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Protonation Constants of Valine, Serine, and *β*-Alanine in Artificial Seawater at 25 °C

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The protonation constants of some amino acids commonly found in natural waters, namely valine, $(CH_3)_2CHCH_2CH(NH_2)COOH$; serine, $OHCH_2CH(NH_2)COOH$; and *β*-alanine, $NH_2CH_2CH_2COOH$, have been studied at different ionic strength of artificial seawater and at 25 °C by potentiometry with a commercial glass electrode. The results have been interpreted by the Pitzer interaction model.

Introduction

Much experimental effort has been devoted to the evaluation of the dissociation constants in seawater (e.g., Khoo et al., 1977; Dickson and Riley, 1979; Millero, 1983; Roy et al., 1993a,b). In particular, the amino acid protonation data are potentially useful, especially given the lack of information available on the effects of ionic medium at higher concentrations. In continuation of some previous studies (Fiol et al., 1995a,b; Vilariño et al., 1997) on the protonation constants of amino acids in artificial seawater (ASW), and being the last one of a series, in this work we report the ionization constants for valine, serine, and *β*-alanine in ASW. These amino acids, together with glycine, α-alanine, leucine, threonine, methionine, and cysteine, whose ionization constants in ASW have been previously reported (Fiol et al., 1995a,b; Vilariño et al., 1997), make up the neutral group of amino acids commonly found in natural waters, according to Thurman (1985).

Experimental Section

As described in previous works (e.g., Herrero et al., 1992; Fiol et al., 1995a), we used the potentiometric technique to determine equilibrium constants. The apparatus, procedure and conditions used have been described in detail elsewhere (Herrero et al., 1992, 1993a; Fiol et al., 1995a,b). The amino acids used were supplied by Merck (for biochemistry, >99%). The artificial seawater samples were prepared from NaCl, KCl, $CaCl_2$, $MgCl_2$, and Na_2SO_4 (Merck, p.a.) as electrolytes following the seawater recipe of Millero (1986), which gives the molal concentration of dissolved species—mol/(kg solvent). A stock solution of salinity 35 ‰ seawater was prepared with deionizated distilled water. The different ionic media were prepared by dilution of the stock solution. The ionic strength on the molality scale can be derived from a function that depends exclusively on salinity (Khoo et al., 1977).

The experimental sets of data, emf (mV ± 0.1) vs volume of added titrant (mL ± 0.1), were processed with the program MINIQUAD (Sabatini et al., 1974), which computes the apparent ionization constants of the amino acids on the molar scale. Since the Pitzer formalism (Pitzer, 1973) has been developed on the molal scale, pK^{app} values have to be converted from molarity to molality (Herrero et al., 1993b) which involves the previous knowledge of the

Table 1. Experimental pK^{app} Values (Molal Scale) for the Amino Acids Studied in Artificial Seawater at Variable Ionic Strength and 25 °C[a]

	I/	serine		valine		*β*-alanine	
salinity	mol-kg^{-1}	pK_1^{app}	pK_2^{app}	pK_1^{app}	pK_2^{app}	pK_2^{app}	$K_1^{app}pK_2^{app}$
5	0.10	2.292	9.072	2.341	9.480	3.495	9.923
9	0.18	2.252	9.011	2.276	9.465		
10.6	0.21					3.448	9.907
14.5	0.29	2.247	8.951	2.297	9.431	3.515	9.853
17.5	0.35	2.186	8.773	2.286	9.393	3.472	9.866
20	0.41	2.210	8.791	2.315	9.287	3.451	9.905
21	0.43		8.883	2.298	9.340	3.465	9.852
22.6	0.46	2.186	8.858	2.276	9.361		
24.7	0.50		8.845	2.304	9.341	3.498	9.884
29	0.59	2.172	8.727	2.281	9.338	3.488	9.837
32	0.69	2.124	8.821	2.332	9.378		
35	0.72	2.177	8.793	2.316	9.319	3.476	9.836

[a] Standard deviations range between ±.001 and ±.02.

Table 2. Single Electrolyte Solution Pitzer Parameter Values for Each Cation−Anion Pair in the Ionic Medium (Harvie et al., 1984)[a]

electrolyte (ca)					
cation	anion	$\beta_{ca}^{(0)}$	$\beta_{ca}^{(1)}$	$\beta_{ca}^{(2)}$	C_{ca}^{ϕ}
Na	Cl	0.0765	0.2644		0.001 27
Na	SO_4	0.01958	1.113		0.004 97
Na	HSO_4	0.0454	0.398		
K	Cl	0.04835	0.2122		−0.000 84
K	SO_4	0.04995	0.7793		
K	HSO_4	−0.0003	0.1735		
Ca	Cl	0.3159	1.614		−0.000 34
Ca	SO_4	0.20	3.1973	−54.24	
Ca	HSO_4	0.2145	2.53		
Mg	Cl	0.35235	1.6815		0.005 19
Mg	SO_4	0.2210	3.343	−37.23	0.025
Mg	HSO_4	0.4746	1.729		
MgOH	Cl	−0.10	1.658		
MgOH	SO_4				
MgOH	HSO_4				

[a] Emtpy cells indicate zeros.

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Table 3. Fitting Parameters Obtained by the Applying Pitzer Model to $pK^{app}-I$ Data of Each Amino Acid Studied

	serine		valine		β-alanine	
	$C \neq 0$	$C = 0$	$C \neq 0$	$C = 0$	$C \neq 0$	$C = 0$
pK_1^θ	2.32 ± 0.13	2.35 ± 0.05	2.44 ± 0.1	2.38 ± 0.04	3.57 ± 0.13	3.50 ± 0.05
A_1	-0.27 ± 1.4	0.04 ± 0.1	0.89 ± 1.1	0.22 ± 0.09	0.84 ± 1	0.06 ± 0.1
B_1	-0.12 ± 3	0.50 ± 0.40	2.08 ± 2.2	0.74 ± 0.32	1.76 ± 3	0.22 ± 0.5
C_1	0.31 ± 1.4		-0.66 ± 1.1		-0.78 ± 1.5	
σ	0.02	0.02	0.02	0.02	0.03	0.03
pK_2^θ	9.30 ± 0.3	9.44 ± 0.11	9.56 ± 0.18	9.77 ± 0.07	10.15 ± 0.13	10.09 ± 0.05
A_2	-1.2 ± 3	0.39 ± 0.3	-2.2 ± 2	0.25 ± 0.18	0.68 ± 1	-0.03 ± 0.1
B_2	1.93 ± 7	-1.2 ± 0.9	4.6 ± 4	-0.34 ± 0.6	-0.62 ± 3	0.79 ± 0.4
C_2	1.5 ± 3		2.4 ± 2		-0.71 ± 1	
σ	0.06	0.06	0.04	0.04	0.03	0.02

Table 4. Comparison of the Extrapolated and Literature Values of the Thermodynamic pK's of the Different Amino Acids

	literature			$pK_i^\theta(\text{calcd}) - pK_i^T(\text{ref})$			
	pK_1^T	pK_2^T	ref	$\Delta pK_1{}^a$	$\Delta pK_1{}^b$	$\Delta pK_2{}^a$	$\Delta pK_2{}^b$
serine	2.186	9.208	Smith et al., 1939	0.13	0.16	0.09	0.23
valine	2.286	9.719	Smith et al., 1937	0.15	0.09	0.16	0.05
β-alanine	3.55	10.29	Boyd et al., 1967	0.02	0.05	0.08	0.14

[a] pK_i^θ calculated with the quadratic term. [b] pK_i^θ calculated without the quadratic term.

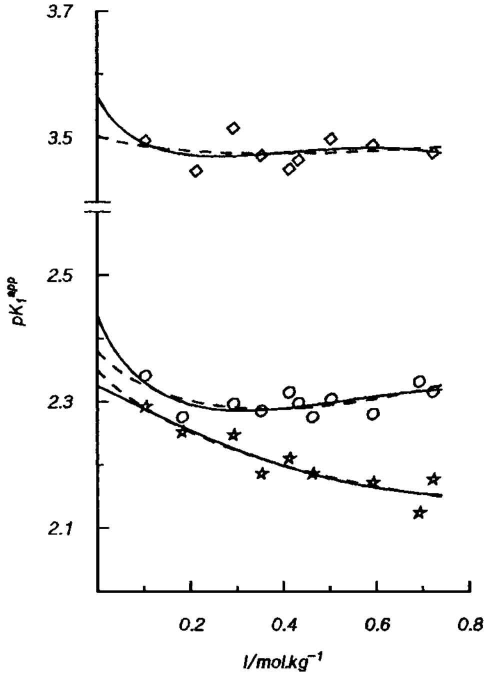

Figure 1. Experimental $pK_1^{app}-I$ data of the amino acids: serine (☆), valine (○), and β-alanine (◇). Fitting curves obtained by applying the Pitzer model: — including quadratic term; --- not including quadratic term.

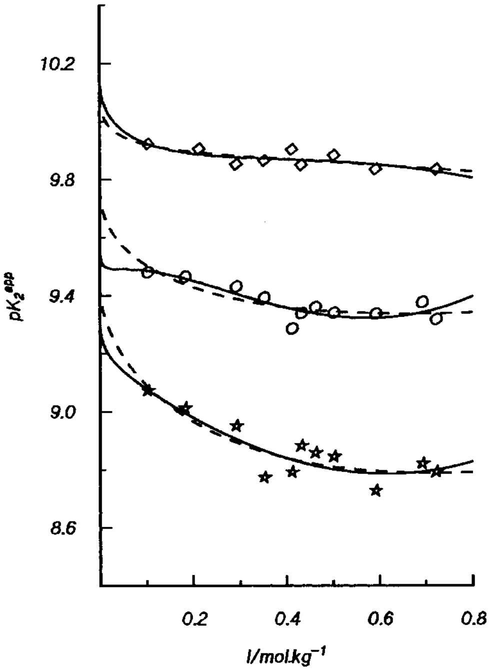

Figure 2. Experimental $pK_2^{app}-I$ data of the amino acids: serine (☆), valine (○), and β-alanine (◇). Fitting curves obtained by applying the Pitzer model: — including quadratic term; --- not including quadratic term.

density of solutions. A relationship between salinity and density at 1 atm pressure has been given by Millero (1982).

In Table 1 the stoichiometric equilibrium constants, which are the average of at least two different experiments, are given on the molal scale.

Results and Discussion

The Pitzer equations have been used to derive an equation that enables the stoichiometric dissociation constants to be calculated for seawater solutions of different compositions. Application of the Pitzer model (Pitzer, 1973) to the apparent ionization constants of an amino acid with two ionizable groups in artificial seawater (Fiol et al., 1995a,b) leads to the expressions

$$pK_1^{app} = pK_1^\theta + A_1{\cdot}2I + B_1{\cdot}[-1 + (1 + 2\sqrt{I})e^{-2\sqrt{I}}] + C_1{\cdot}I^2 \quad (1)$$

$$pK_2^{app} - \frac{2}{\ln 10} f^{(\gamma)} - \frac{2}{\ln 10} f^{(\text{med})} = pK_2^\theta + A_2{\cdot}2I + B_2{\cdot}[-1 + (1 + 2\sqrt{I})e^{-2\sqrt{I}}] + C_2{\cdot}I^2 \quad (2)$$

where $f^{(\gamma)}$ is the Debye–Hückel term

$$f^{(\gamma)} = -0.392\left[\frac{\sqrt{I}}{1 + 1.2\sqrt{I}} + \frac{2}{1.2}\ln(1 + 1.2\sqrt{I})\right] \quad (3)$$

and $f^{(\text{med})}$ just includes interaction parameters between the electrolytes that make up the ionic medium (Pitzer, 1991)

$$f^{(\mathrm{med})} = \sum_c \sum_a m_c m_a \left[B'_{ca} + \frac{C^{\phi}_{ca}}{2|z_a z_c|^{1/2}} \right] \qquad (4)$$

where the sums in a and c are over all anions and cations of electrolytic medium, respectively.

The second virial coefficient B'_a is given by the following ionic strength dependence

$$B'_{ca} = \beta^{(1)}_{ca}\, g'(\alpha_1 \sqrt{I}) + \beta^{(2)}_{ca}\, g'(\alpha_2 \sqrt{I}) \qquad (5)$$

$$g'(\alpha_i I) = -2 \frac{[1 - (1 + \alpha_i \sqrt{I} + (\alpha_i^2/2) I\, \mathrm{e}^{-\alpha_i \sqrt{I}}]}{\alpha_i^2 I} \qquad (6)$$

where $\alpha_1 = 2$ kg$^{1/2}$·mol$^{1/2}$ and α_2 is set equal to zero for all kinds of electrolytes except for 2:2 electrolytes, whose satisfactory values are $\alpha_1 = 1.4$ kg$^{1/2}$·mol$^{1/2}$ and $\alpha_2 = 12$ kg$^{1/2}$·mol$^{1/2}$ (Pitzer, 1991).

The complete set of parameters defining the model for the nonideal behavior of electrolyte solutions for each cation–anion pair are given in Table 2, from which the $f^{(\mathrm{med})}$ term is calculated

$$\begin{aligned} f^{(\mathrm{med})} = {} & .3317[1 - (1 + 2\sqrt{I} + 2I)\mathrm{e}^{-2\sqrt{I}}] + \\ & .0141[1 - (1 + 1.4\sqrt{I} + .98I)\mathrm{e}^{-1.4\sqrt{I}}] - \\ & -.0023[1 - (1 + 12\sqrt{I} + 72I)\mathrm{e}^{-12\sqrt{I}}] \end{aligned} \qquad (7)$$

Fitting the apparent pK^{app} according to eqs 1 and 2, A_i, B_i, and C_i parameters together with the thermodynamic ionization constant, pK^{θ}, have been obtained. As shown in Table 3, fits including the quadratic term lead to a much larger error of parameters and the curves show an odd behavior, as can be seen in Figures 1 and 2. Parameter C has been reported to be only significant at high concentrations (Pitzer and Mayorga, 1973, Herrero, 1993); in fact this approximation has been used by Pitzer and Mayorga (1973) for ionic strengths smaller than 2 m, so that can be excluded without an increase in the error of the fit and the estimate parameters.

Comparison of our extrapolated thermodynamic pK's and the literature values, taken from two recent reviews (Sóvágó et al., 1993; Berthon, 1995), are shown in Table 4. Our extrapolated pK_i^{θ} values obtained for serine and β-leucine agree better with the literature ones when the higher-order term of the fit is taken into account; on the contrary, pK_i^{θ} values of valine lead to a better agreement without including the quadratic term. As can be seen from Figure 2, the fitting curve obtained for the second ionization equilibrium of valine shows an odd behavior at low ionic strength, which would explain the above-mentioned difference of agreement.

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