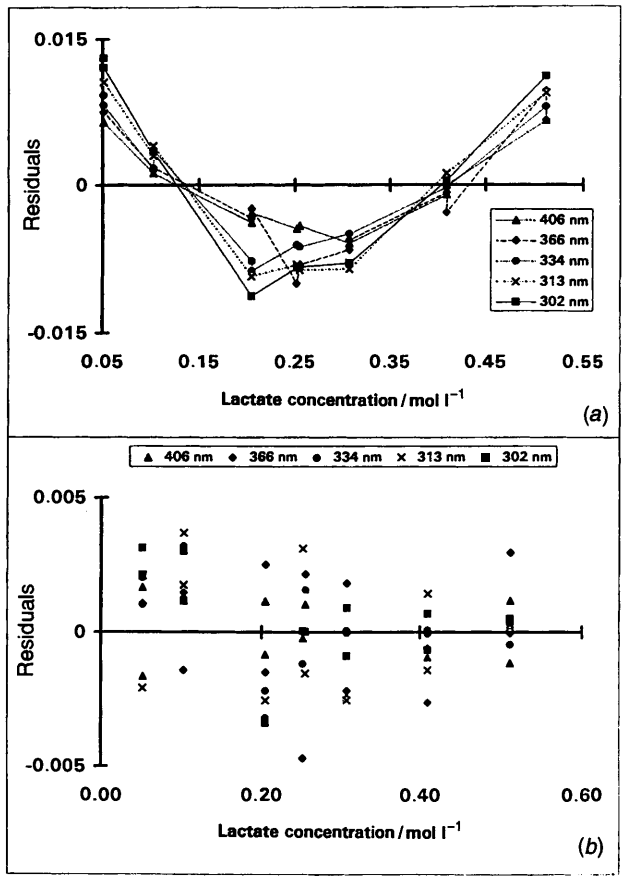

Fig. 2 Differences between observed and calculated rotation values, (*a*) when only monomeric species are considered, and (*b*) when both monomeric and dimeric lactate species are considered

Table 1 Calculated properties of lactate where $[\varphi_{1:0}]$ and $[\varphi_{2:0}]$ are the molar rotation of lactate monomer and dimer, respectively

	When monomer lactate is considered	When monomer and dimer species are both considered	
λ/nm	$[\varphi_{1:0}]$	$[\varphi_{1:0}]$	$[\varphi_{2:0}]^a$
406	−30.9 ± 0.10	−32.9 ± 0.08	−56.9 ± 0.29
366	−39.6 ± 0.11	−42.2 ± 0.13	−72.9 ± 0.47
334	−49.3 ± 0.11	−52.2 ± 0.09	−91.7 ± 0.34
313	−58.2 ± 0.14	−61.5 ± 0.13	−107.8 ± 0.47
302	−63.6 ± 0.17	−67.5 ± 0.12	−117.2 ± 0.44

[a] K (the formation constant of the lactate dimer) = 1.12 ± 0.01 dm^3 mol^{-1}.

and points calculated from the best fitting linear parameters are tendentiously paraboloid [Fig. 2(*a*)] and in many cases significantly exceed the experimental error. The paraboloid distribution indicates that the best fitting function is a polynomial of higher order.

Calculations when both monomeric and dimeric species were taken into account resulted in a significantly improved fit. The residual distribution after quadratic fitting [Fig. 2(*b*)] shows that differences between observed and calculated values are non-tendentious and never exceed the ±0.005 degree limit. Calculation results for the homoassociation are listed in Table 1.

Upon addition of guanidinium ions, the rotation values change a few-hundredths of a degree only. A difference plot of 'lactate with guanidinium minus lactate' *vs.* total lactate concentration can be seen in Fig. 3(*a*) and (*b*). With decreasing wavelength, the maximum of the difference plot is shifted towards the low lactate concentrations. This indicates that

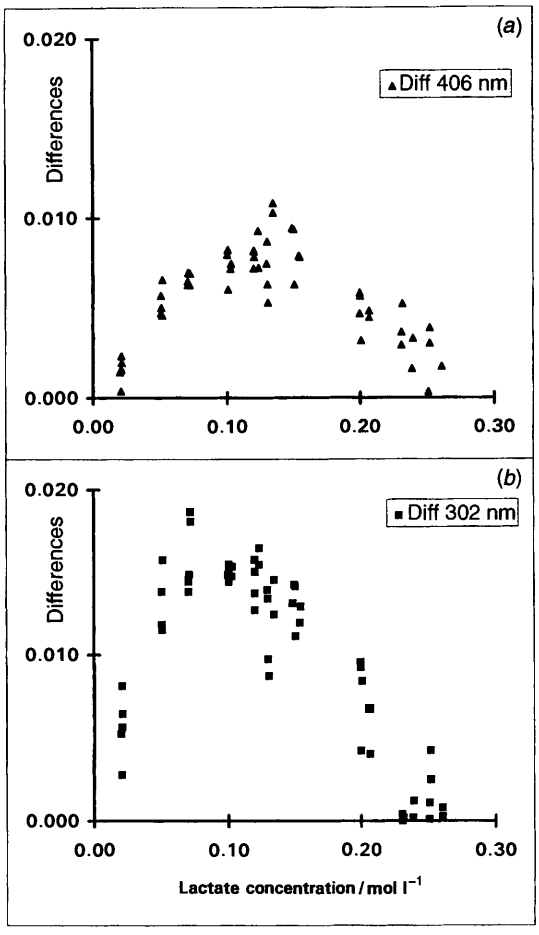

Fig. 3 Difference plot of 'lactate with guanidinium minus lactate' rotation as a function of analytical lactate concentration at λ (*a*) 406 and (*b*) 302 nm

Table 2 Parameters of the lactate–guanidinium complex, where $K_{1:1}$ (the formation constant for the species of 1:1 composition) = 6.11 ± 0.017 dm^3 mol^{-1} and $[\varphi]_\lambda$ is the molar rotation at wavelength λ

λ/nm	$[\varphi]$
406	−29.7 ± 0.08
366	−37.4 ± 0.12
334	−47.6 ± 0.09
313	−55.6 ± 0.10
302	−61.2 ± 0.10

besides the 1:1 stoichiometry, a 2:1 (guanidinium:lactate) species may also exist. Calculations, however, could not prove this assumption. This is partly due to the principle that the system should be described in terms of as few species as possible. Even by taking into account 1:1 composition species only, minimizing the sum of difference squares, the residual analysis provided sufficiently good results [Fig. 4(*a*) and (*b*)], with no tendency and small error. Hence, an attempt to determine the formation constant of a 2:1 species would have yielded results of high uncertainty. The formation constant and the calculation parameters are listed in Table 2.

Conclusions

The formation constant $K_{1:1}$ = 6.11 dm^3 mol^{-1} is high taking into account that it is in an aqueous medium. The difference between the heteroassociation and homoassociation constants might, at first glance, seem small. In water, however, where an overwhelming excess of dipolar solvent molecules offer alternative bindings, such a difference is of considerable size. The water dipoles may be bridging entities, causing the association of the molecules, as assumed by Bondon *et al.*[21]

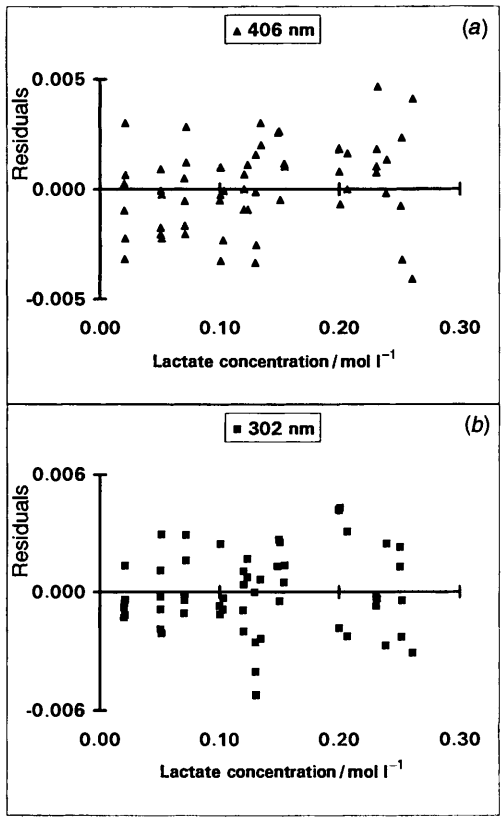

Fig. 4 Differences between calculated and observed rotations for the lactate–guanidinium complex at λ (*a*) 406 and (*b*) 302 nm

Even though the lactate–guanidinium complex formation constant is high, such an association parameter cannot account for the extra stability of lactate–protein complexes. Thus, the invisibility of the lactate in the NMR spectra can only be understood if favourable entropic effects (such as the displacement of bound water), further binding sites and an apolar envelope in the protein also contribute to the lactate binding.

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