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Effect of Solvent on Protonation Equilibria of L-Cystein and L-Threonine in Aqueous Solutions of SLS

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ABSTRACT

The protonation constants of L-Cystein and L-Threonine have been studied pH metrically in various concentrations (0.0-2.50% v/v) of SLS- water mixtures maintaining an ionic strength of 0.16mol L^{-1} at 303 K. The protonation constants have been calculated with the computer program MINIQUAD75 and the best fit chemical models are selected based on statistical parameters. Linear variation of step-wise protonation constants (log K) with reciprocal of dielectric constant of the solvent mixture has been attributed to the dominance of the electrostatic forces.

Keywords: Protonation constants, L-Cystein, L-Threonine, SLS, MINIQUAD75.

INTRODUCTION

Cysteine is an important source of sulfur in human metabolism, and although it is classified as a non-essential amino acid, cysteine may be essential for infants, the elderly, and individuals with certain metabolic diseases or who suffer from malabsorption syndromes [1]. Threonine is thus usually considered aglycine-like monoacid. However, the involvement of the oxygen atom of its hydroxy group in metal chelation can make it lose its proton at more accessible pH values [2-4].

Sodium Lauryl Sulphate (SLS) or sodium dodecyl sulphate (SDS) is anionic surfactant used in many cleaning and hygiene products. It is used in preparing proteins for electrophoresis in the SDS-PAGE (Sodium dodecyl sulphate - polyacrylamide gel electrophoresis) technique. This compound works by disrupting non-covalent bonds in the proteins, denaturing them, and causing the molecules to lose their native shape (conformation). Also 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 1.49 g SLS g^{-1} protein). This new negative charge is significantly greater than the original charge of that protein. The electrostatic repulsion that is created by binding of SLS causes proteins to unfold into a rod-like shape there by eliminating differences in shape as a factor of separation in the gel. The CMC in pure water at 25^0C is 0.0082M and aggregation number at this concentration is usually considered to be about 62. The micellization fraction is around 0.3 [5]. A number of studies have been reported on the effect of solvent on protonation equilibria and chemical speciation of α-amino acids in different media in recent times [6-8].

MATERIALS AND METHODS

Materials: 0.05 mol dm^{-3} aqueous solution of L-Cystein and L-Threonine (GR grade, E-Merck, Germany) were prepared by dissolving samples in water. To increase the solubility of ligands, 0.05 mol dm^{-3} hydrochloric acid concentration was maintained in the solutions. The probable errors that may creep into the concentrations of the stock solutions of the ligands were determined by the computer program COSWT [9]. The pessimistic errors in the preparation of the ligand solutions by weight method did not exceed 0.1%. G R Sample of Sodium lauryl sulphate (SLS, Qualigens, India.) was used as such and its purity was checked by determining critical micellar concentration (CMC) Conductometrically. CMC values of SLS, were 0.0084 M, at 303K. Sodium hydroxide of 0.4mol L^{-1} was prepared. The strengths of alkali and mineral acid were determined using the Gran plot method [10-11].

Procedure: The titrimetric data were obtained by using calibrated ELICO (Model LI-120) pH-meter (readability 0.01). The glass electrode was equilibrated in a well stirred solvent solution containing inert electrolyte. The effects of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor [12]. For the determination of protonation constants of Cys and Thr, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with solvent solution of equivalent composition as that of the titrand. The titrations were carried out in media containing varying amounts of surfactants maintaining an ionic strength of 0.16 mol dm^{-3} with NaCl at 303K. In these titrations, the titrand consisted of mineral acid and ligand, in the presence and absence of metal ion, in a total volume of 50 cm^3. Titrations were performed by adding each time 0.1cm^3 portions of sodium hydroxide (0.4 mol dm^{-3}) to the titrand. The pH meter reading was recorded only after a constant value was displayed. Typical duplicate titrations showed that equilibration is fast and titration data do not differ by more than 0.02 units.

Alkalimetric Titration Assembly: The glass electrode was equilibrated in well-stirred SLS- water mixtures containing inert electrolyte for several days. At regular intervals titration of acid with alkali was carried out to check whether complete equilibration was achieved or not. Typical Alkalimetric titrations are given in fig. 1.

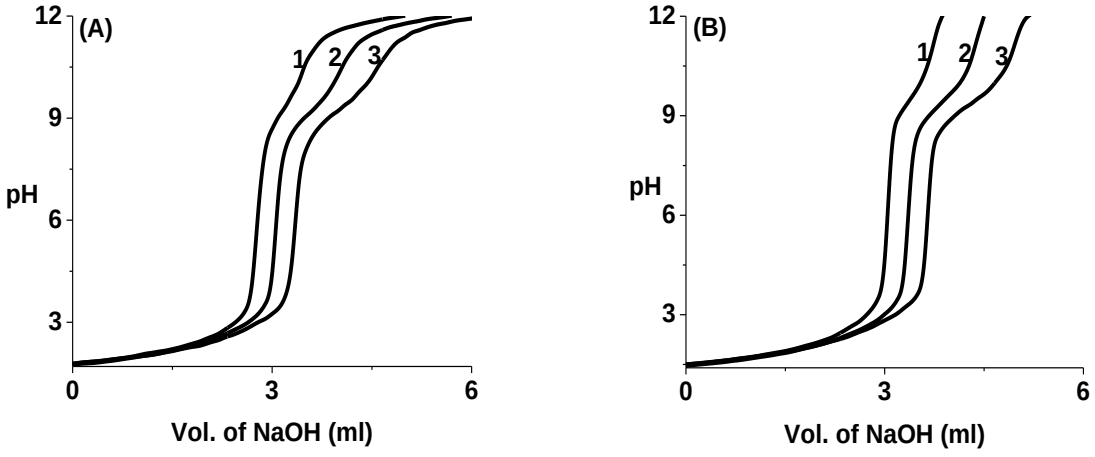

Fig. 1: Alkalimetric titration curves of SLS in 2.0% v/v (A) Cys and (B) Thr- water mixtures; 1, 2 and 3 indicate 0.25, 0.375 and 0.50 mmol of ligand, respectively.

RESULTS AND DISCUSSION

Modeling strategy: The approximate protonation constants of L-Cys and L-Thr were calculated with the computer program SCPHD [13] and they were refined using non-linear least-squares computer program MINIQUAD75 [14]. The variation of overall protonation constants were analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions. The best fit models that contain the type of species and overall protonation constants (log β) along with some of the important statistical parameters are given in table 1.

A very low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} (sum of squares of deviations in the concentrations of ligand and hydrogen ion at all experimental points corrected for degree of freedom) indicate that the experimental data can be represented by the models. 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 as well as platykurtic patterns. The values of skewness are between -0.71 and 2.65. These data evince that the residuals from 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-Cys and L-Thr in SLS- water mixtures

Table 1: Parameters of the best fit chemical models of protonation equilibria of L-Cys and L-Thr in SLS-water mixtures at 303.0 K and ionic strength, μ = 0.16 mol L^{-1}

% v/v	log β_{mlh} (SD) 11	12	13	NP	U_{corr} *10^8	χ^2	Skew-ness	Kurt-osis	R-factor
Cys (pH range 2.0-11.5)									
0.0	10.55(2)	18.51(10)	20.30(1)	150	1.67	92.11	-0.12	5.45	0.016293
0.5	10.43(8)	20.44(17)	22.44(16)	166	6.04	74.75	-0.71	5.67	0.055456
1.0	9.96(3)	18.59(6)	23.99(14)	106	4.47	57.96	2.65	11.17	0.046660
1.5	9.85(13)	19.15(11)	26.41(11)	118	2.53	63.55	2.22	6.58	0.065385
2.0	9.96(6)	18.97(5)	26.62(5)	79	4.51	55.28	2.15	17.36	0.026856
2.5	10.53(7)	20.86(4)	28.61(3)	91	3.84	22.17	0.88	12.62	0.020767
Thr (pH range 2.0-11.5)									
0.0	10.73(12)	20.99(13)	-------	66	1.39	3.64	0.29	3.83	0.080140
0.5	11.05(6)	20.15(6)	-------	30	0.76	5.64	0.96	3.38	0.014309
1.0	11.16(23)	22.15(6)	-------	72	1.08	11.11	1.48	5.44	0.072574
1.5	11.40(2)	19.70(2)	-------	50	1.68	20.64	-0.77	2.62	0.039641
2.0	11.49(4)	20.25(4)	-------	36	2.43	1.78	-0.19	3.32	0.008756
2.5	11.42(6)	20.56(6)	--------	39	0.41	1.18	0.88	3.63	0.018840

In order to rely upon the best fit 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 ligand. 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 those in the ligand and log F.

Table 2: Effect of systematic errors in influential parameters on the protonation constants of L-Cystein and L-Threonine in SLS-water mixtures.in 1.5 w/v

Ingredient	% Error	log β_{mlh} (SD)				
		Cys			Thr	
		11	12	13	11	12
	0	9.85(12)	19.15(11)	26.41(27)	11.40(2)	19.70(2)
	-5	9.81(21)	19.15(38)	26.33(45)	11.61(30)	19.98(47)
Alkali	-2	9.55(11)	19.68(20)	26.79(24)	11.29(12)	19.36(21)
	+2	9.22(8)	19.09(14)	27.10(17)	11.90(6)	19.62(11)
	+5	9.97(18)	19.65(32)	26.60(39)	11.59(14)	19.07(23)
	-5	9.02(18)	19.64(33)	26.33(42)	11.75(14)	19.28(25)
Acid	-2	9.25(8)	19.09(15)	26.00(19)	11.96(6)	19.70(12)
	+2	9.53(10)	19.68(19)	27.88(23)	11.23(11)	19.27(20)
	+5	9.74(19)	19.13(36)	26.56(43)	11.44(26)	19.71(44)
	-5	9.33(8)	19.38(15)	26.59(18)	11.94(5)	19.77(10)
Ligand	-2	9.37(7)	19.58(13)	26.50(16)	11.03(5)	19.90(9)
	+2	9.41(6)	19.28(12)	26.38(15)	11.15(7)	19.07(12)
	+5	9.44(7)	19.38(13)	26.30(15)	11.23(9)	19.19(16)
	-5	9.38(7)	19.37(13)	26.40(16)	11.09(6)	19.98(11)
log F	-2	9.39(7)	19.38(13)	26.43(15)	11.09(6)	19.98(10)
	+2	9.39(6)	19.38(12)	26.45(14)	11.09(6)	19.99(11)
	+5	9.39(6)	19.39(11)	26.48(14)	11.10(6)	19.99(11)
	-5	9.39(4)	19.38(9)	26.53(10)	11.09(10)	19.97(19)
Volume	-2	9.39(5)	19.38(11)	26.48(13)	11.09(7)	19.98(12)
	+2	9.39(9)	19.38(14)	26.40(17)	11.09(6)	19.99(12)
	+5	9.39(9)	19.38(18)	26.34(21)	11.09(10)	19.99(17)

Effect of solvent: Effect of solvent on protonation constant depends upon electrostatic and non-electrostatic factors. Born's classical treatment holds good in accounting for the electrostatic contribution [15] which is related to dielectric constant. Hence, the logarithm of step-wise protonation constant (log K) should vary linearly as a function of the reciprocal of dielectric constant (1/D) of the medium. The log K values in present study are linearly increasing (Fig. 2) with decreasing dielectric constant of the medium in both the Ligands (Cys and Thr).

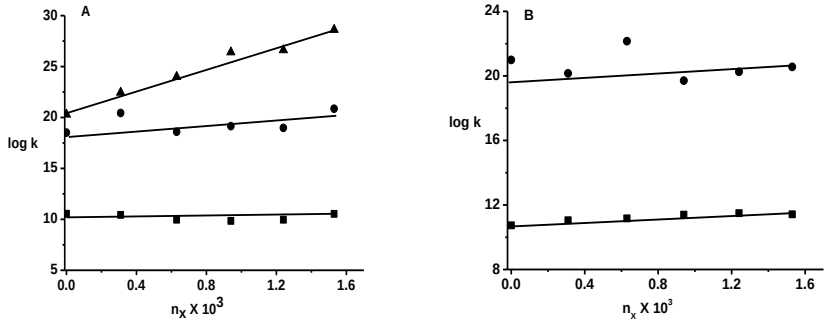

Fig. 2: Variation of stepwise protonation constant (log K) with mole fraction of solvent.(A) Cys and (B) Thr in ctab-water mixtures ; (■) log K_1, (●) log K_2.

L-Cyestein exists as anion (L^-), zwitterion (LH) and cation (LH_2^+) and(LH_3^{2+}) (Fig. 3) at different pH values. L-Thronine exists as anion (L^-), zwitterion (LH) and cation (LH_2^+) (Fig. 4) at different pH values. The cation stabilizing nature of co-solvent, specific solvent-water interactions, charge dispersion and specific interactions of co-solvent with solute account for the linear relationship of log K with mole fraction of solvent.

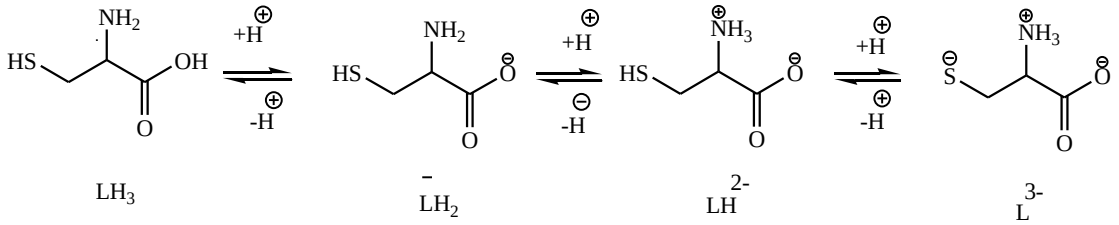

Fig. 3: Protonation-deprotonation equilibria of L-Cystein

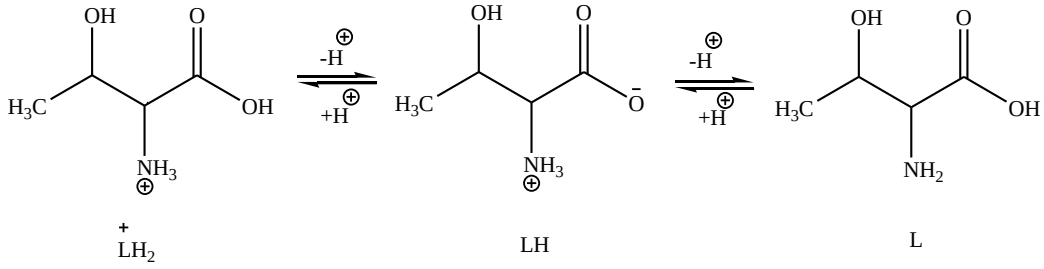

Fig. 4: Protonation-deprotonation equilibria of L-Threonine

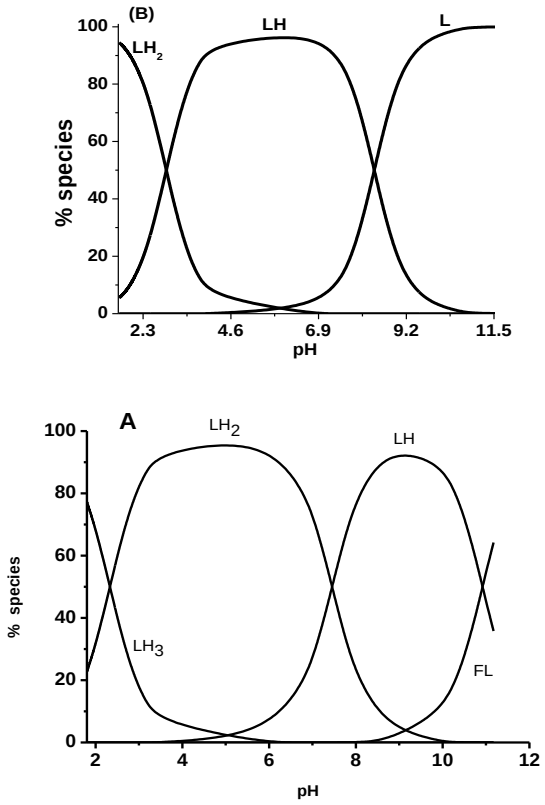

Fig. 5: distribution diagrams of L-Cys in 0.5% w/v (A), L-Thr in 0.5% w/v (B) in SLS- water mixture

DistributionDiagrams: L-Cystein as anion (L^-), zwitterion (LH) cation and (LH_3^{2+}) (Fig. 5A) at different values. (LH_3^{2+}) exists at pH 2.0-5.0, (LH_2^+) exists at pH 2.0-9.0, (LH) exists at pH 6.0-11.0 and (L^-) exists at pH 9.0-11.6.

L- Thronine exists as anion (L^-), zwitterion (LH) and cation (LH_2^+) (Fig. 5b) at different pH values. (LH_2^+) exists at pH 2.0-6.0, (LH) exists at pH 2.0-11.0 and (L^-) exists at pH 6.0-11.5.

APPLICATIONS

Speciation determines the behavior of trace elements in a system, and in the human organism speciation has a great effect on bioavailability, distribution and toxicity. The studies carried out on these systems under the present experimental conditions are useful to understand the role played by the active site cavities in biological molecules. Hence, the speciation studies on the protonation equilibria of L-Cys and L-Thr in varying compositions of SLS – water mixtures have been carried out.

CONCLUSIONS

1. CL-Cys exists as LH_3^{2+} at low pH and gets deprotonated with the formation of LH_2^+ and LH successively with increase in pH. L-Thr exists as LH_2^+ at low pH and gets deprotonated with the formation and LH successively
2. The log K values of protonation constants increase linearly with decreasing dielectric constant of Cys and Thr in SLS-water mixtures. This trend indicates the dominance of electrostatic forces over the non-electrostatic forces in the protonation-deprotonation equilibria.
3. The effect of systematic errors in the influential parameters shows that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand and log F.

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