Temperature Dependence of the Dissociation Constants of Several Amino Acids

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The apparent dissociation constants of the amino acids lysine, histidine, arginine, glutamic acid, tyrosine, phenylalanine, tryptophan, and threonine were determined at 283.1 K, 298.1 K, 313.1 K, and 333.1 K at various ionic strengths by potentiometric titration. The Davies equation was used to extrapolate the dissociation constants to zero ionic strength. The pK values of the carboxylic acid attached to the α-carbon and the pK of the carboxylic acid on the side chain of glutamic acid were almost independent of temperature. Conversely, the pK values of the amino groups attached to the α-carbon and those on the side chain of the basic amino acids varied substantially with temperature. van't Hoff type plots of the pK values showed linear relationships indicating that the standard enthalpy changes of reaction are constant over this range of temperatures.

Introduction

Amino acids are important industrial products manufactured on the scale of 1 million metric tons per year.[1] They find applications in many areas including foods, animal feed supplements, and intravenous formulations, as well as chemical intermediates in pharmaceutical manufacturing. As is well-known, amino acids are amphoteric and dissociate in aqueous solution forming positively charged, zwitterionic, and negatively charged forms. Knowledge of the speciation of amino acids among these different forms is critical for many practical calculations. For example, modeling and design of unit operations for the industrial separation and purification of amino acids by ion exchange require a description of the dissociation behavior in solution to predict ion exchange equilibrium (e.g., see Saunders et al.,[2] Wang et al.,[3] Helfferich,[4] Bellot et al.,[5] and Nagai and Carta[6]). Crystallization modeling and design also require knowledge of speciation into the different ionized forms to predict solubility as a function of solution composition.[7]

The dissociation constants for most amino acids are generally available in the literature.[8,9] However, most literature values are limited to room temperature (298 K) and low ionic strength conditions that deviate substantially from those frequently encountered in practical industrial manufacturing processes. The effect of ionic strength on the dissociation constant of several amino acids has been reported by Rey et al.[10] although only at 298 K. Various measurements of thermodynamic properties of aqueous solutions of amino acids have also been reported as a function of temperature in the geochemical literature, including molar volumes,[11] densities,[12] and molar heat capacities.[13] Amino acid dissociation constants have also been reported as a function of temperature. However, most of these measurements are limited to only a few, neutral amino acids or are for temperature ranges that fall outside those used in the industrial processing of amino acids. For example, Robinson and Stokes[14] reported the temperature dependence of the dissociation constants of aspartic acid, glycine, leucine, iso-leucine, proline, serine, threonine, and valine. These authors used the integrated Gibbs–Helmholtz equation with a temperature-dependent enthalpy function to fit the measured apparent dissociation constants. Other authors[15–17] have also investigated the effect of temperature, but these studies have also been limited to glycine, alanine, and aminobutyric acid. More recently, Borst et al.[18] measured the temperature dependence of the dissociation constants of phenylalanine. These authors found that the pK of the carboxyl group of this amino acid is nearly independent of temperature while the pK of the amino group decreases substantially with temperature. The effect of temperature was described accurately with the integrated Gibbs–Helmholtz equation using a constant enthalpy over the temperature range from (278 to 358) K. Finally, Clarke et al.[19] have reported the temperature dependence of the carboxylic acid dissociation constant for glycine, alanine, and proline at temperatures between (150 and 250) °C.

The objectives of this contribution are 2-fold. The first is to extend the measurement of the effect of temperature on the dissociation constants to the amino acids lysine, histidine, arginine, glutamic acid, tyrosine, and tryptophan. Data for threonine and phenylalanine are also obtained to provide a consistent set of data and for comparison with previous work. The second objective is to determine the effect of ionic strength varied up to about 1 mol·kg^{-1} on the dissociation constants at different temperatures. Apparent dissociation constants are obtained by potentiometric titrations with activity coefficient corrections based on the Davies equation. Values of the enthalpy of dissociation are obtained by fitting the data with the Gibbs−Helmoltz equation.

Experimental Section

Chemicals. The amino acids lysine, histidine, arginine, glutamic acid, tyrosine, phenylalanine, tryptophan, and threonine were obtained from Ajinomoto Co., Inc. (Tokyo, Japan) with purity in excess of 99 %. Since lysine and histidine form hydrochloride salts, both free amino acids and HCl forms were used in the titration experiments. Titrants were certified hydrochloric acid normal solution and sodium hydroxide normal solution purchased from Wako Junyaku Chemicals (Osaka, Japan).

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Table 1. Apparent Dissociation Constants for Lysine at Different Temperatures and Ionic Strengths

			lysine solution			titrant				
T			Lys conc.	HCl conc.	volume	HCl conc.	NaOH conc.	volume	solution density	ionic strength
K	apparent dissociation constant		mol·L^{-1}	mol·L^{-1}	mL	mol·L^{-1}	mol·L^{-1}	mL	g·mL^{-1}	mol·kg^{-1}
283.1	$pK_{1,app}$	2.03	0.05	0.00	50	0.1000	0.0000	47.935	1.000	0.0611
283.1	$pK_{2,app}$	9.42	0.05	0.00	50	0.1000	0.0000	12.095	1.000	0.0199
283.1	$pK_{3,app}$	11.06	0.05	0.00	50	0.0000	0.1001	12.175	1.000	0.0205
298.1	$pK_{1,app}$	2.04	0.05	0.00	50	0.1000	0.0000	48.450	0.997	0.0611
298.1	$pK_{2,app}$	9.08	0.05	0.00	50	0.1000	0.0000	11.920	0.997	0.0199
298.1	$pK_{3,app}$	10.66	0.05	0.00	50	0.0000	0.1001	12.480	0.997	0.0204
313.1	$pK_{1,app}$	2.05	0.05	0.00	50	0.1000	0.0000	47.515	0.992	0.0613
313.1	$pK_{2,app}$	8.64	0.05	0.00	50	0.1000	0.0000	12.500	0.992	0.0203
313.1	$pK_{3,app}$	10.31	0.05	0.00	50	0.0000	0.1001	12.635	0.992	0.0204
333.1	$pK_{1,app}$	2.07	0.05	0.00	50	0.1000	0.0000	48.990	0.983	0.0616
333.1	$pK_{2,app}$	8.33	0.05	0.00	50	0.1000	0.0000	12.370	0.983	0.0205
333.1	$pK_{3,app}$	9.74	0.05	0.00	50	0.0000	0.1001	11.730	0.983	0.0202
313.1	$pK_{1,app}$	2.16	0.10	0.10	50	0.1000	0.0000	25.235	0.992	0.1376
313.1	$pK_{2,app}$	8.72	0.10	0.10	50	0.0000	0.1001	24.780	0.992	0.0676
313.1	$pK_{3,app}$	10.13	0.10	0.10	50	0.0000	0.1001	75.280	0.992	0.0607
313.1	$pK_{1,app}$	2.19	0.50	0.50	50	0.5000	0.0000	24.850	1.011	0.6634
313.1	$pK_{2,app}$	8.73	0.50	0.50	50	0.0000	0.5000	24.870	1.013	0.3312
313.1	$pK_{3,app}$	10.04	0.50	0.50	50	0.0000	0.5000	74.870	1.008	0.2989
313.1	$pK_{1,app}$	2.38	1.00	1.00	50	1.0000	0.0000	24.200	1.029	1.3061
313.1	$pK_{2,app}$	8.76	1.00	1.00	50	0.0000	1.0000	24.990	1.033	0.6502
313.1	$pK_{3,app}$	9.97	1.00	1.00	50	0.0000	1.0000	74.990	1.022	0.5900
298.1	$pK_{1,app}$	2.14	0.10	0.10	50	0.1000	0.0000	25.325	0.997	0.1370
298.1	$pK_{2,app}$	9.10	0.10	0.10	50	0.0000	0.1000	25.290	0.997	0.0839
298.1	$pK_{3,app}$	10.66	0.10	0.10	50	0.0000	0.1000	74.790	0.997	0.0906
298.1	$pK_{1,app}$	2.17	0.50	0.50	50	0.5000	0.0000	24.850	1.020	0.6577
298.1	$pK_{2,app}$	9.09	0.50	0.50	50	0.0000	0.5000	24.675	1.025	0.3266
298.1	$pK_{3,app}$	10.68	0.50	0.50	50	0.0000	0.5000	75.175	1.014	0.2966
298.1	$pK_{1,app}$	2.21	1.00	1.00	50	1.0000	0.0000	25.010	1.036	1.2896
298.1	$pK_{2,app}$	9.07	1.00	1.00	50	0.0000	1.0000	25.010	1.039	0.6445
298.1	$pK_{3,app}$	10.51	1.00	1.00	50	0.0000	1.0000	75.010	1.029	0.5849
333.1	$pK_{1,app}$	2.13	0.10	0.10	50	0.1000	0.0000	24.975	0.983	0.1394
333.1	$pK_{2,app}$	8.35	0.10	0.10	50	0.0000	0.1000	24.880	0.983	0.0681
333.1	$pK_{3,app}$	9.78	0.10	0.10	50	0.0000	0.1000	75.285	0.983	0.0612
333.1	$pK_{1,app}$	2.16	0.50	0.50	50	0.5000	0.0000	24.200	1.008	0.6720
333.1	$pK_{2,app}$	8.35	0.50	0.50	50	0.0000	0.5000	24.880	1.020	0.3284
333.1	$pK_{3,app}$	9.78	0.50	0.50	50	0.0000	0.5000	74.880	1.006	0.2992
333.1	$pK_{1,app}$	2.23	1.00	1.00	50	1.0000	0.0000	24.820	1.028	1.3016
333.1	$pK_{2,app}$	8.33	1.00	1.00	50	0.0000	1.0000	25.040	1.035	0.6476
333.1	$pK_{3,app}$	9.67	1.00	1.00	50	0.0000	1.0000	75.040	1.021	0.5898

Procedure. A model AT-310J potentiometric automatic titrator obtained from Kyoto Electronics Manufacturing Co., Ltd. (Kyoto, Japan) was used for the measurements in conjunction with a model H-171 pH glass electrode with inner silver chloride polar and a model R-171 reference electrode also from Kyoto Electronics Manufacturing. Both electrodes had ceramic wick junctions and were filled with 3.3 M KCl. Titration end points were determined at the inflections of the first derivative of the potentiometric function. Half-equivalence points were then used to obtain the apparent dissociation constants.

The procedure was as follows. Free amino acids or HCl salts for lysine or histidine were first dissolved in pure water in a 300 mL magnetically stirred vessel immersed in a thermostatted water bath. The pH meter of the automatic titrator was calibrated with two buffers at each temperature using 0.05 M potassium hydrogen phthalate (pH 4.00) and 0.025 M phosphate buffer (pH 6.86) for the acidic region and 0.01 M sodium borate (pH 9.18) and 0.025 M sodium phosphate (pH 6.86) for the alkaline region. Potentiometric titrations were then conducted adding either standardized HCl or NaOH. At the half-equivalence point, the apparent pK values were recorded along with the volume of titrant added to calculate the ionic strengths. Temperature was maintained constant by immersing the titrator syringe and the titrant vessels in the same thermostatic bath. Solution densities were determined with a model DA-30 (Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan) electrical density meter and used to convert molarities into molalities for the calculation of ionic strength. However, because of the relatively small temperature difference and fairly dilute conditions, the difference between molarity and molality was negligible.

Theory. Dissociation of the basic amino acids considered in this work (lysine, histidine, and arginine) is described by the following equations

$$AH_3^{2+} \leftrightarrow AH_2^+ + H^+ \tag{1}$$

$$AH_2^+ \leftrightarrow AH^\pm + H^+ \tag{2}$$

$$AH^\pm \leftrightarrow A^- + H^+ \tag{3}$$

where

$$K_1 = \frac{a_{AH_2^+} a_{H^+}}{a_{AH_3^{2+}}} = \frac{\gamma_{AH_2^+}\gamma_{H^+}}{\gamma_{AH_3^{2+}}} \frac{[AH_2^+][H^+]}{[AH_3^{2+}]} \tag{4}$$

$$K_2 = \frac{a_{AH^\pm} a_{H^+}}{a_{AH_2^+}} = \frac{\gamma_{AH^\pm}\gamma_{H^+}}{\gamma_{AH_2^+}} \frac{[AH^\pm][H^+]}{[AH_2^+]} \tag{5}$$

$$K_3 = \frac{a_{A^-} a_{H^+}}{a_{AH^\pm}} = \frac{\gamma_{A^-}\gamma_{H^+}}{\gamma_{AH^\pm}} \frac{[A^-][H^+]}{[AH^\pm]} \tag{6}$$

Table 2. Apparent Dissociation Constants for Histidine at Different Temperatures and Ionic Strengths

			histidine solution			titrant				
T			His conc.	HCl conc.	volume	HCl conc.	NaOH conc.	volume	solutiondensity	ionic strength
K	apparent dissociation constant		mol·L^{-1}	mol·L^{-1}	mL	mol·L^{-1}	mol·L^{-1}	mL	g·mL^{-1}	mol·kg^{-1}
283.1	$pK_{1,app}$	1.72	0.05	0.00	50	0.1000	0.0000	48.100	1.000	0.0659
283.1	$pK_{2,app}$	6.18	0.05	0.00	50	0.1000	0.0000	8.955	1.000	0.0182
283.1	$pK_{3,app}$	9.53	0.05	0.00	50	0.0000	0.1001	10.778	1.000	0.0192
298.1	$pK_{1,app}$	1.78	0.05	0.00	50	0.1000	0.0000	48.705	0.997	0.0648
298.1	$pK_{2,app}$	6.00	0.05	0.00	50	0.1000	0.0000	8.965	0.997	0.0183
298.1	$pK_{3,app}$	9.07	0.05	0.00	50	0.0000	0.1001	10.850	0.997	0.0193
313.1	$pK_{1,app}$	1.84	0.05	0.00	50	0.1000	0.0000	48.210	0.992	0.0641
313.1	$pK_{2,app}$	5.83	0.05	0.00	50	0.1000	0.0000	8.970	0.992	0.0184
313.1	$pK_{3,app}$	8.80	0.05	0.00	50	0.0000	0.1001	11.175	0.992	0.0195
333.1	$pK_{1,app}$	1.83	0.05	0.00	50	0.1000	0.0000	48.225	0.983	0.0648
333.1	$pK_{2,app}$	5.53	0.05	0.00	50	0.1000	0.0000	8.948	0.983	0.0185
333.1	$pK_{3,app}$	8.31	0.05	0.00	50	0.0000	0.1001	11.030	0.983	0.0196
313.1	$pK_{1,app}$	1.90	0.10	0.10	50	0.1000	0.0000	24.930	0.992	0.1408
313.1	$pK_{2,app}$	5.85	0.10	0.10	50	0.0000	0.1000	24.920	0.992	0.0672
313.1	$pK_{3,app}$	8.84	0.10	0.10	50	0.0000	0.1000	74.740	0.992	0.0605
313.1	$pK_{1,app}$	1.90	0.20	0.20	50	0.2000	0.0000	25.120	1.000	0.2724
313.1	$pK_{2,app}$	5.89	0.20	0.20	50	0.0000	0.2000	24.990	1.001	0.1332
313.1	$pK_{3,app}$	8.98	0.20	0.20	50	0.0000	0.2000	74.780	0.999	0.1202
298.1	$pK_{1,app}$	1.85	0.10	0.10	50	0.1000	0.0000	25.000	0.997	0.1408
298.1	$pK_{2,app}$	5.98	0.10	0.10	50	0.0000	0.1000	24.780	0.997	0.0669
298.1	$pK_{3,app}$	9.09	0.10	0.10	50	0.0000	0.1000	74.860	0.997	0.0602
298.1	$pK_{1,app}$	1.86	0.20	0.20	50	0.2000	0.0000	24.935	1.004	0.2726
298.1	$pK_{2,app}$	6.02	0.20	0.20	50	0.0000	0.2000	25.185	1.005	0.1326
298.1	$pK_{3,app}$	9.10	0.20	0.20	50	0.0000	0.2000	75.055	1.003	0.1196
333.1	$pK_{1,app}$	1.89	0.10	0.10	50	0.1000	0.0000	25.050	0.983	0.1421
333.1	$pK_{2,app}$	5.55	0.10	0.10	50	0.0000	0.1000	25.220	0.983	0.0678
333.1	$pK_{3,app}$	8.46	0.10	0.10	50	0.0000	0.1000	74.965	0.983	0.0610
333.1	$pK_{1,app}$	1.90	0.20	0.20	50	0.2000	0.0000	25.205	1.000	0.2725
333.1	$pK_{2,app}$	5.55	0.20	0.20	50	0.0000	0.2000	24.680	1.001	0.0667
333.1	$pK_{3,app}$	8.41	0.20	0.20	50	0.0000	0.2000	75.165	0.999	0.0601

Table 3. Apparent Dissociation Constants for Arginine at Different Temperatures and Ionic Strengths

			arginine solution			titrant				
T			Arg conc.	HCl conc.	volume	HCl conc.	NaOH conc.	volume	solution density	ionic strength
K	apparent dissociation constant		mol·L^{-1}	mol·L^{-1}	mL	mol·L^{-1}	mol·L^{-1}	mL	g·mL^{-1}	mol·kg^{-1}
283.1	$pK_{1,app}$	2.15	0.05	0.00	50	0.1000	0.0000	42.550	1.000	0.0603
283.1	$pK_{2,app}$	9.38	0.05	0.00	50	0.1000	0.0000	12.450	1.000	0.0248
283.1	$pK_{3,app}$	11.88	0.05	0.00	50	0.0000	0.1001	12.225	1.000	0.0237
298.1	$pK_{1,app}$	2.16	0.05	0.00	50	0.1000	0.0000	42.190	0.997	0.0604
298.1	$pK_{2,app}$	9.05	0.05	0.00	50	0.1000	0.0000	13.930	0.997	0.0231
298.1	$pK_{3,app}$	11.80	0.05	0.00	50	0.0000	0.1001	12.385	0.997	0.0232
313.1	$pK_{1,app}$	2.17	0.05	0.00	50	0.1000	0.0000	42.550	0.992	0.0605
313.1	$pK_{2,app}$	8.64	0.05	0.00	50	0.1000	0.0000	14.950	0.992	0.0213
313.1	$pK_{3,app}$	11.78	0.05	0.00	50	0.0000	0.1001	12.140	0.992	0.0230
333.1	$pK_{1,app}$	2.13	0.05	0.00	50	0.1000	0.0000	42.310	0.983	0.0615
333.1	$pK_{2,app}$	8.34	0.05	0.00	50	0.1000	0.0000	12.650	0.983	0.0204
333.1	$pK_{3,app}$	11.60	0.05	0.00	50	0.0000	0.1001	12.085	0.983	0.0222

are the thermodynamic dissociation constants. In these equations the a_i's are activities [mol·L^{-1}]; the γ_i's are activity coefficients; and the terms in brackets are molarities [mol·L^{-1}]. The activity coefficients can be related to ionic strength using a suitable activity coefficient model for electrolytes. The Davies equation[20] was used in this work

$$\log \gamma_i = -z_i^2\left(\frac{A\sqrt{I}}{1+\sqrt{I}} - bI\right) \tag{7}$$

where A, the Debye–Hückel constant, incorporates temperature effects, $b = 0.1$, and z_i is the ion charge. A is approximately 0.51 at 298 K or, more precisely, 0.5091 at 298.15 K according to Fernandez et al.[21] The ionic strength is defined as

$$I = \frac{1}{2}\sum_k m_k z_k^2 \tag{8}$$

where m_k is the molality [mol·kg^{-1}] of ion k. A can be estimated from[22]

$$A = 1.8252 \cdot 10^6\left(\frac{\rho_W}{\epsilon^3 T^3}\right)^{1/2} \tag{9}$$

where ρ_W and ϵ are the density [g·cm^{-3}] and dielectric constant of water, respectively. The latter is expressed as a function of temperature by the equation[23]

$$\epsilon = \frac{5321}{T} + 233.76 - 0.9297T + 1.417 \cdot 10^{-3}T^2 - 8.292 \cdot 10^{-7}T^3 \tag{10}$$

while ρ_W is readily available in standard handbooks. We also compared predictions based on this equation with those from the NIST database[24] and found that the results were in nearly perfect agreement (maximum deviation $< \pm 0.1$ %) over the range of temperatures of interest in this work.

It should be noted that other models that are thought to be more accurate at higher ionic strengths exist, notably the Pitzer ion interaction model.[25,26] The latter takes into account ion association effects but requires experimental data specific to the types of ions

Table 4. Apparent Dissociation Constants for Glutamic Acid at Different Temperatures and Ionic Strengths

			glutamic acid solution			titrant				
T			Glu conc.	NaOH conc.	volume	HCl conc.	NaOH conc.	volume	solution density	ionic strength
K	apparent dissociation constant		mol·L^{-1}	mol·L^{-1}	mL	mol·L^{-1}	mol·L^{-1}	mL	g·mL^{-1}	mol·kg^{-1}
283.1	$pK_{1,app}$	2.13	0.05	0.00	50	0.1000	0.0000	19.300	1.000	0.0267
283.1	$pK_{2,app}$	4.20	0.05	0.00	50	0.0000	0.1000	11.610	1.000	0.0196
283.1	$pK_{3,app}$	9.82	0.05	0.00	50	0.0000	0.1001	36.360	1.000	0.0476
298.1	$pK_{1,app}$	2.19	0.05	0.00	50	0.1000	0.0000	19.340	0.997	0.0263
298.1	$pK_{2,app}$	4.23	0.05	0.00	50	0.0000	0.1000	11.610	0.997	0.0197
298.1	$pK_{3,app}$	9.63	0.05	0.00	50	0.0000	0.1001	36.130	0.997	0.0477
313.1	$pK_{1,app}$	2.18	0.05	0.00	50	0.1000	0.0000	19.500	0.992	0.0266
313.1	$pK_{2,app}$	4.24	0.05	0.00	50	0.0000	0.1000	12.050	0.992	0.0200
313.1	$pK_{3,app}$	9.32	0.05	0.00	50	0.0000	0.1001	36.100	0.992	0.0480
333.1	$pK_{1,app}$	2.14	0.05	0.00	50	0.1000	0.0000	19.560	0.983	0.0272
333.1	$pK_{2,app}$	4.27	0.05	0.00	50	0.0000	0.1000	12.030	0.983	0.0198
333.1	$pK_{3,app}$	9.11	0.05	0.00	50	0.0000	0.1001	36.180	0.983	0.0495
298.1	$pK_{2,app}$	4.21	0.20	0.20	50	0.2000	0.0000	24.680	1.004	0.1331
298.1	$pK_{3,app}$	9.66	0.20	0.20	50	0.0000	0.2000	24.640	1.005	0.2217
313.1	$pK_{2,app}$	4.18	0.20	0.20	50	0.2000	0.0000	24.790	1.000	0.1336
313.1	$pK_{3,app}$	9.24	0.20	0.20	50	0.0000	0.2000	24.750	1.001	0.2224
333.1	$pK_{2,app}$	4.21	0.20	0.20	50	0.2000	0.0000	25.020	1.000	0.1335
333.1	$pK_{3,app}$	8.86	0.20	0.20	50	0.0000	0.2000	24.790	1.001	0.2223
298.1	$pK_{2,app}$	4.21	0.50	0.50	50	0.5000	0.0000	25.060	1.018	0.3278
298.1	$pK_{3,app}$	9.61	0.50	0.50	50	0.0000	0.5000	25.310	1.012	0.5477
313.1	$pK_{2,app}$	4.17	0.50	0.50	50	0.5000	0.0000	25.070	1.01	0.3304
313.1	$pK_{3,app}$	9.17	0.50	0.50	50	0.0000	0.5000	24.680	1.012	0.5503
333.1	$pK_{2,app}$	4.19	0.50	0.50	50	0.5000	0.0000	24.980	1.012	0.3298
333.1	$pK_{3,app}$	8.76	0.50	0.50	50	0.0000	0.5000	24.730	1.015	0.5484

Table 5. Apparent Dissociation Constants for Tyrosine at Different Temperatures and Ionic Strengths

			tyrosine solution			titrant				
T			Tyr conc.	HCl conc.	volume	HCl conc.	NaOH conc.	volume	solution density	ionic strength
K	apparent dissociation constant		mol·L^{-1}	mol·L^{-1}	mL	mol·L^{-1}	mol·L^{-1}	mL	g·mL^{-1}	mol·kg^{-1}
283.1	$pK_{1,app}$	2.03	0.0003	0.0000	50	0.0010	0.0000	5.029	1.000	0.003850
283.1	$pK_{2,app}$	9.42	0.0003	0.0000	50	0.0000	0.0010	0.222	1.000	0.000094
283.1	$pK_{3,app}$	11.06	0.0003	0.0000	50	0.0000	0.0010	0.545	1.000	0.000232
298.1	$pK_{1,app}$	2.04	0.0003	0.0000	50	0.0010	0.0000	2.170	0.997	0.003780
298.1	$pK_{2,app}$	9.08	0.0003	0.0000	50	0.0000	0.0010	0.225	0.997	0.000087
298.1	$pK_{3,app}$	10.66	0.0003	0.0000	50	0.0000	0.0010	0.560	0.997	0.000152
313.1	$pK_{1,app}$	2.05	0.0003	0.0000	50	0.0010	0.0000	4.975	0.992	0.003723
313.1	$pK_{2,app}$	8.64	0.0003	0.0000	50	0.0000	0.0010	0.220	0.992	0.000085
313.1	$pK_{3,app}$	10.31	0.0003	0.0000	50	0.0000	0.0010	0.530	0.992	0.000124
333.1	$pK_{1,app}$	2.07	0.0003	0.0000	50	0.0010	0.0000	4.490	0.983	0.003592
333.1	$pK_{2,app}$	8.33	0.0003	0.0000	50	0.0000	0.0010	0.241	0.983	0.000085
333.1	$pK_{3,app}$	9.74	0.0003	0.0000	50	0.0000	0.0010	0.570	0.983	0.000093

Table 6. Apparent Dissociation Constants for Phenylalanine at Different Temperatures and Ionic Strengths

			phenylalanine solution			titrant				
T			Phe conc.	HCl conc.	volume	HCl conc.	NaOH conc.	volume	solution density	ionic strength
K	apparent dissociation constant		mol·L^{-1}	mol·L^{-1}	mL	mol·L^{-1}	mol·L^{-1}	mL	g·mL^{-1}	mol·kg^{-1}
283.1	$pK_{1,app}$	2.32	0.10	0.00	50	0.1000	0.0000	25.205	1.000	0.03578
283.1	$pK_{2,app}$	9.54	0.10	0.00	50	0.0000	0.1000	24.665	1.000	0.03329
298.1	$pK_{1,app}$	2.28	0.10	0.00	50	0.1000	0.0000	24.770	0.997	0.03602
298.1	$pK_{2,app}$	9.19	0.10	0.00	50	0.0000	0.1000	24.765	0.997	0.03339
313.1	$pK_{1,app}$	2.32	0.10	0.00	50	0.1000	0.0000	25.200	0.992	0.03585
313.1	$pK_{2,app}$	8.90	0.10	0.00	50	0.0000	0.1000	24.860	0.992	0.03357
333.1	$pK_{1,app}$	2.33	0.10	0.00	50	0.1000	0.0000	24.300	0.983	0.03612
333.1	$pK_{2,app}$	8.32	0.10	0.00	50	0.0000	0.1000	24.140	0.983	0.03371

in solution to determine the ion interaction parameters[27] which are not available for our case. The Davies equation is generally believed to be accurate up to $I = 0.5$ mol·kg^{-1}.[27] Nonetheless, even at higher ionic strengths, the accuracy of the Davies model is reasonable. For example, comparing predictions based on eqs 7 to 10 with the mean activity coefficient data and fitted values based on the Pitzer model reported by Samson et al.[28] and, more recently, by Moggia and Bianco[27] for various electrolyte solutions shows that the error of eq 7 on log γ is less than 10 % at ionic strengths as high 1.5 mol·kg^{-1}. Moreover, as shown later in this paper, the Davies model predicts the trends observed in our work within the apparent experimental accuracy of our pK value determinations.

Combining eqs 4 to 7 yields:

$$K_1 = 10^{2(A\sqrt{I}/1+\sqrt{I}-bI)}\frac{[AH_2^+][H^+]}{[AH_3^{2+}]} \quad (11)$$

$$K_2 = \frac{[AH^{\pm}][H^+]}{[AH_2^+]} \quad (12)$$

$$K_3 = 10^{-2(A\sqrt{I}/1+\sqrt{I}-bI)}\frac{[A^-][H^+]}{[AH^{\pm}]} \quad (13)$$

or, alternatively

Table 7. Apparent Dissociation Constants for Tryptophan at Different Temperatures and Ionic Strengths

			tryptophan solution			titrant				
T			Trp conc.	HCl conc.	volume	HCl conc.	NaOH conc.	volume	solution density	ionic strength
K	apparent dissociation constant		mol·L^{-1}	mol·L^{-1}	mL	mol·L^{-1}	mol·L^{-1}	mL	g·mL^{-1}	mol·kg^{-1}
283.1	$pK_{1,app}$	2.32	0.005	0.000	50	0.010	0.000	16.270	1.000	0.004565
283.1	$pK_{2,app}$	9.70	0.005	0.000	50	0.000	0.010	10.500	1.000	0.001927
298.1	$pK_{1,app}$	2.37	0.005	0.000	50	0.010	0.000	16.500	0.997	0.004326
298.1	$pK_{2,app}$	9.32	0.005	0.000	50	0.000	0.010	10.520	0.997	0.001918
313.1	$pK_{1,app}$	2.30	0.005	0.000	50	0.010	0.000	18.700	0.992	0.004815
313.1	$pK_{2,app}$	8.98	0.005	0.000	50	0.000	0.010	10.520	0.992	0.001922
333.1	$pK_{1,app}$	2.35	0.005	0.000	50	0.010	0.000	17.195	0.983	0.004527
333.1	$pK_{2,app}$	8.82	0.005	0.000	50	0.000	0.010	10.500	0.983	0.001937

Table 8. Apparent Dissociation Constants for Threonine at Different Temperatures and Ionic Strengths

			threonine solution			titrant				
T			Thr conc.	HCl conc.	volume	HCl conc.	NaOH conc.	volume	solution density	ionic strength
K	apparent dissociation constant		mol·L^{-1}	mol·L^{-1}	mL	mol·L^{-1}	mol·L^{-1}	mL	g·mL^{-1}	mol·kg^{-1}
283.1	$pK_{1,app}$	2.73	0.10	0.00	50	0.1000	0.0000	13.640	1.000	0.031299
283.1	$pK_{2,app}$	9.89	0.10	0.00	50	0.0000	0.1000	37.680	1.000	0.035791
298.1	$pK_{1,app}$	2.72	0.10	0.00	50	0.1000	0.0000	13.600	0.997	0.031394
298.1	$pK_{2,app}$	9.56	0.10	0.00	50	0.0000	0.1000	37.830	0.997	0.035898
313.1	$pK_{1,app}$	2.75	0.10	0.00	50	0.1000	0.0000	13.790	0.992	0.031536
313.1	$pK_{2,app}$	9.25	0.10	0.00	50	0.0000	0.1000	37.680	0.992	0.036031
333.1	$pK_{1,app}$	2.74	0.10	0.00	50	0.1000	0.0000	13.510	0.983	0.031764
333.1	$pK_{2,app}$	8.97	0.10	0.00	50	0.0000	0.1000	37.505	0.983	0.036330

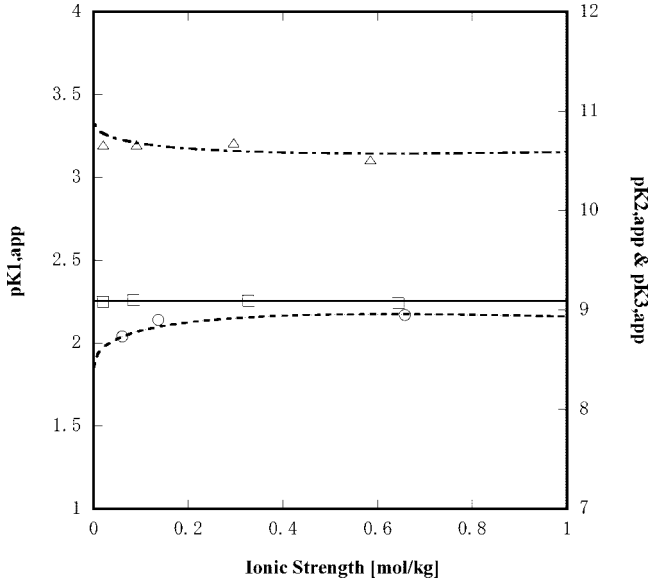

Figure 1. Dependence of ionic strength on apparent lysine dissociation constants at 298.1 K. Symbols are ○, $pK_{1,app}$; □, $pK_{2,app}$; △, $pK_{3,app}$.

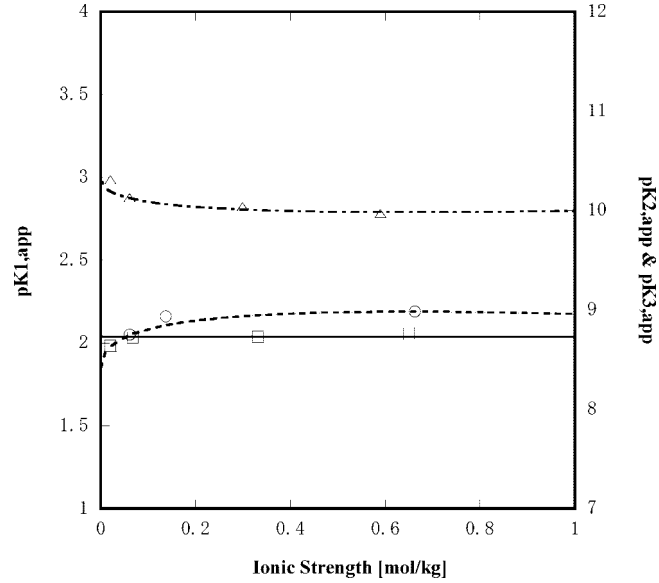

Figure 2. Dependence of ionic strength on apparent lysine dissociation constants at 313.1 K. Symbols are ○, $pK_{1,app}$; □, $pK_{2,app}$; △, $pK_{3,app}$.

$$pK_{1,app} = pK_1 + 2\left(\frac{A\sqrt{I}}{1+\sqrt{I}} - bI\right) \quad (14)$$

$$pK_{2,app} = pK_2 \quad (15)$$

$$pK_{3,app} = pK_3 - 2\left(\frac{A\sqrt{I}}{1+\sqrt{I}} - bI\right) \quad (16)$$

where we assumed that the activity coefficient of the zwitterion is unity. A similar treatment for glutamic acid and tyrosine yields

$$pK_{1,app} = pK_1 \quad (17)$$

$$pK_{2,app} = pK_2 - 2\left(\frac{A\sqrt{I}}{1+\sqrt{I}} - bI\right) \quad (18)$$

$$pK_{3,app} = pK_3 - 4\left(\frac{A\sqrt{I}}{1+\sqrt{I}} - bI\right) \quad (19)$$

Finally, for the neutral amino acids (phenylalanie, tryptophan, and threonine) we have

$$pK_{1,app} = pK_1 \quad (20)$$

$$pK_{2,app} = pK_2 - 2\left(\frac{A\sqrt{I}}{1+\sqrt{I}} - bI\right) \quad (21)$$

Since the ionic strength is known at each point along the potentiometric titration curve, in each case we can calculate the thermodynamic values pK_i from the apparent values $pK_{i,app}$ using these equations.

The temperature dependence of the dissociation constants can be described with the Gibbs–Helmholtz equation

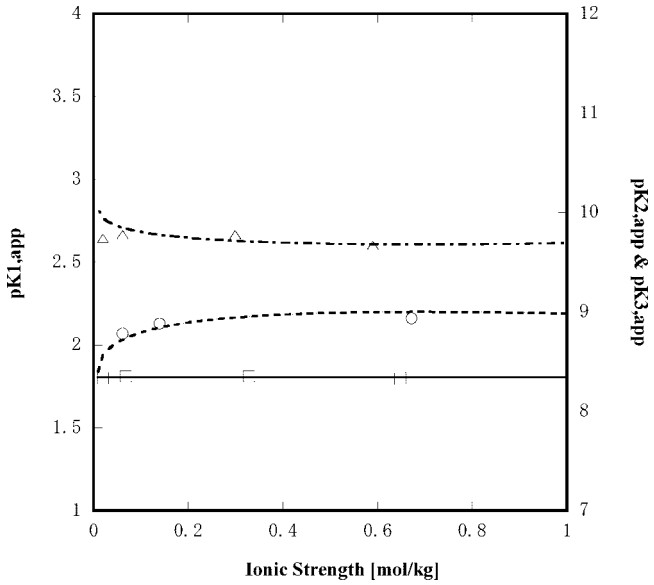

Figure 3. Dependence of ionic strength on apparent lysine dissociation constants at 333.1 K. Symbols are ○, $pK_{1,app}$; □, $pK_{2,app}$; △, $pK_{3,app}$.

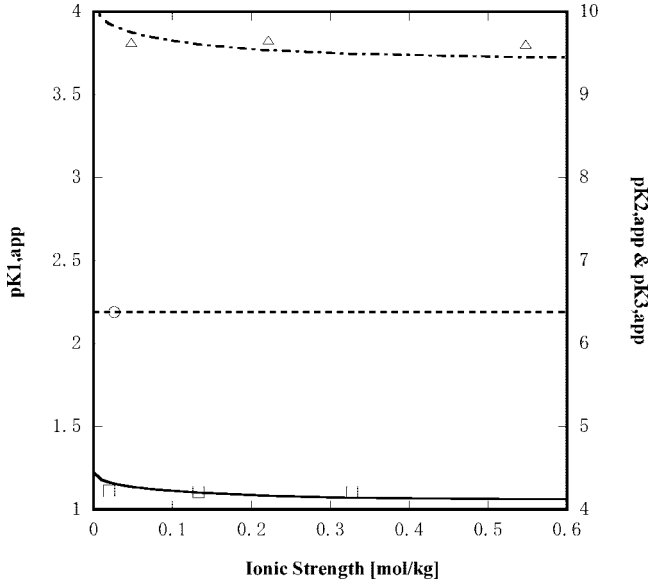

Figure 4. Dependence of ionic strength on apparent glutamic acid dissociation constants at 298.1 K. Symbols are ○, $pK_{1,app}$; □, $pK_{2,app}$; △, $pK_{3,app}$.

$$\frac{d}{dT}\left(\frac{\Delta G^0_{T,i}}{T}\right) = -\frac{\Delta H^0_{T,i}}{T^2} \tag{22}$$

where $\Delta G^0_{T,i} = -RT \ln K_i$ is the Gibbs free energy change; $\Delta H_{T,i}{}^0$ is the standard enthalpy change of reaction; R is the gas constant; and T is temperature. If $\Delta H_{T,i}{}^0$ is constant, we obtain the van't Hoff equation

$$\ln K_i = -\frac{\Delta H^0_{T,i}}{RT} - q_i \tag{23}$$

or

$$pK_i = \frac{1}{2.30}\left(\frac{\Delta H^0_{T,i}}{RT} + q_i\right) \tag{24}$$

where q_i is a dimensionless integration constant.

It should be noted that in addition to the treatment discussed above, a general theoretical framework for the prediction of the effects of temperature on thermodynamic constants for aqueous solutions of organic species, including amino acid dissociation constants, has been presented by Shock and Helgeson.[29] While the simplified treatment discussed above does not permit the level of generalization afforded by the model of Shock and Helgeson, it will be shown to be adequate for the correlation of our experimental data.

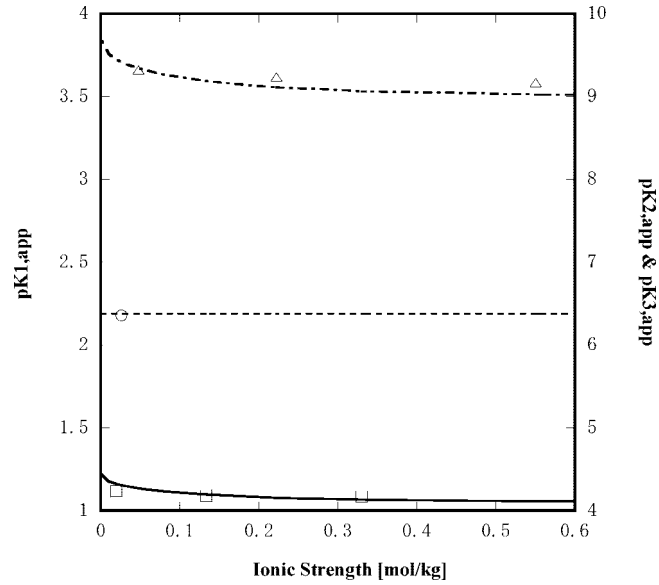

Figure 5. Dependence of ionic strength on apparent glutamic acid dissociation constants at 313.1 K. Symbols are ○, $pK_{1,app}$; □, $pK_{2,app}$; △, $pK_{3,app}$.

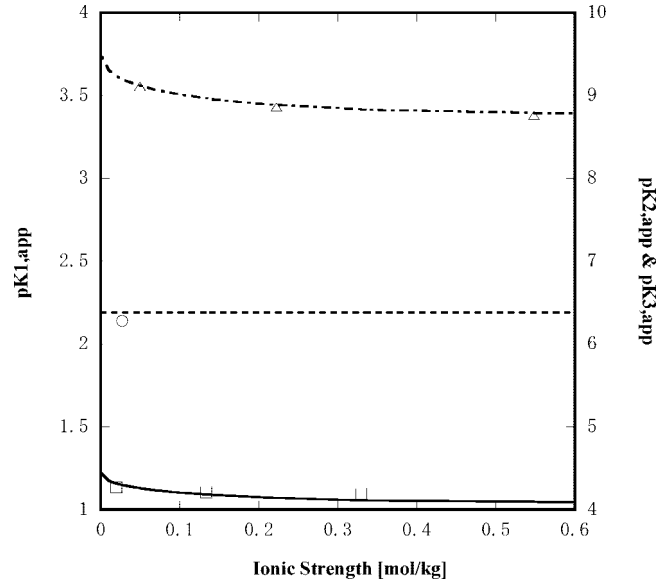

Figure 6. Dependence of ionic strength on apparent glutamic acid dissociation constants at 333.1 K. Symbols are ○, $pK_{1,app}$; □, $pK_{2,app}$; △, $pK_{3,app}$.

Results

The experimentally determined $pK_{i,app}$ values obtained at different temperatures and ionic strengths are summarized in Tables 1 to 8. The ionic strength was calculated from eq 8 based on the solution composition at each half-equivalence point. In this calculation, we took into account the volume and concentration of titrant added, the measured density of the solution, and the concentration of all positively and negatively charged amino acid species calculated using the pK_{app} values and the total

Table 9. Summary of Dissociation Constants for Lysine, Histidine, Arginine, Glutamic Acid, and Tyrosine Extrapolated to Zero Ionic Strength

	pK_1	pK_2	pK_3
Lys			
283.1 K	1.85	9.42	11.18
298.1 K	1.85	9.09	10.90
313.1 K	1.85	8.73	10.32
333.1 K	1.84	8.34	10.04
$\Delta H_T^0/R$ [K]	40	4726	5226
q	4.12	5.00	7.31
His			
283.1 K	1.54	6.18	9.66
298.1 K	1.56	6.00	9.25
313.1 K	1.60	5.85	9.00
333.1 K	1.60	5.54	8.60
$\Delta H_T^0/R$ [K]	−285	2729	4510
q	4.56	4.63	6.24
Arg			
283.1 K	1.97	9.38	12.01
298.1 K	1.97	9.05	11.94
313.1 K	1.98	8.64	11.92
333.1 K	1.93	8.34	11.74
$\Delta H_T^0/R$ [K]	33	874	209
q	0.74	0.99	4.49
Glu			
283.1 K	2.13	4.32	10.09
298.1 K	2.19	4.45	10.1
313.1 K	2.19	4.45	9.7
333.1 K	2.19	4.45	9.5
$\Delta H_T^0/R$ [K]	−44	−96	536
q	1.09	2.23	2.53
Tyr			
283.1 K	2.03	9.43	11.09
298.1 K	2.04	9.09	10.69
313.1 K	2.05	8.65	10.34
333.1 K	2.07	8.34	9.76
$\Delta H_T^0/R$ [K]	−171	4857	5713
q	5.27	4.53	5.40

Table 10. Summary of Dissociation Constants for Phenylalanine, Tryptophan, and Threonine Extrapolated to Zero Ionic Strength

	pK_1	pK_2
Phe		
283.1 K	2.32	9.69
298.1 K	2.28	9.34
313.1 K	2.32	9.06
333.1 K	2.33	8.48
$\Delta H_T^0/R$ [K]	−90	5155
q	5.61	4.17
Trp		
283.1 K	2.32	9.75
298.1 K	2.37	9.36
313.1 K	2.30	9.03
333.1 K	2.35	8.87
$\Delta H_T^0/R$ [K]	−35	3880
q	5.48	8.59
Thr		
283.1 K	2.73	10.04
298.1 K	2.72	9.72
313.1 K	2.75	9.41
333.1 K	2.74	9.14
$\Delta H_T^0/R$ [K]	−76	3945
q	6.54	9.13

amino acid concentration. Representative trend plots illustrating the effects of ionic strength are shown in Figures 1 to 3 for lysine and in Figures 4 to 6 for histidine. These plots also show curves calculated according to eqs 14 to 16. For these calculations, the pK_i values were determined in each case by a nonlinear least-squares fit of the p$K_{i,\mathrm{app}}$ data, minimizing the sum or residual squares between apparent and calculated values. The

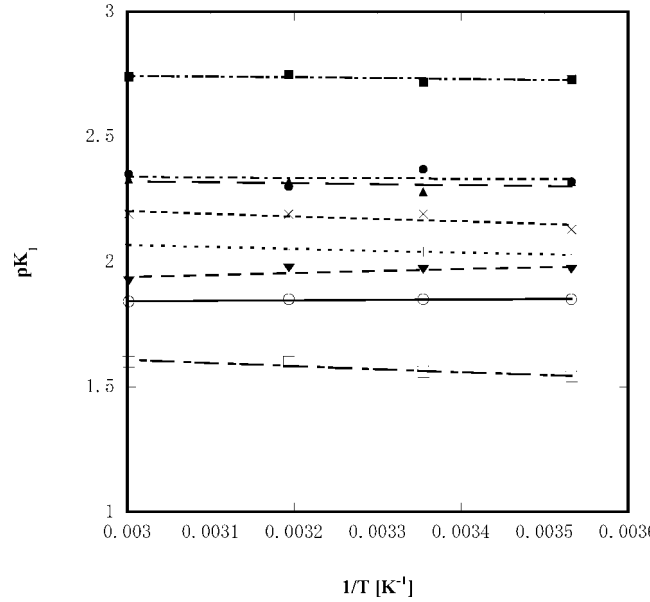

Figure 7. pK_1 values of lysine, histidine, arginine, tyrosine, glutamic acid, phenylalanine, tryptophan, and threonine extrapolated to zero ionic strength at 283.1 K, 298.1 K, 313.1 K, and 333.1 K. Symbols are ○, lysine; □, histidine; ▼, arginine; ×, glutamic acid; +, tyrosine; ▲, phenylalanine; •, tryptophan; and ■, threonine.

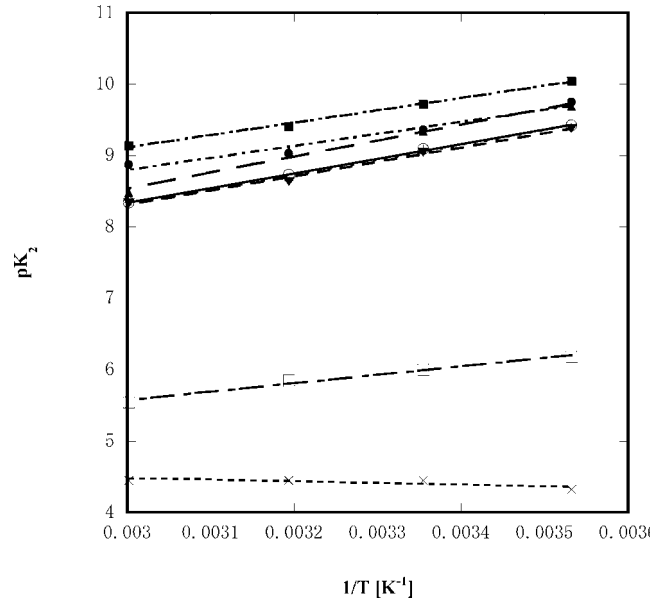

Figure 8. pK_2 values of lysine, histidine, arginine, tyrosine, glutamic acid, phenylalanine, tryptophan, and threonine extrapolated to zero ionic strength at 283.1 K, 298.1 K, 313.1 K, and 333.1 K. Symbols are ○, lysine; □, histidine; ▼, arginine; ×, glutamic acid; +, tyrosine; ▲, phenylalanine; •, tryptophan; and ■, threonine.

corresponding values are summarized in Tables 9 and 10 at different temperatures. As seen in Figures 1 to 6, the Davies equation provides a good fit of the experimental results. As seen, for example, for lysine in Figures 1 to 3, p$K_{1,\mathrm{app}}$ increases with I, p$K_{3,\mathrm{app}}$ decreases with I, and p$K_{2,\mathrm{app}}$ remains essentially constant, consistent with eqs 14 to 16. Analogous results were obtained for the other amino acids considered in this work.

Trend plots illustrating the effect of temperature on the pK_i values are shown in Figures 7 to 9 along with lines fitted to the data according to eq 24. The fitted parameters, $\Delta H_{T,i}^0/R$ and q_i, are also summarized in Tables 9 and 10. With reference to Figure 7, it can be seen that pK_1, corresponding to the

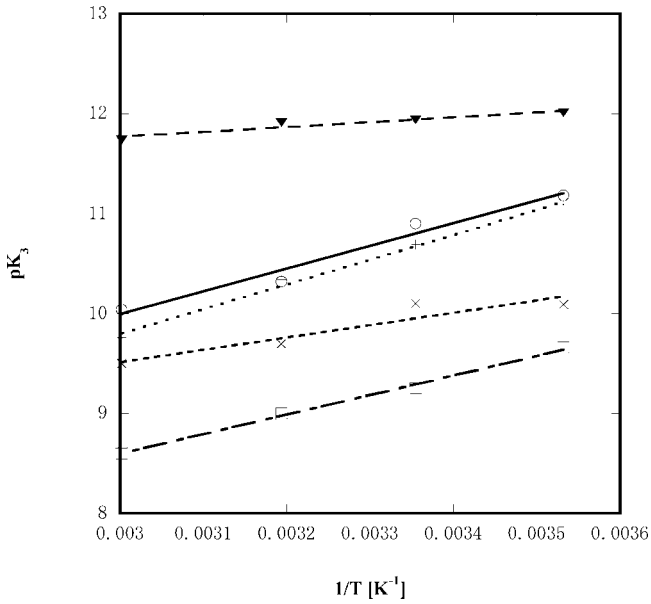

Figure 9. pK_3 values of lysine, histidine, arginine, glutamic acid, and tyrosine extrapolated to zero ionic strength at 283.1 K, 298.1 K, 313.1 K, and 333.1 K. Symbols are ○, lysine; □, histidine; ▼, arginine; ×, glutamic acid; and +, tyrosine.

deprotonation of the carboxylic acid group attached to the α carbon, is nearly independent of temperature in most cases, with $\Delta H_{T,i}^0/R$ varying between (−285 and 40) K. An exception is arginine for which pK_1 decreases significantly with temperature. Even for arginine, however, $\Delta H_{T,i}^0/R$ has a relatively low value (1360 K). This general trend is consistent with prior work. For example, Martel and Smith[8] and Borst et al.[18] have both reported $\Delta H_{T,i}^0/R \sim 0$ for the pK_1 of phenylalanine on overlapping temperature ranges. The reasons why the pK_1 of arginine exhibits a more pronounced temperature dependence are not known. However, it is possible that this behavior is caused by the much more basic side chain in this amino acid.

The effect of temperature on the pK_2 is shown in Figure 8. In the case of glutamic acid, the pK_2 is for the deprotonation of the carboxylic acid group in the side chain. Although its pK value is obviously larger than for the carboxylic acid group attached to the α carbon, its dependence on temperature is only marginally larger ($\Delta H_{T,i}^0/R = -342$ K). In the case of histidine, the pK_2 is for the deprotonation of the imidazole group in the side chain. This dissociation is more sensitive to temperature with an intermediate value $\Delta H_{T,i}^0/R = 2729$ K. Finally, for the remaining amino acids the pK_2 is for the deprotonation of the primary amine group attached to the α carbon. These values are much more strongly dependent on temperature with $\Delta H_{T,i}^0/R$ values between (3380 and 5155) K.

The effect of temperature on the pK_3 is shown in Figure 9. For lysine and arginine, pK_3 is for the dissociation of the basic amine group on the side chain. These values decrease with temperature with $\Delta H_{T,i}^0/R$ values of (5226 and 1604) K, respectively. For histidine and glutamic acid, the pK_3 is for the dissociation of the primary amine group on the α carbon. Its behavior is consistent with that of the other amino acids decreasing with temperature with $\Delta H_{T,i}^0/R$ values of (4510 and 2789) K, respectively. Finally, for tyrosine, pK_3 is for the deprotonation of the aromatic hydroxyl group on the side chain. This value decreases somewhat more dramatically with temperature with $\Delta H_{T,i}^0/R = 5713$ K.

Conclusions

The dissociation constants of amino acids vary with ionic strength, temperature, and the nature of the side chain. The effect of ionic strength at a given temperature is predictable with reasonable accuracy using the Davies equation for the charged amino acid species. The effect of temperature on the pK value extrapolated to zero ionic strength is very small for arginine and practically insignificant for the deprotonation of the carboxylic acid group attached to the α carbon and only marginally more significant for the dissociation of the carboxylic acid group attached to the side chain of glutamic acid. More pronounced effects are seen to the pK value extrapolated to zero ionic strength for the dissociation of the primary amine attached to the α carbon for the basic side chains of lysine and arginine and for the aromatic hydroxyl group of tyrosine. Finally, the temperature dependence of the pK value extrapolated to zero ionic strength for histidine is intermediate. A molecularly based explanation of these effects is outside the scope of this experimental investigation. Nevertheless, the data provided in this paper provide the means of performing speciation calculations for amino acids in solution useful for the design of unit operations for the separation and purification of these molecules. In conclusion, it should be noted that the isoelectric point (pI) of the amino acids also changes with temperature as a result of the different effects of temperature on the various pK's.

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