Solubilities of the common L-α-amino acids as a function of temperature and solution pH

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Abstract: Combining equations of state for the standard molal thermodynamic properties of aqueous amino acids with those for the corresponding properties of their crystalline counterparts permits calculation of the solubilities of these biomolecules at temperatures, pressures, and pHs well beyond those for which experimental data are available. The calculations indicate that the solubilities of all the common amino acids in water increase dramatically with increasing temperature and reach (in molalities) at 100 °C and 1 bar, 0.03 (Tyr), 0.2-0.8 (Trp, Leu, Ile, Cys, and Phe), 1-2 (Val, Met, Asn, and Gln), 2-4 (Thr and Ala), 8-10 (Ser and Gly), and >20 (Pro). In the case of the 5 amino acids that dissociate appreciably in the pH range 3-9 (Asp, Glu, His, Lys, and Arg), the solubilities of Asp and Glu increase with increasing pH above ~3 and approach a 1:1 dependence of log m on pH at pHs above ~5, where the solubilities exceed 3 m at 100 °C and 1 bar. In contrast, the solubilities of His, Lys, and Arg at 100 °C and 1 bar increase with decreasing pH below ~pH 6 (His) and ~8 (Lys and Arg) and reach a 1:1 dependence of log m on -pH at pHs below ~5 (His) and ~7 (Lys and Arg), where the solubilities are greater than 1.5 and 15 m, respectively. Unlike those of Asp and Glu, the solubilities of His, Lys, and Arg minimize with increasing temperature at P_{SAT} (Note a) and constant pH. In unbuffered solutions, the equilibrium pHs for coexisting solid and aqueous Asp and Glu decrease only slightly with increasing temperature from ~2.7 at 25 to ~2.2 at 100 °C. However, the corresponding decrease for His, Lys, and Arg is of the order of a log unit from ~9.7 (His) and ~11.6 (Lys and Arg) to ~8.6 and ~10.3, respectively. The calculated solubilities of the amino acids at elevated temperatures are in close agreement with the bulk of the experimental values reported in the literature.

INTRODUCTION

Amino acids are the major building blocks of all forms of life, regardless of whether the organism thrives under ambient conditions on the Earth, or at the extreme conditions prevailing in environments such as submarine hydrothermal vent systems, continental solfatara fields, or deeply buried sedimentary basins. Life under these conditions is limited by (among other things) the solubilities of biomolecules in solutions of different pH at temperatures ranging up to 100 °C or more. The purpose of the present communication is to report computed solubilities as a function of temperature and solution pH at P_{SAT} for one such group of molecules, the 20 common L-α-amino acids with side chains corresponding to those in proteins.

Many of the experimental measurements of amino acid solubilities in water were carried out in the 1930s (refs. 1-7). In subsequent decades, experimental studies began to focus more on the solubilities of amino acids in aqueous solutions of other amino acids and/or ethanol, electrolytes, urea, and other known protein denaturants (refs. 8-14). Compilations of experimental amino acid solubilities in water have been published by Hutchens (ref. 15) and Cabani and Gianni (ref. 16). Although many of the studies cited in these compilations report experimental solubilities at different low temperatures, the temperature ranges are short, and the solubilities cannot be extrapolated with confidence using polynomial fits of the data (ref. 17). In

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(Note a): P_{SAT} is used throughout this communication to represent pressures corresponding to liquid-vapor equilibrium for the system H_2O, except at temperatures <100 °C where it denotes the reference pressure of 1 bar.

addition, little emphasis has been put on the effect of pH on amino acid solubilities at elevated temperatures. Shortcomings of this kind can be overcome by computing the solubilities of the molecules as a function of temperature and solution pH using equations of state for the crystalline amino acids (ref. 18) and their aqueous counterparts (ref. 17). These equations of state have been widely used to calculate the standard molal thermodynamic properties of a large number of crystalline and aqueous organic and inorganic species over broad ranges of temperature and pressure (refs. 17-25). The theoretical foundations for the equations of state and the evidence supporting their validity have been discussed in detail elsewhere (refs. 26-31).

SOLUBILITIES AS A FUNCTION OF TEMPERATURE AT P_{SAT}

Computed solubilities in water expressed as the logarithms of the molalities (m) of the 20 common L-α-amino acids as a function of temperature at P_{SAT} are depicted as curves in Fig. 1. These curves were generated from the equilibrium constants (K) for the dissolution reactions represented by

$$AA(cr) = AA(aq), \tag{1}$$

where AA(cr) and AA(aq) stand for the crystalline and aqueous amino acid, respectively. Taking account of the standard state for the crystalline amino acid, the law of mass action for reaction (1) can be written as (Note b)

$$a_{AA(aq)} = m_{AA(aq)} \bar{\gamma}_{AA(aq)} = K, \tag{2}$$

where $a_{AA(aq)}$, $m_{AA(aq)}$, and $\bar{\gamma}_{AA(aq)}$ denote the activity, molality, and activity coefficient, respectively, of the aqueous amino acid. For $\bar{\gamma}_{AA(aq)} \approx 1$ (which is the case in dilute and/or ideal solutions), eqn. (2) can be written as

$$m_{AA(aq)} = K. \tag{3}$$

The values of K required to compute those of $m_{AA(aq)}$ from eqns. (2) or (3) can be calculated from

$$K = exp\left(-\Delta G_r^\circ / RT\right), \tag{4}$$

where R represents the gas constant (1.9872 cal mol^{-1}), T stands for the temperature in Kelvins, and ΔG_r° denotes the standard molal Gibbs free energy of reaction for reaction (1), which can be computed from

$$\Delta G_r^\circ = \Delta G_{AA(aq)}^\circ - \Delta G_{AA(cr)}^\circ, \tag{5}$$

where $\Delta G_{AA(aq)}^\circ$ and $\Delta G_{AA(cr)}^\circ$ stand for the apparent standard molal Gibbs free energy of formation of the subscripted species at the temperature and pressure of interest (refs. 19 and 26). Values of ΔG° for the aqueous and crystalline amino acids were computed in the present study from the equations of state cited above using standard molal thermodynamic properties and equations of state parameters for the amino acids taken from Amend and Helgeson (ref. 17) and Helgeson et al. (ref. 18), respectively. Because departures from ideality in amino acid solutions have yet to be documented experimentally as a function of temperature, all of the solubilities represented by the curves in Fig. 1 were computed assuming in a first approximation that the activity coefficients of the aqueous amino acids can be regarded as unity. It should be noted in this regard that the same approximation was used by Amend and Helgeson (ref. 17) to generate values of ΔG° at 25 °C and 1 bar for L-α-amino acids from the experimental solubilities for these species at this temperature and pressure shown in Fig. 1. In contrast, the values of log m computed for other temperatures are in all cases except proline (ref. 17) independent of the experimental data represented by the symbols for these temperatures in Fig. 1.

It can be deduced from Fig. 1 that the solubilities in water of all of the 20 common L-α-amino acids increase substantially with increasing temperature at P_{SAT}. In fact, the calculations indicate that the solubility of arginine at P_{SAT} increases from ~1 m at 25 to >90 m at 200 °C which clearly needs experimental verification! In contrast, the calculated solubility of Gly increases from 3.4 m at 25 °C and 1 bar to 10.0 and 19.1 m at

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(Note b): The standard state for water and the crystalline amino acids adopted in the present study calls for unit activity of pure H$_2$O and the amino acid at any temperature and pressure. The standard state for the aqueous amino acids is one of unit activity of the species in a hypothetical 1 molal solution referenced to infinite dilution at any temperature and pressure.
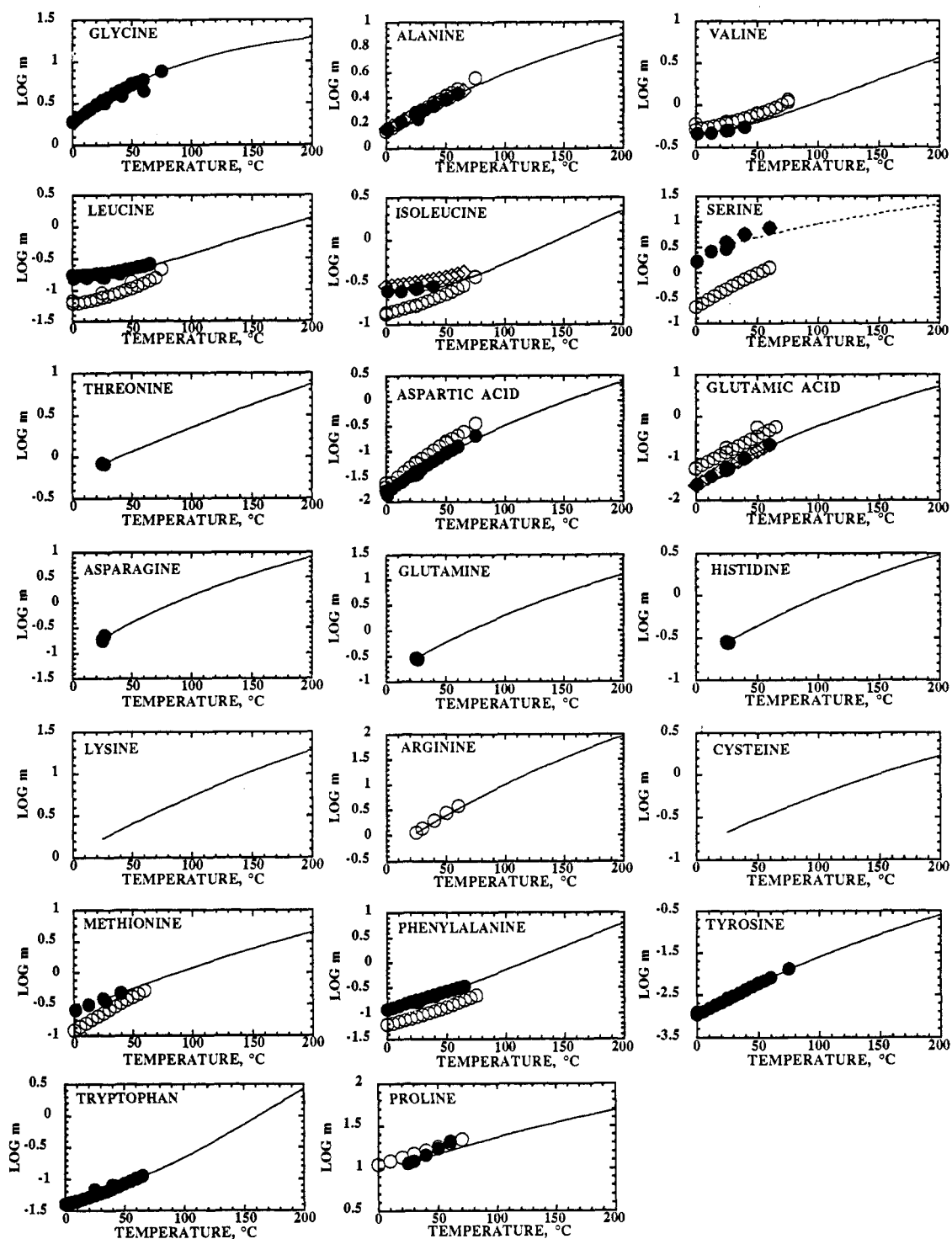
Fig. 1. Solubilities expressed as the logarithms of the molalities (log m) of D, L, and DL forms of the 20 common amino acids in unbuffered solutions as a function of temperature at P_{SAT}. The symbols represent experimental data reported by Dalton and Schmidt (refs. 1, 2), Dunn et al. (ref. 3), Pittz and Bello (ref. 12), Dooley and Castellino (ref. 13), Jin and Chao (ref. 32), Nozaki and Tanford (refs. 8-11), Conio et al. (ref. 14), Hutchens and Hade (ref. 33), and Gekko (ref. 34) for D (◇), L (●), and DL (O) isomers of the amino acids. At temperatures other than 25 °C, the curves represent independent values of log m generated in the present study using equations of state and parameters taken from Amend and Helgeson (ref. 17) and Helgeson et al. (ref. 18), respectively (see text).

P_{SAT} and 100 and 200 °C, respectively. Similarly, the predicted solubility of Ala increases from 1.9 m at 25 °C and 1 bar to 3.9 and 7.9 m at P_{SAT} and 100 and 200 °C, respectively. Note also in Fig. 1 that the solubilities of the amino acids with alkyl side chains (Gly, Ala, Val, Leu, Ile) decrease with increasing carbon number at all of the temperatures considered in the present study. A similar decrease in solubility with increasing carbon number is exhibited by the 3 amino acids with hydroxyl groups in their side chains (Ser, Thr, Tyr). However, this trend does not occur in the case of the amino acids with carboxylic acid (Asp and Glu) or amide (Asn and Gln) side chains. In these cases, the amino acids with the higher carbons number are more soluble. Solubilities consistent with the curves shown in Fig. 1 are listed in Table 1.

TABLE 1. Solubilities expressed as Molalities of the 20 common L-α-Amino Acids as a Function of Temperature at P_{SAT} (see Text).

Amino Acid	Temperature (°C)				
	25	50	100	150	200
Gly	3.370	5.295	10.005	14.932	19.113
Ala	1.867	2.443	3.900	5.750	7.928
Val	0.501	0.621	1.078	1.973	3.600
Leu	0.167	0.204	0.372	0.729	1.378
Ile	0.242	0.292	0.531	1.081	2.228
Ser	3.480	4.952	8.930	14.497	21.478
Thr	0.822	1.154	2.252	4.227	7.383
Asp	0.038	0.086	0.326	0.958	2.336
Glu	0.060	0.142	0.594	1.925	5.157
Asn	0.190	0.402	1.359	3.603	8.033
Gln	0.291	0.596	2.005	5.450	12.500
His	0.281	0.436	0.946	1.798	3.008
Lys	1.687	2.512	5.346	10.568	19.187
Arg	1.125	2.494	10.031	33.012	92.751
Cys	0.211	0.302	0.575	1.018	1.666
Met	0.375	0.551	1.191	2.420	4.489
Phe	0.170	0.259	0.711	2.112	6.201
Tyr	0.003	0.006	0.025	0.088	0.250
Trp	0.065	0.086	0.218	0.727	2.714
Pro	11.298	14.622	23.174	34.435	48.641

Computed values at elevated temperatures for 14 of the amino acids considered in the present study can be compared in Fig. 1 with corresponding experimental values taken from the literature. No experimental solubilities were found in the literature for Lys and Cys, and those represented by the symbols shown in Fig. 1 for Thr, Asn, Gln, and His are restricted to temperatures at or near 25 °C. It can be seen in this figure that the calculated values as a function of temperature are closely consistent with the bulk of the experimental data for the L isomers represented by the solid symbols. Unlike most of the standard molal thermodynamic properties of the amino acids (ref. 17), the experimental solubilities of the D, L, and DL isomers of several of the common amino acids shown in Fig. 1 are appreciably different. Although the extent to which these differences can be attributed to sample impurities is unknown, several lines of evidence indicate that impurities may be a contributing factor to the relatively large differences in some of the isomer solubilities. For example, there is no apparent systematic trend in the solubilities of these different isomers in Fig. 1. The experimental solubility of the DL isomer is highest for 5 of the amino acids (Ala, Val, Asp, Glu, and Pro), but in the case of 3 others (Leu, Met, and Phe) the solubility of the L form is highest, and for Ile the D isomer is apparently the most soluble. It can also be seen in Fig. 1 that the solubilities of the D and L forms are approximately equivalent in the case of Ala, Ser, and Glu.

The experimental data represented by the symbols shown in Fig. 1 for the D, L, and DL isomers exhibit various trends with increasing temperature, either converging, diverging, or remaining quasi parallel. For example, those for the DL isomers of Ala and Val diverge with increasing temperature from the solubilities of the D and L forms, but those of the L and DL isomers of Leu, Ile, Ser, Met, and Pro converge with increasing temperature. Quasi parallel trends are apparent in Fig. 1 for the values of log m represented by the symbols for D- and L-Ile, L- and DL-Asp, D-, L-, and DL-Glu, and L- and DL-Phe.

DEPENDENCE OF SOLUBILITY ON pH

Of the 20 common amino acids, only 5 (Asp, Glu, His, Lys, and Arg) dissociate or protonate appreciably between pHs 3 and 9. The dissociation reactions for Asp and Glu can be written as

$$Asp = Asp^- + H^+ \tag{6}$$

and

$$Glu = Glu^- + H^+, \tag{7}$$
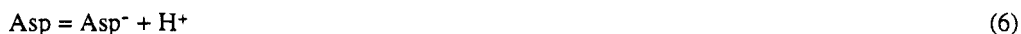
and the protonation reactions of His, Lys, and Arg can be expressed as

$$His + H^+ = His^+, \tag{8}$$
$$Lys + H^+ = Lys^+, \tag{9}$$

and

$$Arg + H^+ = Arg^+. \tag{10}$$

The solubilities of these amino acids as a function of pH can be computed for unit activity coefficients by combining statements of the law of mass action for reactions (6)-(10) with material balance constraints and appropriate statements of eqn. (3). Calculations of this kind resulted in the curves shown in Figs. 2 and 3, where it can be seen that log m (where in this case m refers to the sum of the molalities of the charged and neutral species) for Asp and Glu increases substantially at all temperatures with increasing pH above ~3.
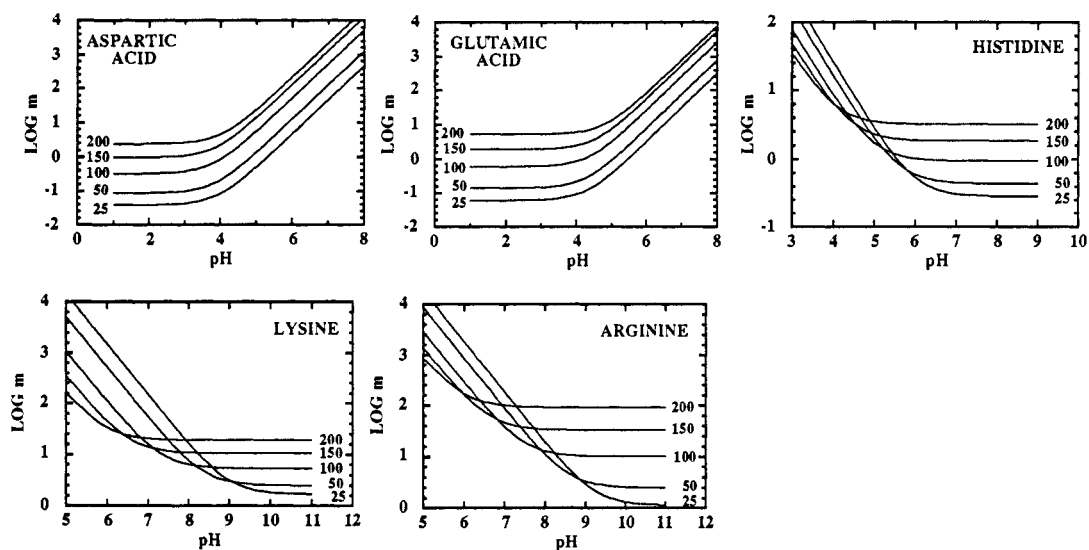

Fig. 2. Computed solubilities expressed as the logarithms of the molalities (log m) of Asp, Glu, His, Lys, and Arg as a function of pH at P_{SAT} and various temperatures corresponding to those indicated in °C.

The increase in solubility corresponding to the increase in log m is particularly striking at the higher temperatures shown in Fig. 2. For example, the solubility of Asp at pH 5 and P_{SAT} exceeds 5, 13, and 23 m at 100, 150, and 200 °C, respectively. At these temperatures, the solubility of Glu is approximately 3, 7, and 13 m. In contrast, it can be deduced from Fig. 2 that the solubilities of His, Lys, and Arg increase dramatically at all temperatures with decreasing pH below ~6 for His and ~8 for Lys and Arg. For example, at pH 4, 150 °C, and P_{SAT} the predicted solubility of His exceeds 6 m and those of Lys and Arg at pH 8, 100 °C, and P_{SAT} exceed, respectively, 6 and 12 m. It can further be seen in Fig. 2 that the onset of the large increase in the solubilities of His, Lys, and Arg occurs at progressively lower pHs with increasing temperature. For example, it takes place at pH ~7 (His) and ~10 (Lys and Arg) at 25 °C and 1 bar, but at pH ~5 (His) and ~7 (Lys and Arg) at 200 °C and P_{SAT}. As a result, the computed solubilities of His, Lys, and Arg decrease with increasing temperature at low pHs.
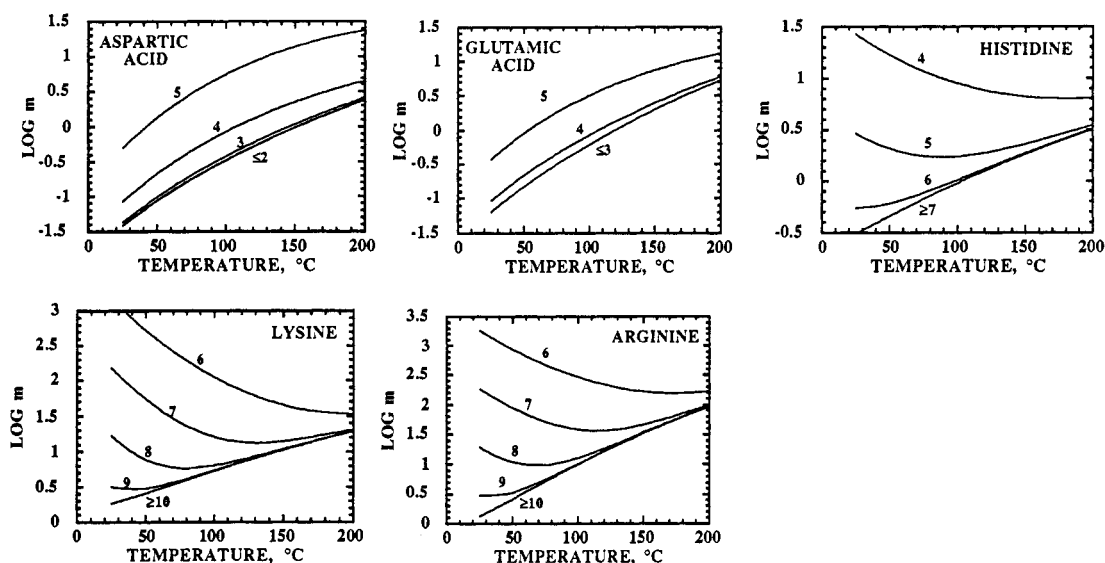
Fig. 3. Computed solubilities expressed as the logarithms of the molalities (log m) of Asp, Glu, His, Lys, and Arg as a function of temperature at P_{SAT} and the various pHs indicated on the curves.

It can be deduced from Fig. 3 that the solubilities of Asp and Glu increase by more than an order of magnitude with increasing temperature from 25 to 200 °C at all of the pHs shown in the figure. Note also that unlike those of Asp and Glu, the solubilities of His, Lys, and Arg minimize with increasing temperature at constant pH. Numerical values of the solubilities (in m) for these 5 amino acids at various temperatures and pHs are given in Table 2.

Unlike the values of log m depicted in Figs. 2 and 3 and those of m listed in Table 2, those shown for Asp, Glu, His, Lys, and Arg in Table 1 and Fig. 1 are unbuffered with respect to pH. The equilibrium pHs for coexisting crystalline and unbuffered aqueous solutions of Asp, Glu, His, Lys, and Arg at 25-200 °C are given in Table 3. It can be deduced from this table that the equilibrium pHs for Asp and Glu are relatively insensitive to temperature at P_{SAT}, decreasing only slightly with increasing temperature from 2.67 and 2.75, respectively, at 25 °C to 2.14 and 2.29 at 100 °C, and with further increase in temperature to 1.84 and 2.05 at 200 °C. In contrast, the equilibrium pHs for His, Lys, and Arg at P_{SAT} decrease substantially with increasing temperature from 9.71, 11.59, and 11.63, respectively, at 25 °C to 8.58, 10.14, and 10.35 at 100 °C, and to 7.90, 9.22, and 9.58 at 200 °C. As a frame of reference, neutral pH at P_{SAT} decreases from 7 at 25 °C to 6.13 and 5.64 at 100 and 200 °C, respectively (ref. 26).

UNCERTAINTIES

Uncertainties in the solubilities computed in the present study are difficult to judge because in most cases the calculated solubilities are independent of the experimental data represented by the symbols for the L-α-amino acids shown in Fig. 1 only at temperatures other than 25 °C (see above). Nevertheless, the computed and experimental values of log m at these temperatures are in most cases closely consistent with one another. Taking account of the scatter in the experimental data shown in Fig. 1, it appears that the uncertainties associated with most of the calculated solubilities are of the order of ±0.05 log units or less. Serine is a notable exception.

CONCLUDING REMARKS

Assuming the activity coefficients of the aqueous amino acids to be unity, the revised HKF equations of state, together with the equations of state parameters and standard molal thermodynamic properties at 25 °C and 1 bar discussed above permit calculation with the aid of SUPCRT92 (ref. 31) of the solubilities of the 20 common L-α-amino acids as a function of temperature, pressure, and solution pH. The results of these calculations provide a provisional framework for thermodynamic investigation of biochemical systems. However, more experimental solubility measurements over broad ranges of temperature and solution pH are required to verify and/or refine the algorithms by Amend and Helgeson (ref. 17) that were used to generate

the predictions summarized above. Of particular interest in this regard is experimental determination of the activity coefficients of amino acids as a function of temperature and amino acid and electrolyte concentrations.

TABLE 2. Solubilities expressed as Molalities of Asp, Glu, His, Lys, and Arg as a Function of Temperature and pH (see Text).

Amino Acid	pH	Temperature (°C)				
		25	50	100	150	200
Asp	1	0.038	0.086	0.327	0.967	2.398
	2	0.038	0.087	0.331	0.978	2.417
	3	0.042	0.098	0.379	1.094	2.608
	4	0.084	0.212	0.850	2.252	4.521
	5	0.499	1.346	5.561	13.824	23.642
Glu	1	0.060	0.141	0.592	1.934	5.271
	2	0.060	0.142	0.595	1.939	5.278
	3	0.063	0.149	0.618	1.988	5.350
	4	0.091	0.217	0.856	2.478	6.064
	5	0.374	0.903	3.227	7.382	13.208
His	4	26.708	16.789	8.875	6.599	6.437
	5	2.923	2.071	1.739	2.309	3.534
	6	0.545	0.600	1.025	1.879	3.244
	7	0.307	0.452	0.954	1.837	3.215
	8	0.283	0.438	0.947	1.832	3.212
	9	0.281	0.436	0.946	1.832	3.212
Lys	8	16.727	7.773	6.419	10.917	19.317
	9	3.191	3.040	5.453	10.607	19.187
	10	1.838	2.567	5.356	10.576	19.174
	11	1.702	2.519	5.347	10.573	19.173
Arg	8	19.449	11.110	12.858	34.361	93.694
	9	2.958	3.356	10.317	33.166	93.003
	10	1.309	2.581	10.063	33.046	92.934
	11	1.144	2.503	10.038	33.034	92.927

TABLE 3. Equilibrium pHs as a Function of Temperature at P_{SAT} for coexisting solid and aqueous Asp, Glu, His, Lys, and Arg (see Text).

Amino Acid	Temperature (°C)				
	25	50	100	150	200
Asp	2.67	2.45	2.14	1.95	1.84
Glu	2.75	2.56	2.29	2.13	2.05
His	9.71	9.24	8.58	8.15	7.90
Lys	11.59	11.00	10.14	9.58	9.22
Arg	11.63	11.10	10.35	9.88	9.58

The equations, properties, and parameters used above to calculate amino acid solubilities are being incorporated in a computer code to be called BIOTHERM97. This code will constitute a biochemical version of the SUPCRT92 (Johnson et al., 1992) software package. BIOTHERM97 should be available at no cost by mid-1997 from the Laboratory of Theoretical Biogeochemistry at the University of California, Berkeley, otherwise known as Prediction Central.

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