Protonation Equilibria of *L*-Aspartic, Citric and Succinic Acids in Anionic Micellar Media

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Abstract: The impact of sodium lauryl sulphate (SLS) on the protonation equilibria of *L*-aspartic acid, citric acid and succinic acid has been studied in various concentrations (0.5-2.5% w/v) of SLS solution maintaining an ionic strength of 0.16 mol dm⁻³ at 303 K. The protonation constants have been calculated with the computer program MINQUAD75 and the best fit models have been calculated based on statistical parameters. The trend of log values of step-wise protonation constants with mole fraction of the medium has been explained based on electrostatic and non-electrostatic forces operating on the protonation equilibria. The effects of errors on the protonation constants have also been presented.

Keywords: Protonation equilibria, MINQUAD75, Sodium lauryl sulphate.

Introduction

L-Aspartic acid (Asp) is a non-essential amino acid found in abundance in plant proteins. It plays an important role in maintaining the solubility and ionic character of proteins¹. It assists the liver in removing excess ammonia and other toxins from the blood stream. It is also very important in the functioning of RNA and DNA, and in immunoglobulin and antibody synthesis. Asp is popular as a drug for chronic fatigue from the crucial role it plays in generating cellular energy, moves the coenzyme nicotinamide adenine dinucleotide (NADH) molecules from the main body of the cell to its mitochondria, where it is used to generate adenosine triphosphate (ATP)².

Citric acid (Cit) is one of a series of compounds involved in the physiological oxidation of fats, proteins, and carbohydrates to carbon dioxide and water. This series of enzyme catalyze chemical reactions of central importance in all living cells that use oxygen as part of cellular-respiration. In aerobic organisms, the citric acid cycle is part of a metabolic pathway involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water to generate a form of usable energy.

Succinic acid (Suc) plays a significant role in intermediary metabolism (Krebs cycle) in the body. Krebs cycle (also called citric acid cycle or tricarboxylic acid cycle) is a sequence process of enzymatic reactions in which a two carbon acetyl unit is oxidized to carbon dioxide and water to provide energy in the form of high-energy phosphate bonds. It is an exchanger of dicarboxylic and tricarboxylic Krebs cycle intermediates³.

Sodium lauryl sulfate (SLS) is an anionic surfactant and profoundly influences the bulk properties of physiological systems. They can solubilise, concentrate and compartmentalize ions and molecules⁴. Hence, the influence of anionic micellar media on the protonation equilibria of Asp, Cit and Suc are investigated in the presence of SLS.

Experimental

Solutions (0.05 mol dm^{-3}) of *L*-aspartic acid, citric acid and succinic acid (GR, E-Merck, Germany) were prepared in triple distilled water by maintaining 0.05 mol dm^{-3} acid (HNO_3) concentration to increase the solubility. Analytical reagent grade sodium lauryl sulphate was obtained from Qualigens and was used as received. Sodium nitrate was prepared to maintain the ionic strength in the titrant. Sodium hydroxide of 0.4 mol dm^{-3} was prepared. The strengths of alkali and mineral acid were determined using the Gran plot method^{5,6}.

Alkalimetric titration assembly

The glass electrode was equilibrated in a well stirred SLS-water mixture containing inert electrolyte for several days. At regular intervals titration of strong acid with alkali was carried out to check whether complete equilibration had been achieved or not. The calomel electrode was refilled with SLS-water mixture of equivalent composition as that of the titrant. The details of experimental procedure and titration assembly were given elsewhere⁷.

Modeling strategy

The approximate protonation constants of Asp, Cit and Suc were calculated with the computer program SCPHD⁸. The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINQUAD75⁹, which exploit the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. The variation of stepwise protonation constants was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

Results and Discussion

The best fit models that contain the type of species and overall formation constants along with some of the important statistical parameters are given in Table 1. A very low standard deviation in $\log \beta$ values indicates the precision of these parameters. The small values of U_{corr} (the sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points) corrected for degrees of freedom, indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around a zero mean with little dispersion. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that the residuals form leptokurtic patterns. The values of skewness given in the table are between -2.55 and 2.11. These data evince that the residuals form a part of normal distribution; hence, least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values. The statistical parameters thus show that the best fit models portray the acido-basic equilibria of *L*-aspartic acid, citric acid and succinic acid in SLS-water mixtures.

Table 1. Best fit chemical models of acido-basic equilibria of *L*-aspartic acid, citric acid and succinic acid in SLS-water mixtures. Temp= 303 K, Ionic strength=0.16 mol dm⁻³.

% w/v of SLS	logβ _{mlh} , (SD)			NP	U _{corr}	Skew-ness	χ ²	R-factor	Kurtosis	pH-Range
011	012	013								
L-Aspartic acid										
0.0	9.67(1)	13.65(1)	15.53(1)	131	0.110	2.11	54.89	0.0153	23.14	1.75-10.5
0.5	9.86(1)	13.85(1)	15.78(1)	151	0.478	-0.10	62.08	0.0097	8.74	1.72-11.0
1.0	10.32(1)	14.70(1)	17.30(2)	190	1.093	0.30	125.14	0.0178	7.30	1.65-11.5
1.5	10.31(1)	14.80(2)	17.89(1)	104	0.799	0.51	21.88	0.0283	4.40	2.25-11.0
2.0	10.05(1)	14.28(2)	17.03(2)	116	0.507	0.55	30.76	0.0281	4.80	2.05-10.8
2.5	9.48(1)	13.30(1)	15.02(1)	94	1.151	0.17	25.16	0.0279	4.24	2.05-10.5
Citric acid										
0.0	5.69(1)	10.07(2)	2.97(1)	114	0.076	-0.80	13.68	0.0073	4.98	2.0-6.8
0.5	5.92(1)	10.46(1)	13.53(2)	107	0.371	0.12	18.67	0.0086	4.40	2.0-6.2
1.0	5.90(1)	10.38(1)	13.40(2)	140	3.607	-1.16	42.51	0.0050	5.75	1.73-6.0
1.5	6.20(1)	10.65(2)	13.68(3)	100	1.569	-1.80	33.28	0.0224	11.33	2.25-8.0
2.0	5.78(2)	10.11(2)	13.21(3)	96	0.775	-1.56	10.83	0.0145	11.79	2.25-8.0
2.5	5.45(1)	9.53(1)	12.23(1)	73	2.331	-0.33	35.72	0.0226	6.00	2.5-6.5
Succinic acid										
0.0	5.41(1)	9.60(2)	-	92	0.076	1.37	22.78	0.0160	11.06	1.95-6.0
0.5	5.46(1)	9.72(1)	-	41	0.371	1.00	11.85	0.0176	3.64	2.9-5.15
1.0	5.50(2)	9.62(3)	-	97	3.607	-0.42	4.30	0.0147	3.62	1.95-6.5
1.5	5.77(1)	9.86(1)	-	52	1.569	-1.72	5.38	0.0480	10.90	3.0-7.5
2.0	5.40(1)	9.52(2)	-	58	0.775	-1.38	11.86	0.0285	7.80	2.7-7.9
2.5	4.86(1)	8.49(1)	-	46	2.331	-2.35	17.38	0.0157	12.75	2.4-8.0

U_{corr} = U/(NP-m)X10⁸, Where, m = number of species; NP=Number of experimental points

Effect of systematic errors on best fit model

MINIQUAD75 does not have provision to study the effect of systematic errors in the influential parameters like concentrations of ingredients and electrode calibration on the magnitude of protonation constant. In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid and the ligands. The results of a typical system given in Table 2 emphasize that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand.

Effect of micelles

The primary factor in the micellar effect on lower alkylamines^{10,11} is the electrostatic interaction of the amine cation and anionic surface of SLS micelle while the hydrophobic interaction plays only a secondary role. Similar situation prevails for Asp, Cit and Suc under the present experimental conditions.

The protonation equilibria of these acids have significant influence on their metabolism. Anions of SLS bind to the main peptide chain at a ratio of one SLS anion for every two amino acid residues. This effectively imparts a negative charge on the protein that is proportional to the mass of that protein (about 1.4 g SLS/g protein). The electrostatic repulsion created by binding of SLS causes proteins to unfold into a rod-like shape thereby eliminating the differences in shape as a factor for separation in the gel.

Table 2. Effect of errors in influential parameters on the protonation constants in 2.5% w/v SLS-water mixture.

Ingredient	%	$\log\beta_{\text{mlh}}(\text{SD})$		
	Error	011	012	013
<i>L</i> -Aspartic acid				
Alkali	0	9.67(1)	13.65(1)	15.53(1)
	-5	10.65(2)	15.23(5)	18.76(4)
	-2	10.15(1)	14.54(4)	17.63(2)
	+2	9.53(1)	12.90(4)	16.24(3)
	+5	9.04(2)	11.82(6)	15.23(3)
Acid	-5	9.26(1)	12.39(3)	15.47(2)
	-2	9.59(1)	13.05(3)	16.29(2)
	+2	10.07(1)	14.08(2)	17.54(2)
	+5	10.45(1)	14.87(2)	18.53(5)
Ligand	-5	9.50(1)	12.88(1)	16.49(1)
	-2	9.16(2)	11.88(2)	16.06(4)
	+2	10.48(1)	14.61(2)	17.64(2)
	+5	10.00(1)	13.83(1)	17.09(2)
Citric acid				
Alkali	0	5.69(1)	10.07(1)	12.97(1)
	-5	5.38(3)	9.85(2)	12.48(2)
	-2	4.72(1)	9.23(2)	11.66(2)
	+2	4.55(1)	9.10(1)	11.52(1)
	+5	5.62(3)	10.13(2)	12.92(2)
Acid	-5	5.42(2)	9.62(4)	12.72(3)
	-2	4.52(1)	9.42(4)	11.82(1)
	+2	4.92(1)	9.38(2)	11.68(2)
	+5	5.58(3)	9.44(2)	12.24(5)
Ligand	-5	5.24(1)	9.37(1)	11.99(1)
	-2	4.34(1)	9.54(1)	11.86(1)
	+2	4.65(1)	9.15(1)	11.32(2)
	+5	5.28(1)	10.53(1)	12.63(2)
Succinic acid				
Alkali	0	5.41(1)	9.60(1)	-
	-5	6.43(3)	11.04(2)	-
	-2	5.94(1)	10.23(2)	-
	+2	5.29(1)	9.32(1)	-
	+5	4.94(3)	8.74(2)	-
Acid	-5	4.51(2)	7.92(4)	-
	-2	4.75(1)	8.42(1)	-
	+2	5.12(1)	9.05(2)	-
	+5	5.38(3)	9.56(2)	-
Ligand	-5	4.63(1)	8.87(2)	-
	-2	4.29(1)	8.19(1)	-
	+2	5.50(1)	9.35(1)	-
	+5	5.07(1)	8.87(1)	-

The apparent shift in the magnitude of protonation constants in micellar media compared to aqueous solution (Figure 1) was attributed to the creation of concentration gradient of protons between the interface and the bulk solution¹². Further the presence of micelles is known to alter the dielectric constant of the medium, which has direct influence on the protonation-de protonation equilibria¹³⁻¹⁵.

Figure 1. Variation of stepwise protonation constant ($\log K$) with mole fraction of SLS in SLS-water mixtures. (A) Aspartic acid, (B) Citric acid (C) Succinic acid (■) $\log K_1$, (♦) $\log K_2$, (▲) $\log K_3$.

Lower alkyl amines and carboxylic acids in their deprotonated state (RNH_2 and RCOO^-) stay in the bulk of the solution and protonated amines (RNH_3^+) will be located both in the bulk and on the surface of the anionic micelles¹⁶. Their incorporation in the SLS micelle is not probable because of low hydrophobicity of simple alkyl chains of the ligands. In addition, they have negatively charged carboxylate groups at high pH range. Hence, these species are expected to stay in the bulk and/or on the surface of the SLS micelles.

Distribution diagrams

The distribution diagrams drawn using the protonation constants from the best fit models are given in Figure 2. The corresponding protonation equilibria are shown in Figure 3. The protonation equilibria of Suc were given in our earlier publication¹⁷.

Secondary formation functions

Secondary formation functions like number of moles of alkali consumed per mole of ligand (a) and average number of protons bound per mole of ligand ($\bar{n}_H$) are useful to detect the number of equilibria. Plots of a with pH (Figure 4) have three and two plateaus, respectively, for Asp and Cit; and Suc indicating the existence of three and two equilibria. Plots of $\bar{n}_H$ versus pH for different concentrations of the ligand should overlap if there is no formation of polymeric species.

Figure 2. Species distribution diagrams of (A) Asp, (B) Cit, (C) Suc in 1.5% w/v SLS-water mixture.

Figure 3. Protonation-deprotonation equilibria of *L*-aspartic acid and citric acid.

Figure 4. Variation of $\overline{n_H}$ and a with pH : (A) Asp (B) Cit (C) Suc.

Conclusions

1. *L*-Aspartic and Citric acids form LH_3 at low pH and get deprotonated with the formation of LH_2 , LH and L successively with increase in pH.
2. Succinic acid forms LH_2 at low pH and gets deprotonated with the formation of LH and L^{2-} with increase in pH.
3. The linear variation of log values of stepwise protonation constants with the mole fraction of SLS in SLS-water mixtures indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria. The non-linear part is due to the contributions from non-electrostatic / hydrophobic interactions between the solute and the solvent.
4. The effect of errors in the influential parameters on the protonation constants shows that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand.

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