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Infrared spectrum analysis of the dissociated states of simple amino acids

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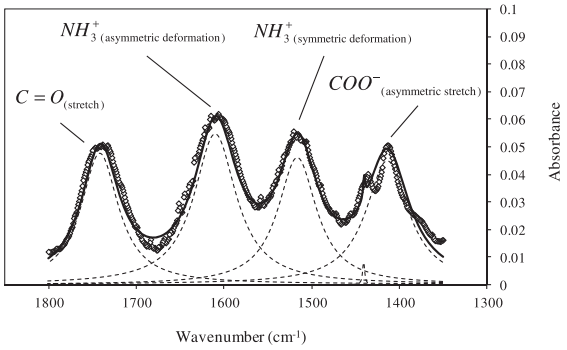
HIGHLIGHTS

- Systematic subtraction of solution spectrum to create solvated amino acid spectrum.
- pH effects on solvated amino acid spectra.
- Peak deconvolution to interpret apparent peak shift with increasing pH.
- pK_a value evaluation from deconvoluted absorption-peak areas at selected pH.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, we present detailed analyses of the dissociation of dilute aqueous solutions of glycine and of lysine over the range 1 < pH < 12. Using appropriate spectrum subtraction methods, we obtained ATR-IR spectra of the solvated species as a function of pH. Discernible changes in the ionic species were identified in the absorption region between 1800 and 1100 cm^{-1}. By applying peak deconvolution techniques to the spectra, we correctly interpret the apparent peak shift from 1615 to 1600 cm^{-1} as being due to the receding NH_3^+ asymmetric deformation alongside the appearing COO^- asymmetric stretching. The effect of aqueous solution environment was also investigated in terms of 10 and 100 mmol/L NaCl. Salt solution spectra at each pH were also subtracted from each solution phase spectrum. Analysis of the deconvoluted peak areas due to C=O and COO^- at pH ranges < 4.5 and those due to NH_2 and NH_3^+ for pH > 8 resulted in consistent pK_a values for the amino acids.

Introduction

A considerable body of published literature presents details of amino acid solubility properties in aqueous solution and in aqueous electrolyte solution. The focus on these properties is due to their increasing relevance in industrial processes. Further optimisation of such processes and understanding the finer details of the solution properties, is complicated by the dissociation of the amino acid as a function of solution pH, which, in turn, is also affected by the presence of dissolved electrolyte. Many of these industrial processes involve adsorption. Amino acid adsorption by inorganic oxide surfaces has recently been reviewed by Lambert [1] and has come under investigation as both a foundation for understanding prebiotic evolution [2–4] and protein–surface interactions [5,6]. Many natural waters contain free amino acids and hydrolysable, combined amino acids, contributing to the total dissolved nitrogen content [7]. Natural waters also contain a wide variety of dissolved salts, adding to the overall ionic strength of the medium [8,9]. To improve adsorption purification processes and

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Nomenclature

a	activity, mol/L
C	third virial coefficient
dx	half-width at half maximum, cm^{-1}
f	an expression of the long-range electrostatic forces
h	peak amplitude
I_m	ionic strength, mol/L
k	number of protonated species within molecule
K_a	thermodynamic equilibrium dissociation constant
K_n	apparent equilibrium dissociation constant for nth dissociation
m_0	unit molarity, 1 mol/L
x	wavenumber value, cm^{-1}
x_0	wavenumber position of peak maximum, cm^{-1}
z_M, z_X	cation or anion charge

Greek symbols

β	interaction parameter between two ions of opposite charge
θ	interaction between two ions of like charge
ξ	an expression consisting of the Debye–Hückel coefficient α and the solution ionic strength
ψ	triple interaction parameter

enhance our comprehension of prebiotic life, in terms of simple amino acid interactions with inorganic surface, it is important to understand how dissolved ions affect amino acid dissociation. Clay, silica, and alumina surfaces have been suggested to promote amino acid polymerisation, which may have resulted in the chemical evolution of early-earth life [10]. Although some of these reactions may have occurred in gas or elevated thermal conditions, some may have occurred in solution phase. How simple amino acids exist in solution phase in the presence of electrolytes at different pH and temperature is an important question to be answered.

Max et al. used a factor analysis methodology applied to an IR titration of aqueous glycine to determine the abundance of each ionic species in solution [11]. The experimental results showed good correlation with the calculated theoretical abundance. Their analyses focussed solely on pure aqueous solutions of glycine. Kitadai et al. showed the effects of temperature on the ATR-IR spectra of glycine at constant pH [12]. Their results described changes in the structure of glycine, with no mention of dissociation. Neither of these papers addressed ionic solution effects on glycine dissociation.

Kitadai et al. also expanded on the work of Max et al. by examining the adsorption of lysine by both amorphous silica and montmorillonite, using the ATR-IR analytical technique [13,14]. They produced detailed spectra in the pH range of 1.9–11.8 for silica, and 2–11.4 for montmorillonite. For each system, they used a graphical method of analysis to define the percentage of each ionic species present, and applied these to the interpretation of the adsorbed phase. Their work contained no discussion of dissolved ion influences.

Vlasova and Golovkova made similar pH dependent studies for lysine adsorption by silica, and also considered solution phase ionic strength [15]. Solution of I_m = 10 mmol/L showed measurable effects on pK_a and amount adsorbed, whereas I_m = 100 mmol/L suppressed solution concentration changes to levels below their detection limits, interpreted as no adsorption. Their work made no analysis of the pH-dependent species interacting with the surface.

Max and Chapados gave detailed explanations for the subtraction of solvent contributions to solution IR spectra resulting in solvated molecule spectra [16]. Their work subtracted acidic, basic, saline or pure water as the solvent phase with the methods resulting in flat baselines. These authors also employed the method of relating symmetric and asymmetric COO^- stretch to pK_a as described by Cabaniss and McVey [17]. Wolpert and Hellwig analysed the infrared spectra of 20 solvated alpha amino acids [18]. Their primary focus was on the spectrum range 1200–500 cm^{-1}, mainly accounting for C—H vibrations. Our work uses ATR-IR spectroscopy to analyse the dilute aqueous solutions of glycine and of lysine to make the following contributions to the understanding of effects of solvent pH and presence of dissolved sodium chloride on the spectra. Firstly, the effect of sodium chloride addition is examined via speciation calculations using Pitzer's single ion evaluation of the activity coefficient contribution to changes in the amino acid pK_a values. Secondly, well-resolved, pH-dependent spectra of the dissolved amino acids are analysed in detail in the wavenumber range 1800–1100 cm^{-1} using peak deconvolution methods to show how apparent shifts in peak maxima are due to the weakening of one and strengthening of another adjacent vibration. Such detail can only be defined using peak analysis. Thirdly, for the first time, we show how the selection of appropriate peak area ratio analyses lead to an equivalence of pK_a evaluation.

Materials and methods

Materials

Glycine and lysine of purity > 99% (*ex.* Sigma Aldrich, Sydney, Australia) were used as received to prepare stock solutions in Milli-Q water (18 MΩ cm) detailed in Table 1. Sodium chloride, cited as 99.99 Suprapur® (*ex.* Merck, Melbourne, Australia) was used without further purification. Standard solutions of NaOH and HCl (as both 100 and 10 mmol/L solutions, *ex.* ASIS Scientific, Adelaide, Australia) were used as received.

Equipment

FTIR spectra were collected on a Shimadzu 8400S spectrometer with an MCT detector. A Pike HATR apparatus fitted with a trough-type zinc selenide crystal was used for all ATR experiments. The measurement parameters varied for each amino acid and are summarised in Table 1, employing solution concentrations and instrument parameters as described by Kitadai et al. [12–14].

Table 1
Parameters used to obtain the solvated amino acid IR spectra.

Amino acid	Concentration (mmol/L)	pH range	Wavenumber range (cm^{-1})	Resolution (cm^{-1})	Scans per measurement
Glycine	400 ± 2	2.0–10.5	800–3800	2	50
Lysine	200 ± 2	1.6–11.8	1000–1800	4	100