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# Calorimetric Studies of the Interactions of Guanidinium Hydrochloride and Potassium Iodide with Model Amides in Aqueous Solution

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## Synopsis

The enthalpies of transfer, $\Delta H_{tr}$, of a series of amides from water to aqueous solutions of either guanidinium hydrochloride (GuHCl) or potassium iodide were obtained from calorimetric measurements at 25°C. The amides were studied at molalities around $10^{-2}$ *m* while salt molalities ranged from 0–10 *m*. The amides investigated were Ac-Gly-NHMe, Ac-Gly-Gly-NHMe, Ac-Ala-NHMe, and Ac-Leu-NHMe. Use of an additivity assumption allowed the calculation of group contributions to $\Delta H_{tr}$ in these two salt systems for the methyl group, leucyl side chain, and the peptide backbone unit. Values of the entropy of transfer were also obtained. The great ability of GuHCl to randomize protein structures appears to arise from effects on polar and nonpolar groups, which are characterized by enthalpies and entropies of transfer not substantially different from those with KI, a salt comprised of ions of comparable size and polarizability. The difference in the sign of the free energies of transfer of nonpolar groups from water to *M*X solutions, negative for GuHCl and positive for KI, is the result of these small differences in enthalpies and entropies of transfer. Variations in water structure produced by differences in ionic properties rather than a mode of action for GuHCl very different from that of other salts characterizes its superior denaturing ability.

## INTRODUCTION

The extensive studies of Tanford and his collaborators[1] have demonstrated that of all the neutral salts that have been studied as perturbants of protein structure, GuHCl produces the conformation with the least residual order. The question arises as to why this salt differs from other electrolytes in its denaturing properties. While there have been speculations regarding the mechanism of GuHCl action,[1,2] there is agreement primarily on the experimental results,[3] which indicate that GuHCl solubilizes nonpolar residues as well as interacting favorably with amide groups. Although extensive data exist for the free energies of transfer of polar and nonpolar groups from water to salt solutions for both GuHCl[3] and other electrolytes,[4,5] enthalpies of transfer obtained by high-precision calorimetry for these groups are practically nonexistent.[6,7] The gathering of such data for selected systems of interest was the aim of the present study. In addition, we have attempted to take the measured enthalpies and calculated

entropies for the transfer of peptide backbone units and nonpolar groups from water to GuHCl and compare them with similar quantities obtained for an "ordinary" electrolyte, potassium iodide, comprised of ions of a comparable size and polarizability. Inferences regarding the mechanism of action of GuHCl can be drawn from this comparison.

## MATERIALS AND METHODS

Ac-Gly-NHMe,[8] Cyclo Chemical Corporation, was recrystallized twice from hot methyl acetate solutions, filtered, ground to a fine powder, and stored for several weeks in a vacuum dessicator over $P_2O_5$ until all traces of methyl acetate and water were removed. The amide melted at 159.5°C, under a nitrogen atmosphere. Ac-Gly-Gly-NHMe, Ac-Ala-NHMe, and Ac-Leu-NHMe were purchased from Fox Chemical Company. These materials were dried *in vacuo* for at least 24 hr and used without further purification.

Eastman GuHCl was purified before use in the manner described by Nozaki and Tanford.[9] Potassium iodide was J. T. Baker reagent grade. Pretreatment consisted of drying at 110°C for 24 hr. Doubly distilled water was used throughout in making up solutions. All solutions were made up on a molality basis, i.e., moles of solute/1000 g water.

Since the calorimeter and the experimental procedure have been described recently,[7] only some brief comments will be given here. Measurements of enthalpies of solution of each compound in water and in salt solutions of various molalities were required to calculate enthalpies of transfer. These enthalpies were obtained using the LKB model 8700-1 precision calorimeter. This calorimeter is of the isoperibol type. The temperature of the thermostatting bath was maintained at 25.00° ± 0.01°C during the runs. Reactions were initiated and completed within ±0.05°C of this reference temperature.

A measure of the accuracy of the calorimetric measurements was obtained by measuring the enthalpy of solution of the test substance, tris-(methylhydroxy)aminomethane in 0.1 *M* hydrochloric acid. The mean value obtained for a series of measurements, −7.114 ± 0.005 kcal/mol, compared favorably to that recently determined by Prozen and Kilday,[10] −7.115 ± 0.001 kcal/mol. We would estimate the uncertainty in enthalpy of solution values on the basis of this result and other consideration of possible systematic error as ±5 cal/mol.

## RESULTS

### Enthalpies of Solution of the Amides in Water

Table I gives values of the enthalpies of solution, $\Delta H_{soln}^{w}$, at 25°C for the amides in water. The superscript, w, designates enthalpies derived from measurements made in water alone as opposed to results obtained in water–salt solutions, given below, for which the enthalpies of solution will

TABLE I
Enthalpies of Solution of Amides in Water at 25°C

| Compound | Molecular Weight | Enthalpy of Solution, $\Delta H_{soln}{}^{w}$ (kcal/mol) |
|---|---|---|
| Ac-Gly-NHMe | 130.15 | 0.4194 ± 0.0013 |
| Ac-Gly-Gly-NHMe | 187.20 | 4.557 ± 0.001 |
| Ac-Ala-NHMe | 144.17 | −0.6568 ± 0.0002 |
| Ac-Leu-NHMe | 186.26 | −2.340 ± 0.001 |

be identified with the superscript, ws. The number of determinations for each amide varied from two to four. The uncertainty limits given in the table are average deviations. The amide molalities of the final solutions achieved in these runs were approximately 0.01 *m*. In previous experiments,[7] it was shown that at amide molalities below 0.05 *m*, the enthalpy of solution is independent of the amide molality. As far as we can tell, these are the first enthalpy of solution measurements on these compounds so that no comparison with the literature is possible.

### Enthalpies of Solution of the Amides in Salt Solution

The enthalpies of solution, $\Delta H_{soln}{}^{ws}$, of the amides in solutions of GuHCl and KI were measured at 25°C. The results of these measurements are given in Table II. Where uncertainty limits are given, they are the average deviation of duplicate runs. Otherwise, the value given is the result of a single measurement. The dependence of the enthalpy of solution on salt molality was studied over the widest possible salt molality range. As before, keeping the amide molality below 0.01 *m* in the final solution produced values of $\Delta H_{soln}{}^{ws}$ that were independent of amide molality.

## DISCUSSION

### Enthalpies of Transfer of Amides from Water to Salt Solution

The trends in the data are best investigated using the enthalpy of transfer. This quantity is defined as

$$\Delta H_{tr} = \Delta H_{soln}{}^{ws} - \Delta H_{soln}{}^{s} \quad (1)$$

and is the enthalpy gained or lost when a mole of amide is transferred from water to salt solution of a particular salt molality. No specification of the amide molality is required if the experimental conditions are such that the individual terms on the right-hand side of Eq. (1) are independent of amide molality. The uncertainties in the enthalpies of transfer are the sum of the individual uncertainties of the terms comprising them.

Figure 1a shows the enthalpies of transfer of Ac-Gly-NHMe from water to GuHCl solution and from water to KI solutions, respectively, as a function of salt molality. The $\Delta H$ values for the transfer of Ac-Gly-NHMe to GuHCl are more negative at all molalities than are the values for the transfer of the same compound to KI solutions. Figure 1b shows the value of

$\Delta H_{tr}$ for the transfer of Ac-Gly-Gly-NHMe solutions of each of the salts. The presence of the added glycine residue appears to produce a more negative $\Delta H_{tr}$ for Ac-Gly-Gly-NHMe solutions with KI than with GuHCl at least up to 3.5 *m*.

TABLE II
Enthalpies of Solution of Amides in Aqueous Salt Solutions at 25°C

| Salt | Salt Molality $m_s$ | $\Delta H_{soln}^{ws}$ (kcal/mol) |
|---|---|---|
| | *Ac-Gly-NHMe* | |
| GuHCl | 0.1309 | 0.3552 ± 0.0019 |
| | 0.2002 | 0.3234 ± 0.0010 |
| | 0.5163 | 0.2072 ± 0.0065 |
| | 0.5207 | 0.2032 |
| | 1.2170 | −0.0076 |
| | 1.8211 | −0.1279 |
| | 2.2080 | −0.1927 ± 0.0011 |
| | 3.6637 | −0.2442 |
| | 6.1229 | −0.4398 ± 0.0009 |
| KI | 0.2523 | 0.3367 ± 0.0019 |
| | 0.5331 | 0.2560 ± 0.0006 |
| | 0.7798 | 0.2038 ± 0.0015 |
| | 1.0519 | 0.1717 ± 0.0003 |
| | 1.6103 | 0.1260 ± 0.0005 |
| | 2.2088 | 0.0827 ± 0.0006 |
| | 4.5708 | 0.2212 ± 0.0018 |
| | 6.9355 | 0.4733 ± 0.0020 |
| | *Ac-Gly-Gly-NHMe* | |
| GuHCl | 0.5509 | 4.255 |
| | 1.1459 | 4.005 |
| | 1.6580 | 3.834 |
| | 3.6967 | 3.269 |
| | 3.9385 | 3.141 |
| | 10.9708 | 2.829 |
| KI | 0.5020 | 4.187 |
| | 1.2698 | 3.863 |
| | 1.5053 | 3.756 |
| | 2.7035 | 3.405 |
| | 4.3468 | 3.278 |
| | 6.2319 | 3.275 |
| | *Ac-Ala-NHMe* | |
| GuHCl | 0.5431 | −0.7890 |
| | 1.1987 | −0.8652 |
| | 2.6637 | −0.9457 |
| | 6.1229 | −0.8774 |
| KI | 0.2523 | −0.6676 |
| | 0.7844 | −0.6674 |
| | 1.0684 | −0.6374 |
| | 1.6430 | −0.5881 |
| | 2.8497 | −0.4593 |

*Table II (continued)*

TABLE II (*Continued*)

| Salt | Salt Molality $m$, | $\Delta H_{soln}^{ws}$ (kcal/mol) |
|---|---|---|
| | *Ac-Leu-NHMe* | |
| GuHCl | 0.5431 | −2.393 |
| | 1.1987 | −2.364 |
| | 2.6637 | −2.266 |
| | 6.1229 | −1.998 |
| KI | 0.2523 | −2.305 |
| | 0.5442 | −2.258 |
| | 0.7844 | −2.201 |
| | 1.0684 | −2.112 |
| | 1.6430 | −1.923 |

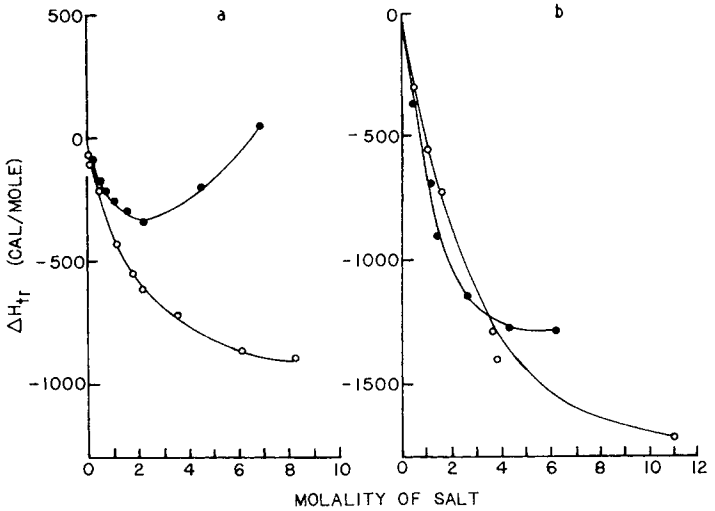



Fig. 1. (a) The enthalpies of transfer $\Delta H_{tr}$ of Ac-Gly-NHMe from water to GuHCl (○) and KI (●) solutions as a function of salt molality. (b) The enthalpies of transfer $\Delta H_{tr}$ of Ac-Gly-Gly-NHMe from water to GuHCl (○) and KI (●) solutions as a function of salt molality.

Figure 2a shows the enthalpies of transfer for Ac-Ala-NHMe from water to respective solutions of GuHCl and KI while Figure 2b shows similar data for Ac-Leu-NHMe. In both amides, the enthalpies are significantly more positive for transfers to KI solutions than to those containing GuHCl. Noteworthy is the minimum in the enthalpies of transfer of Ac-Ala-NHMe to GuHCl solutions while for Ac-Leu-NHMe the $\Delta H_{tr}$ values become strongly positive with increasing GuHCl molality.

## Group Enthalpies of Transfer of the Peptide Backbone Unit and Nonpolar Groups from Water to Salt Solution

Further consideration and comparison of the transfer data can be done most effectively if we *assume* that the group effects comprising the enthalpy

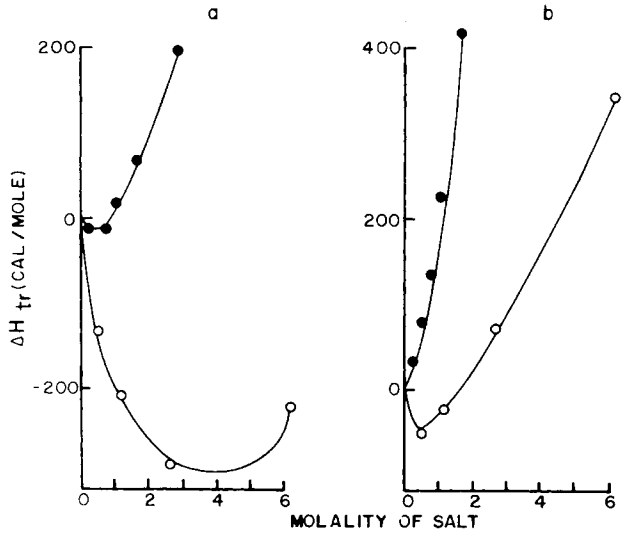
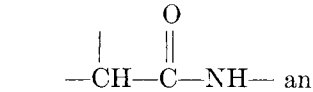



Fig. 2. (a) The enthalpies of transfer $\Delta H_{tr}$ of Ac-Ala-NHMe from water to GuHCl (○) and KI (◑) solutions as a function of salt molality. (b) The enthalpies of transfer $\Delta H_{tr}$ of Ac-Leu-NHMe from water to GuHCl (○) and KI (●) solutions as a function of salt molality.

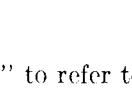
of transfer at a given salt molality are additive, i.e.,

$$\Delta H_{tr} = \sum_i \Delta h_{tr}. \tag{2}$$

In the absence of more data, we consider the groups not as the smallest functional substructures of the molecule,[11] but instead consider both the peptide backbone unit and particular amino acid side chains as distinct entities. We will use the terms "peptide backbone unit" [1] to refer to



and "amide group" to refer to



We then have enough data to evaluate $\Delta h_{tr}$ for the peptide backbone unit and for the alanyl and leucyl side chains, i.e., $CH_3$— and $(CH_3)_2CHCH_2$—. The evaluation is done by subtracting from values taken from the $\Delta H_{tr}$ curves for each of the other compounds, the $\Delta H_{tr}$ of Ac-Gly-NHMe at the particular salt molality. In the case of the nonpolar side chain, as has been pointed out,[1] the effect of an additional hydrogen atom is subtracted from the $\Delta h_{tr}$ for the groups. The resulting $\Delta h_{tr}$ values are given for the peptide backbone unit in Figure 3a and for the alanyl and leucyl side chains in Figure 3b for each of the salts. For the peptide backbone unit, the $\Delta h_{tr}$ values are more negative for transfer to KI solutions than to GuHCl solutions. The transfer enthalpies are more positive for nonpolar side chains passing from water to KI solutions than to solutions containing GuHCl.

Detailed consideration of the interactions of each of the salts with amide groups allows the development of an additional feature of interest. The

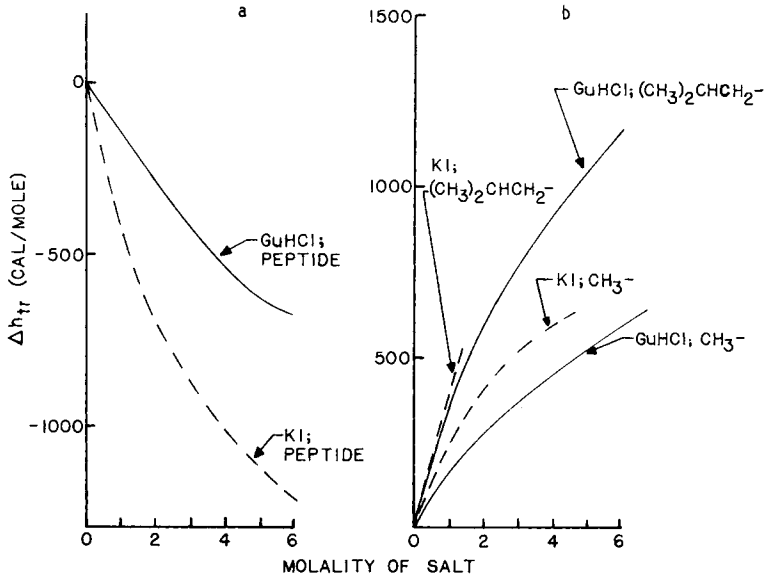



Fig. 3. (a) The calculated enthalpies of transfer $\Delta H_{tr}$ of a peptide backbone unit from water to GuHCl (——) and KI (- - -) solutions. (b) The calculated enthalpies of transfer $\Delta H_{tr}$ of the methyl group and leucyl side chain from water to GuHCl (——) and KI (- - -) solutions.

enthalpies of transfer of formamide,[7] Ac-Gly-NHMe, and Ac-Gly-Gly-NHMe from water to 1.0 *m* GuHCl are −290, −370, and −480 cal/mol, respectively. The same quantities for transfers to 1.0 *m* KI are −270, −350, and −590 cal/mol in the same order. With regard to this latter set of values, the upward curvature of the plot for the KI + Ac-Gly-NHMe makes it necessary to extrapolate $\Delta H_{tr}$ values at lower KI molalities to 1.0 *m* KI, rather than use the experimental value in this comparison.

In order to obtain $\Delta h_{tr}$ values for amide groups from these data we then correct for the effect of the three nonpolar groups in Ac-Gly-NHMe by subtracting from the above values for this compound three-quarters of the enthalpy of transfer of the leucyl side chain from water to the respective salt solution. Similarly, we correct for the four nonpolar groups in Ac-Gly-Gly-NHMe by subtracting the full enthalpy of transfer of the leucyl side chain from water to the salt solutions.

The probable accuracy of these corrections is difficult to assess. The assortment of nonpolar groups on the leucyl side chain is nearly the same as those being corrected for on the molecules of interest, i.e., two methyls and one or two methylenes. The difference between an intact leucyl side chain and the methyls and methylenes scattered throughout the molecules of interest will be the least for the terminal methyl groups, which will contribute the largest share to the correction. The methyl groups are at the ends of the molecules of interest and thus are not as influenced by

proximity to polar groups as the methylenes. While the resulting values are only approximate, the conclusions that will be drawn below will not be vitiated if the corrections are in error by as much as ±100 cal.

Approximate enthalpy values are thus obtained for the transfer of one, two, or three amide groups to the respective salt solutions. These values are −290, −620, and −820 cal/mol for transfer from water to 1.0 *m* GuHCl solutions and −270, −650, and −990 cal/mol for transfer from water to 1.0 *m* KI solutions. The values are 1) roughly the same for the respective salts and 2) there is a relatively smooth progression of the transfer values as a function of the number of amide groups.

We may consider both of these points with respect to the hypothesis of bifunctional hydrogen bonding to amide groups by $GuH^+$, which was proposed by Robinson and Jencks[2] to interpret free energy data in mixtures of GuHCl with Ac-Gly-Gly-Gly-Gly-OEt. Nozaki and Tanford[3] have used the same hypothesis to explain solubility data for mixtures of GuHCl with glycine or larger glycine peptides (diglycine and triglycine). The fact that the enthalpies of transfer of one, two, and three amide groups from water to salt solution are the same for each salt suggests that a similar mechanism of interaction holds in each case. This suggestion is reinforced by the similarity of the enthalpies of transfer of formamide to a number of 1-1 electrolytes[7] including GuHCl and KI. Since direct hydrogen bonding of the ion to the binding site is not possible for any of these ions aside from $GuH^+$, it is difficult to see how the similar transfer enthalpy for $GuH^+$ would arise from a different mode of interaction. Mediation by the aqueous solvent would provide the required element of similarity in all cases, however.

The smooth progression of the enthalpy of transfer values as a function of the number of amide groups in the case of GuHCl solutions may also be considered in terms of the proposed bifunctional hydrogen-bonding mechanism. The making of two hydrogen bonds by one molecule or ion when two sites are available is a process entropically favored over the making of two hydrogen bonds by two separate molecules or ions. Although there need not be any contribution from the enthalpy of binding in order for the process to be favorable, we feel that such a contribution might be expected. In solution, a $GuH^+$ ion might be flexible in order to achieve the most favorable contacts with a continually changing set of solvent nearest neighbors. This flexibility would persist if the ion forms a hydrogen bond at only one site of a bifunctional hydrogen-bond acceptor since the other donor site of the ion would still be solvated. The ion forming hydrogen bonds to both sites of the bifunctional molecules, however, is likely to be rigid. This rigidity facilitates the delocalization of electronic charge in $GuH^+$ in accordance with the well-known resonance formulation. An additional contribution of resonance energy would thereby be manifested only when such a twin hydrogen-bonded structure is formed.

As seen above, the enthalpies of transfer of amide groups from water to GuHCl solutions show only a twofold numerical increase when the number

of amide groups is increased from one to two. On this basis, it is unlikely that bifunctional hydrogen bonding occurs since the postulated additional resonance energy would produce a larger than twofold increase in the same comparison.

A better defined case against the bifunctional hydrogen-bonding mechanism can be made by comparing recent free energy data from this laboratory[12] for mixtures of sodium chloride with glycine, diglycine, and triglycine with data for GuHCl with the same amino acids obtained by Nozaki and Tanford.[3] The difference between the free energies of transfer from water to 6 *M* guanidine hydrochloride for diglycine and glycine is −185 cal/mol while the difference between triglycine and glycine is −640 cal/mol. The fact that the ratio of these numbers is greater than 2 has been interpreted by Tanford[1] as evidence for the bifunctional mechanism.

Free energies of transfer of the same compounds to 1 *m* sodium chloride solutions can be calculated by extrapolating the parameters obtained at limiting solute molalities to unit molality of salt. The resulting difference between the free energies of transfer from water to sodium chloride solutions at unit molality for diglycine and glycine is −590 cal/mol while the difference between triglycine and glycine is −1490 cal/mol. There is a greater than twofold increase in the free energy of transfer to sodium chloride solution of a structure containing two peptide backbone units as opposed to a structure containing one peptide backbone unit. This suggests that the same mechanism is at work in sodium chloride solutions as in GuHCl solutions.

Nandi and Robinson[5] have shown that the free energies of transfer of one, two, and three peptide backbone units to sodium chloride solutions are in a simple 1:2:3 ratio when the model compounds are uncharged glycine esters. There is no reason to believe, therefore, that sodium ion, even considering it to be hydrated, is acting as a bifunctional hydrogen-bond donor toward triglycine. By inference, this is the conclusion we would draw for $GuH^+$ as well. The triglycine molecule seems to be providing anomalous character to the above data in both cases. To add a final note, Roseman and Jencks[13] have recently obtained steric evidence that also cast doubts on the bifunctional hydrogen-bonding mechanism.

## Other Thermodynamic Quantities of Transfer of the Peptide Backbone Unit and Nonpolar Groups from Water to Salt Solutions

Since some values for the free energies of transfer of the peptide backbone unit and the nonpolar groups studied here are available in the literature or can be calculated from existing data, a calculation of entropies of transfer is possible. As complete a tabulation as possible of existing and newly calculated thermodynamic quantities of transfer of these groups from water to GuHCl and KI solutions is given in Table III. The free energies of transfer for systems involving GuHCl have been obtained from the data of Nozaki and Tanford.[3] It was necessary to convert from the mixed

TABLE III

Estimated Thermodynamic Quantities for the Interaction of the Peptide Backbone Unit and the Alanyl and Leucyl Side Chains with GuHCl and KI Solutions at 25°C

| Group | Salt | $m_s$ | $\Delta g_{tr}$ (cal/mol) | $\Delta h_{tr}$ (cal/mol) | $T\Delta s_{tr}$ (cal/mol) |
|---|---|---|---|---|---|
| $-CH_2-\overset{\overset{O}{\|}}{C}-NH-$ | GuHCl | 1.13 | −110[a] | −160 | −50 |
| | | 2.35 | −180[a] | −330 | −150 |
| | | 5.55 | −310[a] | −660 | −350 |
| | KI | 1.06 | −130[b] | −240 | −110 |
| $CH_3-$ | GuHCl | 1.13 | −30[a] | +200 | +230 |
| | | 2.35 | −70[a] | +330 | +400 |
| | | 5.55 | −150[a] | +570 | +720 |
| | KI | 1.13 | +120[c] | +270 | +150 |
| $(CH_3)_2CHCH_2-$ | GuHCl | 1.13 | −170[a] | +390 | +560 |
| | | 2.35 | −260[a] | +670 | +930 |
| | | 5.55 | −470[a] | +1120 | +1590 |

[a] Data from Ref. 3; see text.
[b] Data from Ref. 5; see text.
[c] Data from Ref. 14; see text.

mole fraction, molarity scale used by these workers to the molality scale used here. Equations derived by Lee[14,15] were utilized to give the relation

$$\Delta g_{tr}\text{ (molality scale)} = \Delta g_{tr}\text{ (mole fraction, molarity scale)} - 0.0360\,RTm_s \quad (3)$$

where $m_s$ is the molality of the salt in the solution. The derivation assumes that the concentration of nonelectrolyte is small and that the logarithm of the ratio of activity coefficient of the nonelectrolyte in the salt solution to that in water depends linearly on the salt concentration or molality. The value for $\Delta g_{tr}$ for the transfer of the peptide backbone unit from water to a 1.06 $m$ KI solution was estimated from the results of Nandi and Robinson.[5] An equation similar to Eq. (3) was used to convert this value from the molarity to the molality scale. The $\Delta g_{tr}$ for the transfer of a $CH_3$- from water to 1.13 $m$ KI was estimated from an equation given by Wilcox and Schrier[16] assuming that the value for KI would be the same as that for NaI. Unfortunately, there are no data in the literature that will allow even an approximation of the $\Delta g_{tr}$ of the leucyl side chain from water to KI solution.

A study of the data in Table III leads to the conclusion that the difference in protein denaturing ability between GuHCl and an "ordinary" salt, KI, resides in fairly minor differences in the thermodynamic quantities of transfer. The enthalpies of transfer of the peptide backbone unit to both salts are negative. For $CH_3-$, enthalpies and entropies of transfer that are of comparable magnitude for each salt combine to produce a negative $\Delta g_{tr}$ for the transfer of the methyl group to GuHCl but a positive $\Delta g_{tr}$ in the case of KI solutions. To be more specific, a 70-cal decrease in the enthalpy of transfer of $CH_3-$ from water to GuHCl solution from the enthalpy value for the transfer from water to KI solutions and an 80-cal

increase in the entropy term in the same order makes the sign of $\Delta g_{tr}$ negative for GuHCl solutions and positive for KI solutions. This is an all important difference since GuHCl thereby possesses the ability to solubilize nonpolar groups while KI does not. A subtle rather than a major difference in the primary thermodynamic quantities of transfer causes it, however.

Consideration of the ions comprising these salts suggests that only $GuH^+$ and $I^-$ will have any appreciable influence on water structure.[17] As regards these ions, certain of their molecular parameters are comparable. The polarizability and radius of $I^-$ are $6.28 \times 10^{-24}$ cm$^3$ [18] and $2.16 \times 10^{-8}$ cm.[18] We employed the density data of Kielley and Harrington[19] to calculate the partial molar volume of GuHCl. From this, the radius of $GuH^+$ was calculated using the equation of Couture and Laidler.[20] The calculated radius was used in conjunction with the refractive index data of Kielley and Harrington[19] and the equation of Bottcher[21] to obtain the polarizability. The polarizability is $5.43 \times 10^{-24}$ cm$^3$ and the radius is $2.37 \times 10^{-8}$ cm. On the basis of the similarities between these quantities and those for $I^-$ we would expect the work of cavity formation in the transfer of the $CH_3$— group from water to either of the salt solutions to be the same.[18] Additionally, dispersion interactions would likely be similar in the two cases.[18]

The hydrogen-bonding ability of $GuH^+$ and its potential for forming new three-dimensional structures with water must convey properties on its solution different from the more conventional structure breaker, $I^-$, however. Indeed, the greater $\Delta h_{tr}$ for transfer of $CH_3$— between water and KI solutions as opposed to that for transfers to GuHCl solutions and the greater $\Delta s_{tr}$ for transfer to GuHCl solutions versus transfer to KI solutions may be qualitatively rationalized as follows. The extent of breakdown of structure is less in transfers of $CH_3$— to GuHCl solutions than to KI solutions because some of the water structure initially broken by the $GuH^+$ ion is replaced by structure around the $CH_3$— involving participation of $GuH^+$ ions. This leads to the more positive $\Delta H_{tr}$ for transfer to KI solutions versus transfers to the GuHCl solutions. Because of the two-component nature of this new structure, it has a higher entropy than water, leading to the higher entropy of transfer of $CH_3$— to GuHCl solutions than to KI solutions. Obviously, this rationalization is completely speculative. Substantiation of this or any other hypothesis requires consideration of many other properties of these solutions as well as those of solutions containing other solutes. We do feel, however, that variations in water structure produced by differences in ionic properties rather than a mode of action for GuHCl very different from that of other salts characterizes its superior denaturing ability.

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