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Explanation of Ionic Sequences in Various Phenomena. III. Salting-in and -out of Amino Acids

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SUMMARY

Previous developed theories were applied in explaining the mechanism for the salting-in and -out of various amino acids. Glycine is salted-in according to the cationic sequences $Li^+ > Na^+ > K^+ > Rb^+$ and $Ca^{2+} > Ba^{2+} > Sr^{2+}$. The ability of a cation to increase the solubility of an amino acid therefore corresponds to the destruction of the ion-ion bond between the $-CO_2^-$ and the $-NH_2^+$ group of the amino acid by forming an insoluble ion-ion bond between the added cation and the $-CO_2^-$ group. This insolubilizing effect produces a positive charge on the amino acid. If, however, the anion of the added salt forms a relatively insoluble ion-ion bond with the $-NH_2^+$ group of the amino acid, then the effect is minimized because now both charges on the amino acid are reduced. Consequently, the more insoluble the cation amino acid salt and the more soluble the anion amino acid salt (or vice versa), the greater will be the salting-in effect. Titration of either charged group on the amino acid zwitterion has the same effect, since now the ion-ion bond of the amino acid is again destroyed. Aliphatic and carboxylic acid groups also effect the salting-in sequence, since these groups are salted-out by addition of salt when $D_{\pm} < D_{H_2O}$. These mechanisms explain how leucine is first salted-out, then salted-in (at 4 M) and finally salted-out again (at 9 M) in LiCl solutions. Urea salts-in hydrophobic amino acids by increasing the dielectric constant and salts-out polar amino acids by increasing the interaction between the two charge groups on the amino acid. Glycine reverses the salting-in effect of NaCl on asparagine by competing for the Na^+ ion.

INTRODUCTION

In the previous paper [1] in this series, the ability of an ion to "bind" to a polyelectrolyte or to reverse its charge was discussed. In that study it was shown that the greater the solubility of the counter ion, the greater will be its ability to "bind" or associate with the polyelectrolyte. The "binding" therefore involves the formation of an inner layer of counterions such as that described by Katchalsky et al. [2]. This inner layer becomes an integral part of the polyelectrolyte.

In the present study it will be shown how salts effect the aqueous solubility of amino acids. It will be seen that the cationic sequences do not correlate with the solubility of the carboxylate group on the amino acid, but rather the cationic sequence corresponds to its insolubility. The effect is therefore opposite to the previous study on the reversal of charge and ion binding phenomena. Before presenting the data, it is important to realize why this difference exists.

Amino acids are different from most polyelectrolytes in one respect: The position of the carboxylate group is permanently fixed near that of the amino group. Because of this close proximity, the negatively charged carboxylate group and the positively charged amino group can continually form ion-ion bonds. I say "continually" because the process must be dynamic. If this ion-ion interaction or salt bond were not dynamic but rather if it were permanent, then the amino acid would not be any more soluble in water than a nonelectrolyte of comparable size. A permanent ion-ion bond would completely cancel the electrostatic charge of both the carboxylate and the amino group. Hence the charged groups could not interact with water and they would have no effect on the solubility of the amino acid. But as with all ion-ion bonds, the charged groups must interact with water to form hydrated ions and thus must have a certain degree of solubility. However, the formation of such hydrates does not mean that ion-ion bonds cannot reform again in solution. But by reforming again, the solubility of the amino acid will be reduced. Consequently, this dynamic process of destroying and reforming the ion-ion bond limits the solubility of the amino acid, because part of the time the amino acid has a positive and a negative charge and the rest of the time it is neutral. It follows that any agent that can destroy the ion-ion bond will increase the solubility of the amino acid.

When a salt is added to an aqueous solution of an amino acid, then that salt will increase the solubility of the amino acid if one of its ions (but not both) forms an insoluble ion-ion complex with either the carboxylate or the amino group. In other words, if the destruction of the ion-ion complex of the amino acid occurs by forming an insoluble cation-carboxylate salt bond, then the electro-

statically charged amino group will solubilize the amino acid, since now the amino acid has a more permanent electrostatic charge. However, if both the anion and cation of the added salt form ion-ion complexes which are more insoluble than the ion-ion complex of the amino acid, then the solubility of the amino acid should decrease.

Because the solubilization of the amino acid is determined by the ability of an added salt to insolubilize one of the ionic groups of the amino acid, then the ability of the counterion to solubilize the amino acid should increase as the insolubility of its ion-ion bond increases. Thus the ionic sequence will be correlated with the insolubility rather than the solubility of the ion-ion bond, which is the reverse of the ionic sequence studies on ion "binding" and reversal of colloid charge [1].

The previous results [3] on the solubility of ion-ion bonds will be applied here. These results correlate the salt's solubility with the effective dielectric constant ($D_{\pm}$) of an ion and the presence or absence of A regions on these ions. The method for obtaining the value of $D_{\pm}$ and the structure of hydrated ions were given previously [4, 5]. In essence, if both the cation and anion have similar values of $D_{\pm}$, then their solubility will be comparatively low. If they have dissimilar values, then their solubility will be comparatively high. Moreover, if both anion and cation have A regions, then these A regions interact to give extremely insoluble salt bonds [3]. Also the salting-out (or lowering the solubility) of hydrocarbons in aqueous solutions increases as the value of $D_{\pm}$ for the ions decreases [6]. Moreover, it was shown that a hydrocarbon does not form and is not surrounded by a water clathrate structure, but rather it exists in regions containing largely unbonded water molecules [7]. These results will be applied to the study on how the addition of salts can salt-in or salt-out various types of amino acids.

SALTING-IN AND -OUT OF GLYCINE

Pfeiffer and Würgler [8] were the first to extensively examine the effect of various salts on the solubility of different amino acids. Some of their data, together with that of others, is given in Table 1. First, let us consider the effect of salts on the simplest of all amino acids: glycine. The pK of the carboxylate group of glycine is pK = 2.35. Now the formate ion has a pK of 3.75, while that of acetate is pK = 4.76. As seen below and in previous papers [1, 3], the absence or presence of A regions on the carboxylate ion can be determined by a comparison of the pK values. Since the formate ion is positively hydrated [1, 3], then any carboxylate ion having a pK equal to or less than the formate ion will also have A regions. Consequently, the carboxylate ion of glycine has a tightly bound A re-

Table 1. Salting-in Sequences for Amino Acids[a]

Type of sequence	Amino acid	Salting-in sequence	Corresponding S/So for 1 N salt[e]	Explanation of cationic sequences
Acidic	Glycine[b]	$Li^+ > Na^+ > K^+ > Rb^+$	1.105 > 1.066 > 1.036 > 0.991	Insolubilization of carboxylate ion and added coion produces net positive charge on amino acid
	Glycine[b]	$Ca^{2+} > Ba^{2+} > Sr^{2+}$	1.236 > 1.211 > 1.204	
	Glycine[c]	$Cl^- < Br^- < I^-$	1.036 < 1.042 < 1.063	
Intermediate (acidic and nonpolar)	Leucine[c]	$Li^+ > Na^+ = K^+$	0.96 > 0.83 = 0.83	Insolubilization of $R-CO_2^-$ and salting-out of hydrophobic chain
	Leucine[c]	$Ca^{2+} > Ba^{2+} > Sr^{2+}$	1.16 > 1.14 > 1.12	
	Leucine[c]	$Cl < Br^- < I^-$	0.655 < 0.736 < 0.824	
Intermediate (acidic and basic)	Asparagine[d]	$Li^+ = Na^+$	1.17 = 1.17	Salting-out of amide or γ-carboxylic acid group reverses salting-in sequence
Basic	Aspartic acid[c]	$K^+ > Na^+ > Li^+$	1.317 > 1.288 > 1.190	
	Aspartic acid[c]	$Ba^{2+} > Sr^{2+} > Ca^{2+}$	1.683 > 1.554 > 1.546	
	Aspartic acid[c]	$Cl^- < Br^- < I^-$	1.317 < 1.356 < 1.378	
	Glutamic acid[c]	$K^+ > Li^+$	1.312 > 1.057	
Nonpolar	Tyrosine[b]	$Na^+ < K^+ < Li^+$	0.981 < 0.993 < 1.005	Salting-out of hydrophobic group reverses salting-in sequence
	Tyrosine[b]	$Cl^- < Br^- < I^-$	0.993 < 1.157 < 1.362	

[a]The salting-out sequences at high concentrations of salt are the reverse of the salting-in sequences.
[b]Data obtained from Andō [9].
[c]Data obtained from Pfeiffer and Würgler [8].
[d]See Cohn and Edsall [10, p. 241].
[e]S/So represents the solubility of amino acid in salt solution divided by that in water. Ratios were obtained using K^+ or Cl^- as the coion. All concentrations of salt were 1 N except for the anionic sequence of leucine (2 N) and the cationic sequences of glutamic acid (2 N) and asparagine (0.5 N).

gion and thus its solubility sequence is acidic [3]. That is, this comparison shows that the solubility of various cations with respect to glycine's carboxylate ion should be $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$. A similar comparison of the pK of glycine's amino group (pK = 9.78) with that of ammonia (pK = 9.3) shows that this amino group has a dielectric constant less than that of water ($D_+ < D_{H_2O}$), just as in the case of the NH_4^+ ion. Therefore, its solubility sequence should be $Cl^- < Br^- < I^-$. Conversely, the guanidinium ion has the solubility sequence $Cl^- > Br^- > I^-$ because $D_+ > D_{H_2O}$, which is the reverse of that for the amino group [3]. Consequently, the interaction of the $-CO_2^-$ and $-NH_3^+$ groups on amino acids will form strong ion-ion bonds because in both cases $D_\pm < D_{H_2O}$ and because they are fixed in close proximity of each other.

These solubility sequences can be used to explain the salting-in of glycine. First, let us examine the cationic sequence obtained by using a common anion. Because of the Li^+ ion is the most insoluble of the above monovalent cations, it should reduce the charge on the carboxylate ion the most. As seen in the introduction, the $-NH_3^+$ and the $-CO_2^-$ groups will form salt bonds in the absence of salt and thus limit the solubility of the glycine. However, by adding a lithium salt, the glycine now becomes positively charged because of a partial cancellation of the negative charge on the carboxylate ion, and because of the consequent destruction of the existing ion-ion bond. Thus the solubility of glycine is increased in the presence of lithium ions because the glycine has become a charged molecule rather than essentially a neutral molecule. In other words, the addition of Li^+ made it possible for the glycine to now have an electrostatically charged group which can interact with the medium. Thus the sequence obtained in Table 1 is explainable. That is, the more insoluble the ion-ion bond, the greater will be the salting-in of glycine. It will be seen below that the anion also governs the ability of the salt to increase or decrease the solubility of the amino acid.

The effect of salts containing a divalent cation plus a common anion has also been studied and is given in Table 1. This divalent sequence $Ca^{2+} > Ba^{2+} > Sr^{2+}$ for the salting-in of glycine is also the insolubility sequence for anions having A regions such as the glycine carboxylate anion [3]. Therefore, the Ca^{2+} ion increases the solubility of glycine the most because it forms the most insoluble salt, which again is in agreement with the above conclusions.

The relative effectiveness of a salt can be changed by altering the pK of the carboxylate group on the amino acid. If the pK is increased so that the positively hydrated carboxylate ion is changed to a negatively hydrated one, then the solubility sequence will be changed from $Li^+ < Na^+ < K^+ < Cs^+$ to $K^+ < Na^+ < Li^+ < Cs^+$. A change in solvent from water to 80% ethanol-water mixture should increase the pK of the carboxylate group. Thus it is proposed that

the carboxylate group of glycine is changed from a positively hydrated ion to a negatively hydrated ion when the medium is changed from water to 80% ethanol-water mixture. Such a change in hydration would explain the change in the salting-in sequence from $Li^+ > Na^+$ for glycine in water (Table 1) to $Na^+ > Li^+$ for glycine in an 80% ethanol-water mixture (Fig. 15 of Edsall and Wyman [11]).

CORRELATION BETWEEN ADDITION OF SALT AND ACID-BASE TITRATIONS OF AMINO ACIDS

Further proof of the above conclusions concerning the effect of salt on the solubility of amino acids can be obtained by examining the solubility of an amino acid which had had one of its groups titrated. In other words, titration of one of the groups should have the same effect as adding a salt which causes the destruction of the ion-ion bond between the carboxylate and amino groups. Consequently, the neutralization of the carboxylate or amino group by addition of either a base or an acid should also destroy the ion-ion bond of the amino acid and should thus result in an increase in the solubility of the amino acid. In Table 2 the solubilities of glycine, leucine, and

Table 2. Increase in Solubility (moles/liter) of Amino Acids at 20°C by Addition of Acid or Base[a]

Solvent	Solubility of amino acid		
	Glycine	Leucine	Aspartic acid
H_2O	26.12	0.0741	0.0308
0.1 N HCl	—	0.1692	—
2.0 N HCl	46.03	—	—
0.1 N NaOH	—	0.1734	0.1254
0.5 N NaOH	—	0.1099	—

[a]Data from Pfeiffer and Würgler [8].

aspartic acid are given for various media. The solubility of glycine is increased almost twofold by titration of the carboxylate ion. Similarly, for the amino acid leucine, the solubility is increased by titrating the carboxylate ion or the amino group. The solubility decreases in going from 0.1 N NaOH to 0.5 N NaOH because now the salt (NaOH) is salting-out the amino acid. That is, the values of $D_\pm$ for the Na^+ and OH^+ ions are less than that of water [1]. For

aspartic acid, the addition of NaOH titrates not only the amino group but also ionizes the γ-carboxylic acid group. Hence the solubility of aspartic acid is increased fourfold by the addition of base because of the production of a negative electrostatic charge as well as the destruction of the ion-ion bond on the amino acid.

SYNERGISTIC EFFECT OF ANION AND CATION ON SOLUBILIZATION OF AMINO ACIDS

As noted above, the ion-ion bond of the amino acid can be destroyed by either adding a cation that forms an ion-ion complex of low solubility with the carboxylate ion or by adding an anion that forms a low solubility ion-ion complex with the amino group. If both the anion and cation of the added salt form insoluble complexes, then the solubility of the amino acid will be reduced. But if, for example, the cation forms an insoluble ion-ion interaction whereas the anion forms a soluble, then as noted above the solubility will increase.

As shown by the results of Pfeiffer and Würgler (Table 1), the salt NaI increases the solubility of glycine more than NaCl. That is, the precentage increase in the solubility of glycine is 3.93% for 1 M NaCl and 7.80% for 1 M NaI in comparison to its solubility in water. But according to the solubility sequence for the $-NH_3^+$ group, the Cl^- ion should form the most insoluble ion-ion complex and the I^- ion should form the most soluble. But the Na^+ ion with its A regions should react strongly with the carboxylate ion to form an insoluble ion-ion complex. Consequently, the greatest increase in the solubility of the amino acid occurs when the anion forms the most soluble ion-ion complex, because the common cation Na^+ is already forming an insoluble ion-ion complex.

It should be possible to obtain the reverse of the anionic sequence given in Table 1 for glycine, leucine, and aspartic acid. In other words, if a cation is added that forms an extremely soluble salt with the carboxylate ion, then the Cl^- ion rather than the I^- ion should be the most effective in increasing the solubility of the amino acid. The Cl^- would form the most insoluble salt and therefore would be the most effective in destroying the ion-ion bond of the amino acid and consequently in producing a net negative charge on the amino acid. A cation that should produce such an effect must have a value of D_+ greater than that of water, e.g., the guanidinium ion.

The pK of the carboxylate group should also be related to how the added salt effects the solubility of the amino acid. In other words, the pK of the carboxylate group of the amino acid determines the strength of the ion-ion interaction with the added cation. And as seen above, for a relatively soluble anion, the greater the in-

Table 3. Relationships between Relative Solubilities of Amino Acids in 1 N NaCl and the pK_1 Values of Their Carboxylate Groups[a]

	Amino acid									
	Cystine		Asparagine		Aspartic Acid		Glycine		Leucine
S/So for 1 N NaCl	1.41	>	1.31	>	1.29	>	1.07	>	0.83
pK_1 of carboxylate	1.65	<	2.02	<	2.09	<	2.35	<	2.36

[a]Solubility relationships hold for all concentrations of NaCl (see Fig. 1 of Cohn and Edsall [10, p. 238]). Data on pK_1 values were obtained from Haurowitz [12]. Values of S/So (solubility of amino acid in 1 N NaCl divided by its solubility in pure water) were obtained from Pfeiffer and Würgler [8] (aspartic acid and leucine), from Andō [9] (glycine and asparagine), and from Cohn and Edsall [10] (cystine).

solubility of the cation with the carboxylate ion, the greater will be the solubilization effect. The insolubility of the carboxylate ion with a cation having an A region or $D_- < D_1$ will increase as the pK of the carboxylate ion decreases. In other words, the lower the pK of the carboxylate ion, the more fixed and polarized will be the water molecules hydrated to it. The more strongly polarized the water molecules, the greater will be the insolubility of the carboxylate salt with a cation having an A region [3]. In Table 3 the solubility of an amino acid in 1 N NaCl is divided by its solubility in pure water (S/So). This ratio is compared to the pK of the carboxylate ion. It is seen that the salting-in effect is a direct function of the pK of the carboxylate group. Hence the above reasoning concerning the salting-in of amino acids is again substantiated.

EFFECTS OF ALIPHATIC CHAINS AND CARBOXYLIC ACID GROUPS ON THE SALTING-IN SEQUENCES

Other factors do, however, influence the ratio S/So. For example, the pK of the carboxylate ion of glycine is only slightly smaller than that of leucine ($2.35 < 2.36$), yet the value of S/So is substantially greater ($1.07 > 0.83$). The reason for this anomaly is that the hydrocarbon on leucine is salted-out by NaCl more than the

hydrogen atoms on glycine [6]. This hydrocarbon effect also accounts for the intermediate cationic sequence obtained for leucine (Table 1). Thus the Na^+ ion is more effective in salting-in glycine than the K^+ ion. However, in the case of leucine, the value of D_+ must now be considered because of the presence of an aliphatic chain. The value of D_+ for Na^+ is less than that for K^+ [5]. Consequently, Na^+ will salt-out the hydrocarbon on leucine more than the K^+ ion. This salting-out effect for the hydrophobic group reverses the salting-in effect for the ionic groups of leucine. Thus, instead of the salting-in sequence $Na^+ > K^+$ as obtained for glycine, the salting-in sequence is changed to $Na^+ = K^+$, because of the hydrophobic group on leucine.

As seen in Table 1, the salting-in sequence for aspartic acid is basic instead of acidic. Yet the pK of the α-carboxylate group on aspartic acid is equal to 2.09, showing that it must be positively hydrated. The cationic solubility sequence of the α-carboxylate group must therefore be acidic, the same as it is for the carboxylate group of glycine. Consequently, the Li^+ ion with its A regions should form a more insoluble ion-ion complex with the $-CO_2^-$ ion than the K^+ ion. However, the salting-out effect of the A regions on Li^+ and Na^+ on the neutral γ-carboxylic acid group of aspartic acid must also be considered, just as the effect of D_+ on the hydrocarbon chain of leucine was important. As shown in a previous paper [6], the salting-out sequence for a neutral carboxylic acid group is $Li^+ > Na^+ > K^+ > Ca^+$. The reason for this sequence is that the polarized hydroxyl group on the carboxylic acid acts as an A region of another cation [6]. Consequently, the carboxylic acid chain of the aspartic or glutamic amino acid is salted-out of solution by the A region of the Li^+ ion, just as in the case of phthalic acid. That is, both polarized hydroxyl groups repel each other. The acidic salting-in sequence $Li^+ > Na^+ > K^+$ for amino acids such as glycine is therefore changed to the basic sequence $K^+ > Na^+ > Li^+$, because the salting-in sequence is a sum of the interaction of the cation with the neutral γ-carboxylic acid group plus the ionic α-carboxylic acid group.

In the case of asparagine, the hydrogen atoms on the amide group do not repel the A region of the Li^+ ion as effectively as the γ-carboxylic acid group because the partial charge on the hydrogen atoms of $-NH_2$ is less (a smaller dipole) than that on the hydrogen atom of the γ-carboxylic acid group. Consequently, the salting-in sequence is intermediate ($Li^+ = Na^+$) for asparagine, whereas it is basic ($K^+ > Na^+ > Li^+$) for aspartic acid.

It should be emphasized that the salting-out sequences for all amino acids of Table 1 are the reverse of the salting-in sequences. If an anion or cation has the ability to increase the solubility of an amino acid, then the anion or cation should also be able to maintain that amino acid in solution. Consequently, the fact that the salting-

out sequences are the reverse of the salting-in sequences is understandable. The Li^+ ion will salt-out glycine less than the Rb^+ ion, because an ionic species would be more difficult to salt-out than a neutral molecule.

EXPLANATION FOR THE SALTING-IN AND SALTING-OUT EFFECTS OF LEUCINE

Pfeiffer and Würgler [8] studied the salting-in and -out of leucine more extensively than any other amino acid. Their results are shown graphically in Fig. 1. Their ability to repeat the experiment

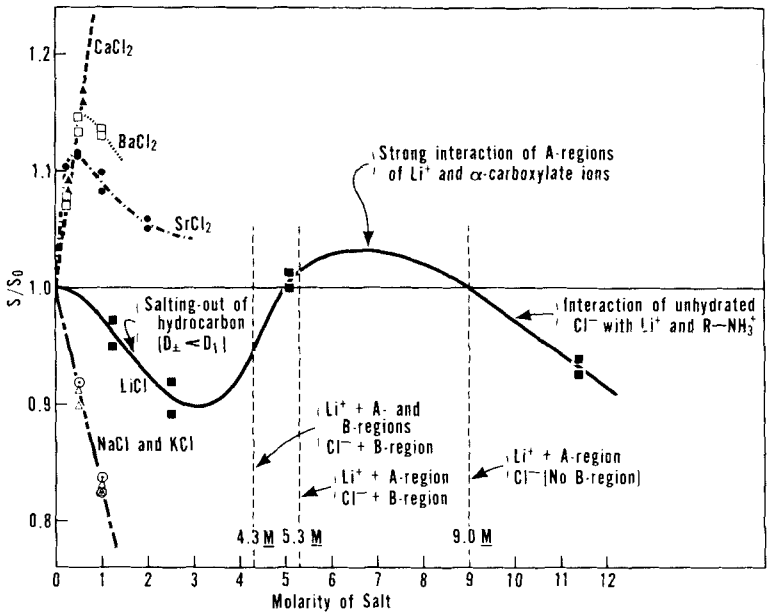

Fig. 1. Salting-in and -out of the amino acid leucine. (Data from Pfeiffer and Würgler [8].)

illustrates that the points are valid and that the curve drawn through these points is also valid. These peculiar salting-in and -out curves can be readily explained if the previous developed theories are applied [1, 3, 5, 6]. As pointed out above, the Na^+ and K^+ ions salt-out leucine to the same degree because the greater salting-out power of the Na^+ ion with respect to the hydrocarbon chain is counteracted by its greater salting-in power with respect to the zwitterion of the leucine molecule.

The A regions of the divalent cations are more polarized than those of the monovalent cations and, therefore, form more insoluble salts with the carboxylate ion of leucine (dipole-dipole interactions [3]). That is, the dipole-dipole interactions which precede any ion-ion interaction also govern the solubility of the salt [3]. In addition, the divalent charge of Ca^{2+}, Ba^{2+}, and Sr^{2+} increases the maximum electrostatic charge of leucine from a +1 to a +2, because the negative carboxylate ion cancels only one charge on the divalent cations. Thus both a decrease in solubility of the byion and carboxylate ion and an increase in the charge of the byion cause the electrostatic charge of the leucine molecule to increase. Consequently, the divalent cations Ca^{2+}, Ba^{2+}, and Sr^{2+} salt-in, whereas the monovalent cations Na^+ and Li^+ initially salt-out the leucine molecule.

As the concentration of LiCl is increased, the leucine molecule is salted-in. By adding a salt which increases the dielectric constant of the medium, one should be able to salt-in leucine because of the favorable action of the salt on both the hydrocarbon chain and the zwitterion. However, this salting-in cannot be due to an increase in the dielectric constant of the medium, because both Li^+ and Cl^- have a value of $D_\pm$ less than that of water [5]. In other words, the initial salting-out of leucine is due to its hydrocarbon chain, because there is a decrease in the solvent dielectric constant. Consequently, the reversal of this salting-out effect cannot be due to a change in the dielectric constant of the medium. It must, therefore, be due to an increase in the destruction of the ion-ion interaction of the $-NH_3^+$ and $-CO_2^-$ groups of the amino acid and a consequent increase in the electrostatic charge of the amino acid. In other words, by increasing the concentration of the Li^+ ion, the solubility of the carboxylate-Li^+ salt is decreased. Hence the salting-in of leucine by 5 M to 9 M LiCl is due to the greater destruction of the ion-ion bond of the amino acid and the consequent formation of a positive net charge on the leucine molecule. This effect now outweighs the salting-out effect of the aliphatic chain.

This salting-in of leucine in LiCl solutions occurs when all the water in the medium has become theoretically hydrated to the Li^+ and Cl^- ions. That is, by using the hydrated radii of Nightengale [13], it can be shown [14] that 4.3 moles of hydrated LiCl occupy 1 liter. At 5.3 moles/liter of LiCl, the B region of the Li^+ ion will have theoretically disappeared because it is the weakest hydration shell. At 9.0 M LiCl the B region of the Cl^- ion will have disappeared, leaving only the A region on the Li^+ ion. Consequently, the solubility of leucine initially increases at around 4 M LiCl, because the hydrated ions now occupy almost all of the volume of the medium. Hence the solubility of the Li^+-carboxylate salt is decreased. At about 9 M LiCl the solubility of leucine again decreases, because now the unhydrated Cl^- ion will begin to associate with the amino group of leucine and hence neutralize its positive electrostatic

charge. Furthermore, the unhydrated Cl^- ion will form ion-ion complexes with the Li^+ ion because at concentrations greater than 9.0 M LiCl, the A regions of the Li^+ ion begin to disappear. These ion-ion interactions will reduce the concentration of Li^+ ions and thus will reduce the interaction of Li^+ ions with the carboxylate ion. Consequently, at concentrations greater than 9.0 M LiCl the leucine is salted-out because of a decrease in its electrostatic charge. The wavy salting-in and -out curve of leucine in LiCl solutions is therefore explainable on the basis of previously developed theories.

SALTING-IN AND SALTING-OUT EFFECT OF UREA

Nozaki and Tanford [15] have studied the effect of urea on the solubility of various amino acids. From their Table II it is seen that the solubility of glycine, alanine, histidine, glutamine, and diglycine decreases with an increase in the molarity of urea. On the other hand, the solubility of amino acids which possess more hydrocarbon in their nature, such as leucine, phenylalanine, tryptophan, methionine, tyrosine, asparagine, carbobenzoxyglycine, and carbobenzoxydiglycine, increases with an increase in the concentration of urea. The increase in solubility of these latter amino acids is due to an increase in the dielectric constant of the medium. That is, the effective dielectric constant of urea (the positive part) is greater than that of water [16].

But why then are the more polar amino acids salted-out with urea? First, it should be pointed out that the maximum solubility of those amino acids salted-in by 8 M urea does not exceed the maximum solubility of those amino acids salted-out by 8 M urea. The salting-out ability of the urea could be due to an increase in the effective concentration of the amino acid. In other words, the urea reduces the amount of available water by its interaction with water (hydration) and by its displacement of water molecules. The urea molecule should be hydrated because it behaves as a zwitterion [17]. By decreasing the amount of available water the urea increases the interaction of the amino and carboxylate ions on the amino acid. This increase in the ion-ion interaction would reduce the charge on the amino acid and hence decrease the solubility of the amino acid.

The same type of mechanism would apply to the amino acids having hydrophobic side chains. However, these amino acids are salted-in because the increase in the solubility of the hydrocarbon side chain is greater than the decrease in the solubility of the amino acid zwitterion. And, as noted above, the solubility of a nonpolar amino acid approaches but does not exceed the solubility of a polar amino acid in 8 M urea. Consequently, the results of Nozaki and Tanford [15] can be explained on the basis of physical

phenomena rather than on the basis of chemical interactions between urea and hydrocarbons.

According to Cohn and Edsall [10, p. 240], the solubility of asparagine in an aqueous NaCl solution is greater than its solubility in an aqueous solution containing the same molarity of NaCl plus the amino acid glycine. The greater the concentration of glycine, the smaller the solvent action of NaCl upon the asparagine. In other words, glycine reduces the effect of the NaCl. The most plausible explanation is that the glycine zwitterion competes for the Na^+ ion. Thus, by being able to associate with the Na^+ ion, the glycine reduces the effective concentration of Na^+ ions and hence decreases the salting-in effect of NaCl on asparagine. Also, the glycine would behave just as in the example of the addition of urea. The glycine would thus not only compete for the Na^+ ion but also would strengthen the ion-ion interaction of the amino acid groups.

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