Effect of tetrabutylammoniumbromide(TBAB) on protonation equilibria of succinic acid and L-glutamine

R. K. Adhikamsetty[1*] and U. S. N. Prasad[2]

Associate Professor, M. R. P. G. College, Vizianagaram, India
Asst. Professor, Adikavi Nannayya University, Rajahmundry, India

gayathria_rk@yahoo.com

Abstract

Effect of tetrabutylammoniumbromide(TBAB) on protonation equilibria of L-glutamine (Gln) and succinic acid (Suc) in varying concentrations (0.0-3.0%, w/v) of TBAB-water mixtures at an ionic strength of 0.16 mol dm^{-3} and temperature 303K has been made using a pH-meter. Various models for the species of these ligards are refined by using the computer programs SCPHD and MINIQUAD75. The active forms of the ligands are LH_2 and LH^- for succinic acid and LH_2^+ and LH for L-glutamine. The trend in the variation of protonation constants with dielectric constant is explained on the basis of electrostatic and non-electrostatic forces. The species distribution with pH at different solvent composition and the plausible equilibria for the formation of the species are also presented.

Key words: complex equilibria, speciation, L-glutamine, succinic acid, TBAB, MINIQUAD75

1. Introduction

Amino acids plays important role in biological functions and L-Glutamine(Gln) and succinic acid (Suc) are biologically important ligands.[1] Gln is normally considered to be a "conditionally essential" amino acid during inflammatory conditions such as infection and injury under appropriate conditions. Glutamine can act as a respiratory fuel and it can enhance the stimulation of immune cells.[2] Gln in the diet increased survival to bacterial challenge[3], is required to support optimal lymphocyte proliferation[4], production of cytokines by lymphocytes and macrophages[5] and it is highly conserved outer sphere residue in the active site of Escherichia coli (E.Coli) manganese superoxide dismutase[6]. Hence, the protonation equilibria of Gln and Suc in urea-water[7], dimethylformamide-water[7], acetonitrile-water[1] mixtures were studied. Suc can be used for manufacture of medicaments or nutritional supplements effective for treating of insulin resistance[8] in mammals. Suc is involved in citric acid or Tricarboxylic acid (TCA) cycle[9] and Glyoxalate cycle. Succinate is obtained by the oxidation of succinic semialdehyde. In neurotransmission, GABA is inactivated by transamination to succinic semialdehyde, which is then oxidised to succinate. The concentration of succinic acid in human blood plasma is 0.1-0.6 mg/dl. Succinate stimulates insulin secretion and proinsulin biosynthesis.[10]

Tetrabutylammoniumbromide (TBAB) is a cationic surfactant and has a positively charged head group which plays important role in modifying the behavior of aqueous media. It is a quaternary ammonium salt with a bromide counter ion commonly used as a phase transfer catalyst. It is used to prepare many other tetrabutylammonium salts via salt metathesis reactions.[11] Protonation and complexation equilibria of Gln and Suc in urea-water[7], dimethylformamide-water[7], ethylene glycol-water[1] and acetonitrile-water[1] media have been studied to thoroughly understand the speciation of its complexes. The protonation constants of Glu and Suc are correlated[1] with the dielectric constant of the medium using various solvents. Similarly, effect of urea on Cobalt(II) and Nickel(II) complexes of Gln and Suc have studied.[12] No such studies were reported in the literature, hence the author has studied the effect of TBAB on the chemical speciation and protonation constants of Glu and Suc.

Material and Methodology

'A' grade glassware were used throughout the experiments. Solutions of L-glutamine and succinic acid (E.Merck, Germany) were prepared in triple distilled water and a pure TBAB (Sigma, Aldrich) was used without further purification. The data were subjected to ANOVA[13] to assess the errors that might have crept into the determination of the concentrations of above solutions. The strength of alkali (NaOH) was determined using the Gran plot method[14]. Alkalimetric titrations were carried out in the medium containing 0.0 – 3.0%, w/vTBAB in water at an ionic strength of 0.16 mol dm^{-3} with NaCl at 303.0±0.1 K using a Control Dynamics-APX 175E/C pH meter. The glass electrode was equilibrated in inert electrolyte. The correction factor log F, was determined using

the computer program SCPHD[15,16] to correct the pH meter dial reading. Other experimental details are given elsewhere.[13] The approximate protonation constants were calculated using SCPHD. By following some heuristics[17] in the refinement of the stability constants and using the statistical parameters of the least squares residuals, the best-fit chemical models for each system were arrived at using the computer program MINIQUAD75.[18]

2. Results and Discussion

Alkalimetric titration curves in TBAB-water mixture revealed that the active forms of Gln and Suc are in the pH ranges 2.0–10.0 and 2.0-7.0 respectively. Models containing various numbers and combination of Gln and Suc are generated using an expert system package CEES.[19] These models were inputted to MINIQUAD75 along with the alkalimetric titration data and the best-fit model were obtained. The final model in TBAB-water mixture for Suc and Gln contains LH and LH_2 are given in Tables 1 and 2, along with the statistical parameters.

Table 1: Protonation constants of succinic acid in %w/vTBAB-water mixtures

Ionic strength = 0.16 mol dm^{-3}; Temperature = 303 K

(pH range = 2.0 to 7.0)									
S. No	%w/v TBAB	$Log\beta_{lh}$(SD)		NP	Skewness	Kurtosis	χ^2	Ucorr x10^6	R- factor
		11	12						
1	0.0	5.17(2)	9.17(3)	71	0.56	5.78	5.04	9.71	0.0019
2	0.5	5.31(2)	9.34(2)	43	0.19	5.76	71.21	2.40	0.0119
3	1.0	5.30(1)	9.31(2)	42	0.55	5.51	51.43	1.45	0.0096
4	1.5	5.28(9)	9.27(1)	41	0.01	4.43	31.76	5.25	0.0058
5	2.0	5.26(1)	9.23(2)	41	0.54	5.34	35.66	9.27	0.0079
6	2.5	5.23(1)	9.18(1)	40	-0.29	4.90	9.80	7.59	0.0069
7	3.0	5.22(4)	9.13(5)	64	0.77	4.17	46.63	1.58	0.0219

Table 2: Protonation constants of L-glutamine in %w/vTBAB-water mixtures

Ionic strength = 0.16 mol dm^{-3}; Temperature = 303 K

(pH range = 2.0 to 10.0)									
S. No	%w/v TBAB	Logβlh(SD)		NP	Skewness	Kurtosis	χ^2	Ucorr x10^6	R- factor
		11	12						
1	0.0	9.10(8)	11.47(9)	37	0.43	2.09	3.4	1.04	0.0515
2	0.5	9.13(1)	11.52(1)	37	0.0	3.38	3.2	2.81	0.0431
3	1.0	9.12(1)	11.46(1)	34	0.0	4.38	4.1	2.61	0.0461
4	1.5	9.11(1)	11.43(1)	37	-0.1	4.88	3.5	3.04	0.0492
5	2.0	9.10(1)	11.40(1)	37	0.0	4.85	3.2	2.85	0.0434
6	2.5	9.08(1)	11.38(1)	36	0.03	5.19	2.5	2.29	0.0391
7	3.0	9.07(1)	11.36(1)	37	0.02	5.11	3.0	2.54	0.0409

Here, skewness is close to zero and kurtosis to six indicates that the residuals follow Gaussian distribution and so least squares technique can be applied. The low standard deviation in the model parameters (log β) illustrates the adequacy of the models.

3.1 Effect of TBAB on protonation equilibria

TBAB is a quaternary ammonium salt act as structure-breaker of pure water due to large hydrophobic group of TBAB and thus forming cages around itself, with empty spaces in the structure.[20,21] The TBAB micelles have positive surface charge and negatively charged complexes are stabilized on the micellar surface and number of micelles increases with the increasing concentration of surfactant.[21] CMC for TBAB is 0.2632 mol/L at 303.16K in aqueous solutions.[22] Dielectric constant is one of the most and prominent solvent properties that could be altered by surfactants in the given titration mixtures. The anisotropic water distribution within micellar structure causes non-uniform micropolarity, microviscosity and degree of hydration within the micellar media. The linear variation of protonation constants (Fig1) of Suc and Gln with %w/vTBAB indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions.

In the present study the stability constants were found to be linearly decreasing as the percentage of surfactants increases progressively for both Gln and Suc. The destabilization of protonation could be attributed mainly to the low dielectric constant of the surfactant mediated solvent compared to aqueous medium. Moreover, the destabilization effect of the low dielectric constant is synergized by the cationic surfactant, TBAB, which causes the log β values to decrease linearly as shown in Fig. 1[A, B]. On the other hand, the proton accepting ability of the ligand increases in acidic environment (in TBAB). This concept is in good agreement with the linearity of plots of log β values vs. %w/vTBAB (low dielectric constant effect of surfactant modified medium).

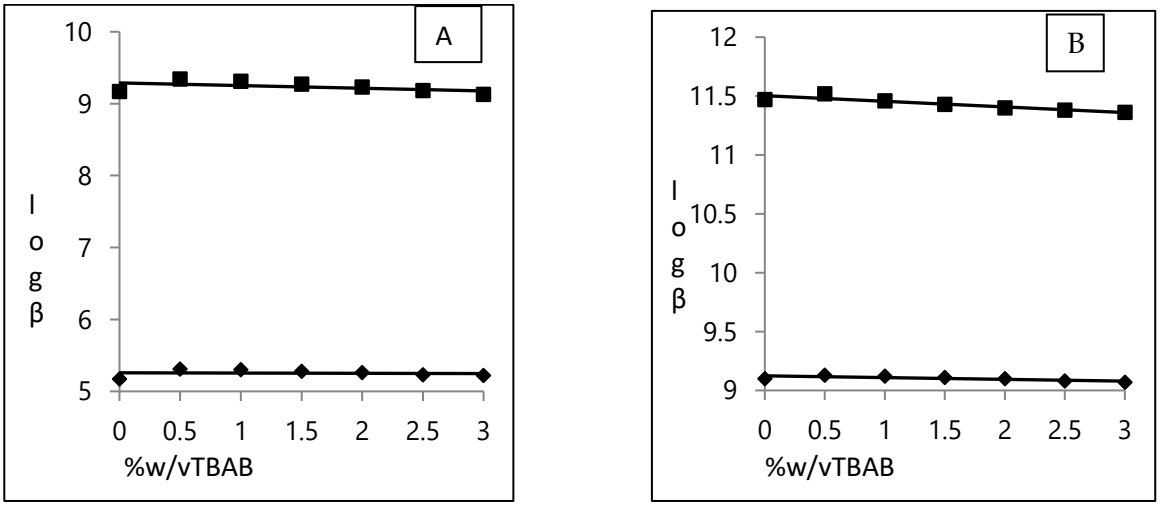
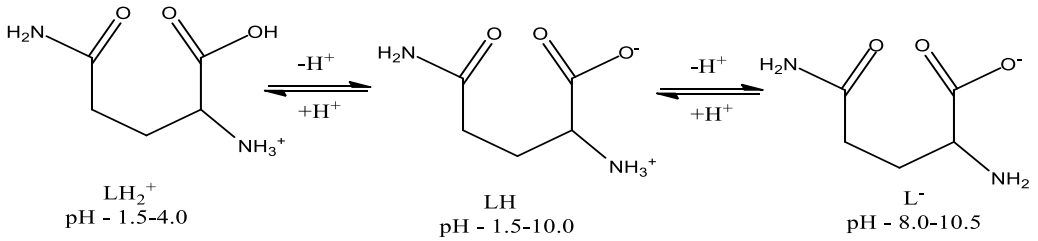
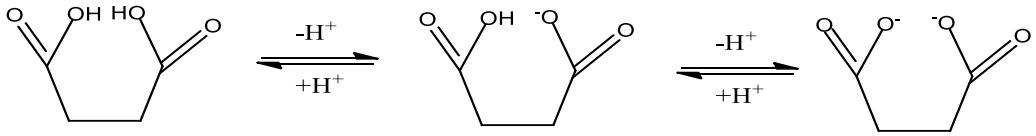


Fig 1: Variation of log β with %w/vTBAB; A) succinic acid B) L-glutamine (♦) log β(LH), (■) logβ(LH_2)

The amino and carboxyl groups of L-glutamine and the two carboxyl groups of succinic acid are protonated. L-glutamine has three functional groups (amino, carboxyl and amido) but only amino and carboxyl groups can associate with protons. Succinic acid has two carboxyl groups and both are protonated. The various forms of ligands exist in the pH range of study (2.0-10.0) are LH_2^+, LH and L^- for Gln (*Scheme 1*) and LH_2, LH^- and L^{2-} for Suc (*Scheme 2*).



Scheme 1: Protonation-deprotonation equilibria of L-glutamine



LH_2 pH - 3.0-6.5 | LH^- pH - 3.0-7.0 | L^{2-} pH - 5.0-7.0

Scheme 2: Protonation-deprotonation equilibria of succinic acid

The models indicates in Fig. 2 that only protonated species are present under the acidic pH (2.0 to 7.0) conditions for Suc systems and species were refined in the pH range 2.0 to 10.0 for Gln. This comparison infers that protonation species (LH) of Gln is highly stable between pH 4 to 8. The zwitterionic form (LH) of Gln is present to an extent of 90% in the pH range 2.5-8.5, which are confirmed by MINIQUAD75. Based on the above observations the formation equilibria are represented below. Equations (a) and (b) for Gln and (c) and (d) are for Suc.

The plausible equilibria

a) $LH + H^+ \rightleftharpoons LH_2^+$

b) $LH_2^+ \rightleftharpoons LH + H^+$

c) $LH^- + H^+ \rightleftharpoons LH_2$

d) $LH_2 \rightleftharpoons LH^- + H^+$

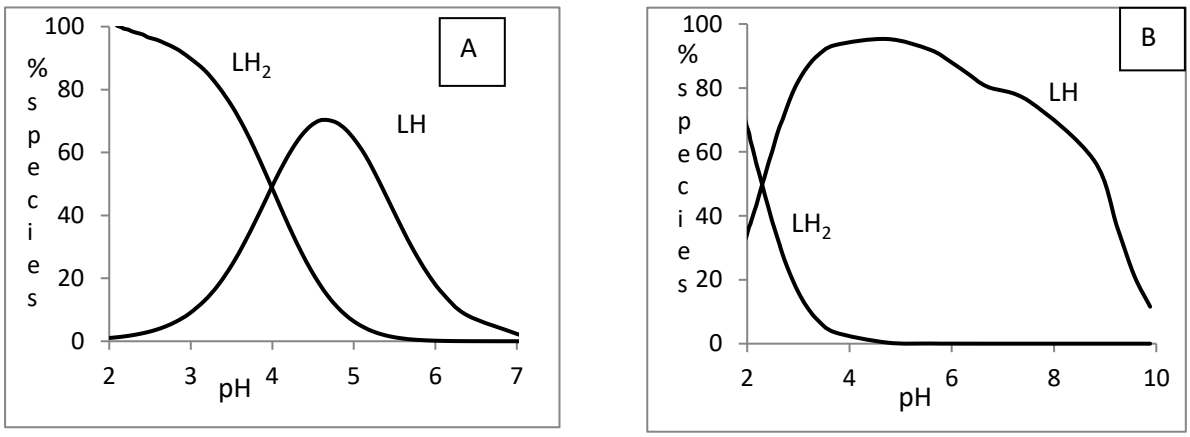


Fig 2: Species distribution diagrams of A) succinic acid and B) L-glutamine with pH in 1.5%w/v TBAB-water mixture

The charges of species are omitted for clarity. The formation of LH and LH_2 for both Gln and Suc are insignificant. The variation of species concentration with pH is shown in Fig. 2 for typical systems. In succinate speciation, concentration of LH_2 decreased with TBAB content and readily converted to LH. In glutamine, the concentration of LH_2 decreased with pH and readily converted to LH. Another notable observation is that the LH concentration of Gln is high about 80% between pH 4 and 8.

Conclusions

L-glutamine and succinic acid are essential amino acids and playing vital role in biological functions. TBAB in aqueous solution considerably decreases the dielectric constant and these solutions are expected to mimic the physiological conditions. The present study is useful to understand.

1. The bonding behaviour of the protein residues with the metal ion in further studies. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of glutamine and succinate residues. The role played by the active site cavities in biological molecules.
2. The biomimetic studies of L-glutamine and succinic acid indicate that the both are protonated (LH_2) under acidic pH 2.0-5.0 and 2.0-4.0 conditions respectively and readily forms deprotonated species (LH).
3. TBAB is a cationic surfactant and has a positively charged head group, which plays important role in modifying the behaviour of aqueous media. As a cumulative effect, the stabilities of the species have decreased with increased TBAB content.

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