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Protonation Constants of Amino Acids in Artificial Sea Water at 25 °C

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In this paper we study the ionic strength dependence of the potentiometrically determined stoichiometric equilibrium constant (K^*) of the amino acids *α-alanine* (CH_3CHNH_2COOH), *leucine* ($(CH_3)_2CHCH_2CHNH_2COOH$), *threonine* ($CH_3CHOHCHNH_2COOH$), and *methionine* ($CH_3SCH_2CH_2CHNH_2COOH$) in artificial sea water at 25 °C and different ionic strengths. The results have been interpreted by the Pitzer interaction model.

Introduction

Although there is great interest on the study of the physicochemical properties of amino acids (*1*), systematic studies on the effect of the ionic strength on the acid–base equilibria are scarce (*2*), except for the contribution of Sammartano's group (see for example refs 3–5 and references therein) and more recently perhaps our own work using different approximations (*6–14*). There are fewer measurements in complex media (i.e., mixtures of electrolytes) and particularly in natural waters (*15*). Taking into account that at low concentration the amino acid and peptide fraction is, next to humic substances, the most studied fraction in natural waters, we have recently initiated a study of the acid–base equilibria in mixtures of electrolytes (*8*). In this paper the equilibrium constants of four neutral amino acids found in natural waters are determined at various ionic strengths. The dependence on the ionic strength is analyzed by using Pitzer equations (*16*) which apply to single electrolytes or mixtures. Previous work for relevant inorganic equilibria in sea water has been carried out by Millero et al. (*17*).

Experimental Section

α-Alanine, leucine, threonine, and methionine were Merck reagent grade. The experimental details are the same as those described in previous papers (*6, 11*) except for the preparation of the electrolytic media. Artificial sea water of salinity 35‰ was prepared according to Millero (*18*), 0.426 64 mol·kg^{-1} NaCl, 0.010 58 mol·kg^{-1} KCl, 0.055 18 mol·kg^{-1} $MgCl_2$, 0.029 26 mol·kg^{-1} Na_2SO_4, and 0.010 77 mol·kg^{-1} $CaCl_2$, and ionic strength was related to salinity (*19*), S (‰), through $I/(\mathrm{mol\cdot kg^{-1}}) = 0.0029 + 0.018575S + 1.639 \times 10^{-5}S^2$. We found it more convenient for further calculations to express the concentration (mol·kg^{-1}) of individual ions, m_i, as a fraction of the total ionic strength (I): $m_{Na} = 0.7130I$, $m_{Cl} = 0.8364I$, $m_{SO_2} = 0.0408I$, $m_K = 0.0156I$, $m_{Mg} = 0.0811I$, and $m_{CA} = 0.0158I$. All the salts were Merck p.a. Stock solutions of $MgCl_2$, $CaCl_2$, and Na_2SO_4 were used for the preparation of artificial sea water. The densities of these solutions were determined by a PAAR DMA 60 densimeter with a PAAR CMA 602 cell at 25 °C.

Results and Discussion

The activity coefficients for ions in artificial sea water can be estimated by using the Pitzer model (*20*) from the equations

$$\ln \gamma_M = z_M{}^2 f^\gamma + 2m_{Cl}(B_{MCl} + EC_{MCl}) + 2m_{SO_4}(B_{MSO_4} + EC_{MSO_4}) + \sum_c \sum_a m_c m_a (z_X{}^2 B_{ca}{}^1 + z_X C_{ca}) \tag{1}$$

$$\ln \gamma_X = z_X{}^2 f^\gamma + 2m_{Na}(B_{NaX} + EC_{NaX}) + 2m_{Mg}(B_{MgX} + EC_{MgX}) + 2m_{Ca}(B_{CaX} + EC_{CaX}) + 2m_K(B_{KX} + EC_{KX}) + \sum_c \sum_a m_c m_a (z_X{}^2 B_{ca}{}^1 + z_X C_{ca}) \tag{2}$$

where $E = \sum|z_c|m_c = \sum|z_a|m_a$ is the equivalent molality, γ_M and γ_X are the activity coefficients of cations and anions, respectively, B, B^1, and C are the interaction terms used by Pitzer, and m_i is the concentration of the individual ions present in solution and expressed in moles per kilogram.

Equations 1 and 2 substituted in the expression of the apparent equilibrium constants for the ionization equilibria of amino acids (*9–11*) and adequately transformed yield the following equations:

$$\mathrm{p}K_{m,1}^{app} = \mathrm{p}K_1^\theta + A_1 f^{(1)} + B_1 f^{(2)} + C_1 f^{(3)} \tag{3}$$

$$\mathrm{p}K_{m,2}^{app} = \mathrm{p}K_2^\theta + A_2 f^{(1)} + B_2 f^{(2)} + C_2 f^{(3)} + D_2 f^{(4)} + f(\mathrm{med}) \tag{4}$$

where $\mathrm{p}K_{m,i}^{app}$ is the stoichiometric equilibrium constant, $\mathrm{p}K_i^\theta$ is the thermodynamic equilibrium constant, A, B, C, and D are the Pitzer interaction terms grouped by the ionic strength functions, $f^{(i)}$

$$\begin{gathered} f^{(1)} = 2I \\ f^{(2)} = -1 + (1 + 2I^{1/2}) \exp(-2I^{1/2}) \\ f^{(3)} = I^2 \\ f^{(4)} = -0.392\left[\frac{I^{1/2}}{1 + 1.2I^{1/2}} + \frac{2}{1.2}\ln(1 + 1.2I^{1/2})\right] \end{gathered} \tag{5}$$

$f^{(4)}$ is the Debye–Hückel term, and $f(\mathrm{MED})$ involves the contribution of the medium to the interaction, contributing only to the second ionization equilibrium:

$$f(\mathrm{med}) = \frac{2}{\ln 10} \sum_c \sum_a m_c m_a (z_X{}^2 B_{ca}{}^1 + z_X C_{ca}) \tag{6}$$

The term $f(\mathrm{med})$ in eq 4 depends only on the electrolytes

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involved in the electrolytic medium, that is, results independent of the amino acid or substance studied. This term, which appears only in the second ionization equilibrium, consists of a set of interaction parameters between the ions and the usual ionic strength functions defined by Pitzer (*21*):

$$f(\mathrm{med}) = m_{\mathrm{NaCl}}m_{\mathrm{Cl}}B^{1}_{\mathrm{NaCl}} + m_{\mathrm{Na}}m_{\mathrm{Cl}}C_{\mathrm{NaCl}} + m_{\mathrm{Na}}m_{\mathrm{SO_4}}B^{1}_{\mathrm{NaSO_4}} + m_{\mathrm{Na}}m_{\mathrm{SO_4}}C_{\mathrm{NaSO_4}} + m_{\mathrm{Mg}}m_{\mathrm{Cl}}B^{1}_{\mathrm{MgCl}} + m_{\mathrm{Mg}}m_{\mathrm{Cl}}C_{\mathrm{MgCl}} + m_{\mathrm{Mg}}m_{\mathrm{SO_4}}B^{1}_{\mathrm{MgSO_4}} + m_{\mathrm{Mg}}m_{\mathrm{SO_4}}C_{\mathrm{MgSO_4}} + m_{\mathrm{Ca}}m_{\mathrm{Cl}}B^{1}_{\mathrm{CaCl}} + m_{\mathrm{Ca}}m_{\mathrm{Cl}}C_{\mathrm{CaCl}} + m_{\mathrm{Ca}}m_{\mathrm{SO_4}}B^{1}_{\mathrm{CaSO_4}} + m_{\mathrm{Ca}}m_{\mathrm{SO_4}}C_{\mathrm{CaSO_4}} + m_{\mathrm{K}}m_{\mathrm{Cl}}B^{1}_{\mathrm{KCl}} + m_{\mathrm{K}}m_{\mathrm{Cl}}C_{\mathrm{KCl}} + m_{\mathrm{K}}m_{\mathrm{SO_4}}B^{1}_{\mathrm{KSO_4}} + m_{\mathrm{K}}m_{\mathrm{SO_4}}C_{\mathrm{KSO_4}} \quad (7)$$

Equation 7 can be rewritten as follows:

$$f(\mathrm{med}) = 0.2882f^{(5)} + 0.0119f^{(6)} - 0.0020f^{(7)} \quad (8)$$

where the new ionic strength functions—$f^{(i)}$—are

$$f^{(5)} = -1 + (1 + 2I^{1/2} + 2I)\exp(-2I^{1/2}) \quad (9)$$

$$f^{(6)} = -1 + (1 + 1.4I^{1/2} + 0.98I)\exp(-1.4I^{1/2}) \quad (10)$$

$$f^{(7)} = -1 + (1 + 12I^{1/2} + 72I)\exp(-12I^{1/2}) \quad (11)$$

The equations obtained for the dependence of $\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},i}$ with the ionic strength are (see also Figure 1):

$$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}(\alpha - \mathrm{Ala}) = 2.548(\pm 0.007) - 0.062(\pm 0.220)f^{(1)} - 0.528(\pm 0.375)f^{(2)} + 0.617(\pm 0.308)f^{(3)} \quad (12)$$

$$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}(\mathrm{Leu}) = 2.568(\pm 0.037) + 0.835(\pm 1.228)f^{(1)} - 2.211(\pm 2.039)f^{(2)} - 0.303(\pm 1.594)f^{(3)} \quad (13)$$

$$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}(\mathrm{Thr}) = 2.443(\pm 0.031) + 0.658(\pm 1.038)f^{(1)} - 2.072(\pm 1.724)f^{(2)} + 0.118(\pm 1.348)f^{(3)} \quad (14)$$

$$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}(\mathrm{Met}) = 2.469(\pm 0.056) + 1.946(\pm 1.850)f^{(1)} - 4.301(\pm 3.072)f^{(2)} - 1.349(\pm 2.403)f^{(3)} \quad (15)$$

$$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},2}(\alpha - \mathrm{Ala}) = 10.075(\pm 0.018) + 2.418(\pm 0.605)f^{(1)} - 4.232(\pm 1.005)f^{(2)} - 2.456(\pm 0.786)f^{(3)} + F(f^{(4)},\mathrm{med}) \quad (16)$$

$$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},2}(\mathrm{Leu}) = 9.924(\pm 0.029) + 1.724(\pm 0.948)f^{(1)} - 3.029(\pm 1.575)f^{(2)} - 1.573(\pm 1.231)f^{(3)} + F(f^{(4)},\mathrm{med}) \quad (17)$$

$$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},2}(\mathrm{Thr}) = 9.274(\pm 0.015) + 0.120(\pm 0.401)f^{(1)} - 0.487(\pm 0.799)f^{(2)} + 0.448(\pm 0.625)f^{(3)} + F(f^{(4)},\mathrm{med}) \quad (18)$$

$$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},2}(\mathrm{Met}) = 9.413(\pm 0.025) + 1.329(\pm 0.834)f^{(1)} - 2.382(\pm 1.385)f^{(2)} - 1.004(\pm 1.083)f^{(3)} + F(f^{(4)},\mathrm{med}) \quad (19)$$

where the numbers in parentheses correspond to the error in the parameters. Even though the error in the parameters is quite large—owing to the ill-conditioned correlation matrix of the explanatory variables in the model arising from the multicolinearity between them (*11, 22, 23*)—the error of the fit falls within the accepted range (Table 2).

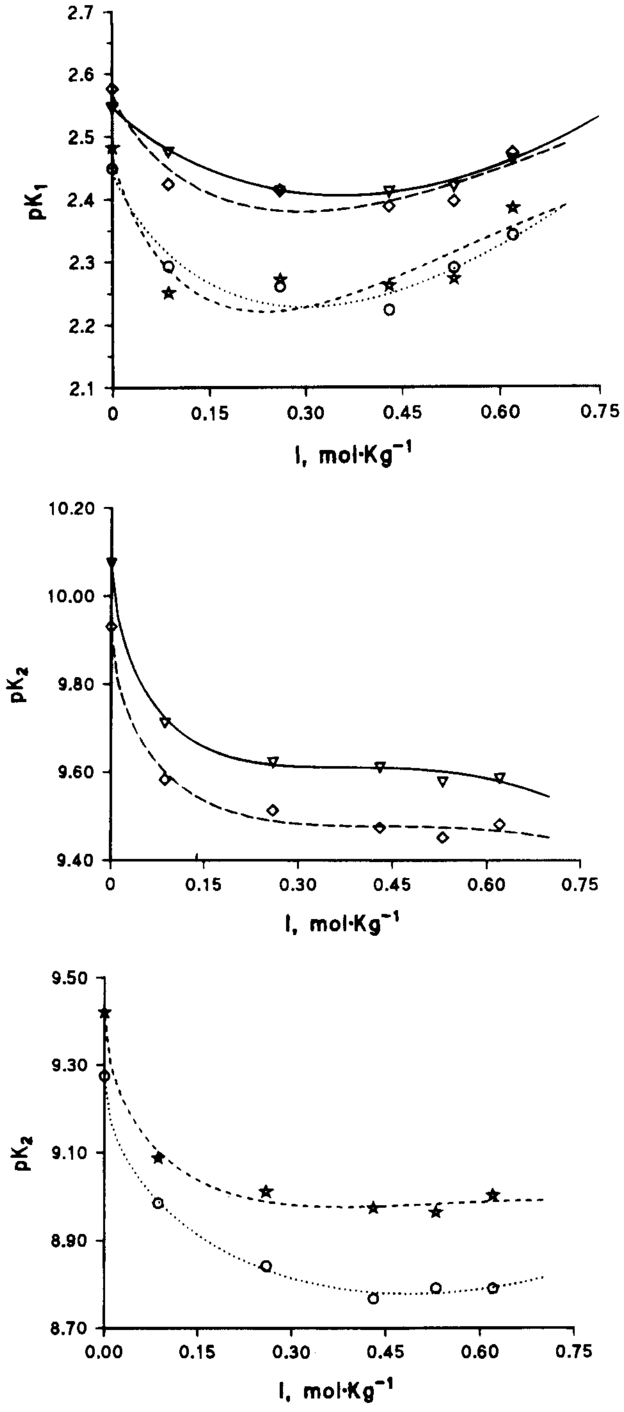

Figure 1. Experimental (a, top) $\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$ and (b, middle, bottom) $\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},2}$ for the amino acids studied (inverted triangle, α-alanine; diamond, leucine; circle, threonine; star, methionine). The lines correspond to the fitting curves of the experimental points to the Pitzer model by an ordinary least-squares method. (—, α-alanine; – – –, leucine; - - -, threonine; ···, methionine).

Since $f(\mathrm{med})$ remains constant for a given medium, we simplified eqs 16–19 using

$$F(f^{(4)},\mathrm{med}) = \frac{2}{\ln 10}f^{(4)} + \frac{2}{\ln 10}f(\mathrm{med}) \quad (20)$$

When we consider the approximation of *additivity in the weighted logarithm of the activity coefficients* [the hypothesis of additivity considers that the logarithm of the activity coefficient of a species, i, in artificial sea water equals the sum of the logarithm of activity coefficients of the ion in the individual electrolytes present in artificial sea water: $\ln \gamma_i$ in artificial sea water $= x_{\mathrm{NaCl}} \ln \gamma_i$ in NaCl $+ x_{\mathrm{KCl}} \ln$

Table 1. Experimental pK_m^{app} for the Amino Acids α-Alanine, Leucine, Threonine, and Methionine at 25 °C and Different Ionic Strengths, *I*, of Artificial Sea Water

I (mol·kg^{-1})	α-alanine	leucine	threonine	methionine
		$pK_{m,1}^{app}$		
0.00^a	2.549	2.576	2.449	2.482
0.087	2.478	2.424	2.293	2.251
0.26	2.415	2.414	2.261	2.271
0.43	2.414	2.388	2.223	2.261
0.53	2.422	2.396	2.290	2.272
0.62	2.465	2.473	2.342	2.384
σ^b	0.01−0.06	0.03−0.06	0.006−0.05	0.006−0.06
		$pK_{m,2}^{app}$		
0.00^a	10.078	9.930	9.275	9.419
0.087	9.715	9.583	8.985	9.087
0.26	9.626	9.514	8.842	9.010
0.43	9.614	9.474	8.767	8.973
0.53	9.581	9.452	8.791	8.963
0.62	9.589	9.482	8.790	9.001
σ^b	0.005−0.03	0.001−0.03	0.001−0.01	0.01−0.02

^{a} Extrapolated values (pK^θ) obtained by us in NaCl. ^{b} Range of variation for the standard deviation of the pK_m^{app}.

Table 2. Error of the Fit (σ)

	σ	
amino acid	first p*K* (eq)	second p*K* (eq)
α-alanine	0.007 (7)	0.019 (11, 15)
leucine	0.038 (8)	0.029 (12, 16)
threonine	0.032 (9)	0.015 (13, 17)
methionine	0.057 (10)	0.026 (14, 18)

γ_i in KCl + ... (x_i is the molar fraction of component i) and it can be considered as a *weighted* sum]—fulfillment of Young's first rule (*24*, *25*), that is, assumption that the properties of a mixture can be approximated by some suitable weighting of the properties of the component single electrolyte solutions—calculated for the individual electrolytes, the new function f(med) has the form

$$f(\mathrm{med}') = 0.2882f^{(5)} \qquad (21)$$

and the function $F(f^{(4)},\mathrm{med}')$ in eqs 16−19 is

$$F(f^{(4)},f(\mathrm{med}')) = 0.8686f^{(4)} + 0.2882f^{(5)} \qquad (22)$$

The coefficients of eqs 12−19 showed only a slight difference compared to those obtained under the hypothesis that the activity coefficient of the species in artificial sea water equals the weighted sum of the activity coefficient in each individual electrolyte. The term which involves the difference is the one with the contribution of the medium f(med′):

$$pK_{m,2}^{app}(\alpha\text{-Ala}) = 10.075(\pm 0.018) + 2.394(\pm 0.639)f^{(1)} - 4.191(\pm 1.060)f^{(2)} - 2.436(\pm 0.828)f^{(3)} + F(f^{(4)},\mathrm{med}') \qquad (23)$$

$$pK_{m,2}^{app}(\mathrm{Leu}) = 9.924(\pm 0.029) + 1.699(\pm 1.000)f^{(1)} - 2.988(\pm 1.661)f^{(2)} - 1.554(\pm 1.297)f^{(3)} + F(f^{(4)},\mathrm{med}') \qquad (24)$$

$$pK_{m,2}^{app}(\mathrm{Thr}) = 9.274(\pm 0.015) + 0.096(\pm 0.508)f^{(1)} - 0.446(\pm 0.844)f^{(2)} + 0.467(\pm 0.659)f^{(3)} + F(f^{(4)},\mathrm{med}') \qquad (25)$$

$$pK_{m,2}^{app}(\mathrm{Met}) = 9.413(\pm 0.025) + 1.304(\pm 0.879)f^{(1)} - 2.341(\pm 1.460)f^{(2)} - 0.984(\pm 1.140)f^{(3)} + F(f^{(4)},\mathrm{med}') \qquad (26)$$

Literature Cited

(1) Lilley, T. H. In *Studies in Modern Thermodynamics & Biochemical Thermodynamics*; Jones, M. N., Ed.; Elsevier: Amsterdam, 1988; Chapter 1.

(2) Rey, F.; Varela, A.; Antelo, J. M.; Arce, F. *J. Chem. Eng. Data* **1989**, *34*, 35−37.

(3) De Robertis, A.; De Stefano, C.; Rigano, C.; Sammartano, S. *J. Solution Chem.* **1990**, *19*, 569−587.

(4) Daniele, P. G.; Rigano, C.; Sammartano, S. *Talanta* **1983**, *30* (2), 81−87.

(5) Casale, A.; De Stefano, C.; Sammartano, S.; Daniele, P. G. *Talanta* **1989**, *36* (9), 903−907.

(6) Brandariz, I.; Fiol, S.; Herrero, R.; Vilariño, T.; Sastre de Vicente, M. *J. Chem. Eng. Data* **1993**, *38*, 531−533.

(7) Brandariz, I.; Arce, F.; Armesto, X. L.; Penedo, F.; Sastre de Vicente, M. *Monatsh. Chem.* **1993**, *124*, 249−257.

(8) Brandariz, I.; Herrero, R.; Sastre de Vicente, M. *J. Chim. Phys. Phys.-Chim. Biol.* **1993**, *90*, 63−77.

(9) Herrero, R.; Brandariz, I.; Fiol, S.; Sastre de Vicente, M. *Collect. Czech. Chem. Commun.* **1993**, *58*, 1269−1278.

(10) Fiol, S.; Brandariz, I.; Armesto, X. L.; Arce, F.; Sastre de Vicente, M. *Ann. Chim. (Rome)* **1993**, *83*, 175−187.

(11) Fiol, S.; Brandariz, I.; Herrero, R.; Vilariño, T.; Sastre de Vicente, M. *Ber. Bunsen-Ges. Phys. Chem.* **1994**, *98* (2), 164−171.

(12) Herrero, R.; Brandariz, I.; Fiol, S.; Vilariño, T.; Sastre de Vicente, M. *An. Quim.* **1993**, *89*, 602−610.

(13) Herrero, R.; Brandariz, I.; Sastre de Vicente, M. *Ber. Bunsen-Ges. Phys. Chem.* **1993**, *97* (1), 59−63.

(14) Herrero, R.; Armesto, X. L.; Arce, F.; Sastre de Vicente, M. *J. Solution Chem.* **1992**, *21* (11), 1185−1193.

(15) Thurman, E. M. *Organic Geochemistry of Natural Waters*; Martinus Nijhoff/Dr. W. Junk Publishers: Dordrecht, 1985; Chapter 6.

(16) Pitzer, K. S. *J. Phys. Chem.* **1973**, *77*, 268−277.

(17) Millero, F. J. *Mar. Chem.* **1990**, *30*, 205−229.

(18) Millero, F. J. *Limnol. Oceanogr.* **1986**, *31* (4), 839−847.

(19) Khoo, K. H.; Ramette, R. W.; Culberson, C. H.; Bates, R. G. *Anal. Chem.* **1977**, *49* (1), 29−34.

(20) Millero, F. J. *Thalassia Jugosl.* **1982**, *18* (1−4), 253−291.

(21) Pitzer, K. S. Ion Interaction Approach: Theory and Data Correlation. In *Activity Coefficients in Electrolyte Solutions*, 2nd ed.; Pitzer, K. S., Eds.; CRC Press: Boca Raton, FL, 1992; Chapter 3.

(22) Van Gaans, P. F. M. *J. Solution Chem.* **1990**, *20* (7), 703−729.

(23) Kendall, M. *Multivariate Analysis*; Ch. Griffin and Co., Ltd.: London, 1980.

(24) Young, T. F. *Rec. Chem. Prog.* **1951**, *12*, 81.

(25) Young, T. F.; Smith, M. B. *J. Phys. Chem.* **1954**, *58*, 716.

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