Noelken, M. E. (1970), *Biochemistry 9*, 4117.
Noelken, M. E., and Timasheff, S. N. (1967), *J. Biol. Chem. 242*, 5080.
Nozaki, Y., and Tanford, C. (1967), *Methods Enzymol. 11*, 732.
Nozaki, Y., and Tanford, C. (1970), *J. Biol. Chem. 245*, 1648.
Privalov, P. L., and Mrevlishvili, G. M. (1967), *Biofizika 12*, 22.
Reisler, E., and Eisenberg, H. (1969), *Biochemistry 8*, 4572.
Riddiford, L. M. (1966), *J. Biol. Chem. 241*, 2792.
Robinson, D., and Jencks, W. P. (1965), *J. Amer. Chem. Soc. 87*, 2460.
Robinson, D., and Jencks, W. P. (1965b), *J. Amer. Chem. Soc. 87*, 2470.
Schrier, E. E., and Schrier, E. B. (1967), *J. Phys. Chem. 71*, 1851.
Simpson, R. B., and Kauzmann, W. (1953), *J. Amer. Chem. Soc. 75*, 5139.
Stefanye, D., Iwamasa, R. T., Shantz, E. J., and Spiro, L. (1964), *Biochim. Biophys. Acta 86*, 412.
St. Pierre, T., and Jencks, W. P. (1969), *Arch. Biochem. Biophys. 133*, 99.
Tanford, C. (1968), *Advan. Protein Chem. 23*, 122.
Tanford, C. (1970), *Advan. Protein Chem. 24*, 1.
Timasheff, S. N. (1970), *Accounts Chem. Res 3*, 62.
Timasheff, S. N., and Inoue, H. (1968), *Biochemistry 7*, 2501.
von Hippel, P. W., and Schleich, T. (1969), *in* Structure and Stability of Biological Molecules, Timasheff, S. N., and Fasman, G. D., Ed., New York, N. Y., Marcel Decker, Chapter 6.
von Hippel, P. W., and Wong, K.-Y. (1965), *J. Biol. Chem. 240*, 3909.
Warren, J. R., and Gordon, J. A. (1970), *J. Biol. Chem. 247*, 4097.
Warren, J. R., and Gordon, J. A. (1971), *Biochim. Biophys. Acta 229*, 216.
Wetlaufer, D. B., Malik, S. K., Stoller, L., and Coffin, R. L. (1964), *J. Amer. Chem. Soc. 86*, 508.
Wong, K.-P., Roxby, R., and Tanford, C. (1971), *Anal. Biochem. 40*, 459.
Woodlock, A. F., and Harrap, B. S. (1968), *Aust. J. Biol. Sci. 21*, 821.
Woods, E. F., Himmelfarb, S., and Harrington, W. F. (1963), *J. Biol. Chem. 238*, 2374.

# Solubility of Amino Acids in Aqueous Guanidinium Thiocyanate Solutions†

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ABSTRACT: The solubilities of amino acids and a model polypeptide, acetyltetraglycine ethyl ester, have been determined in $H_2O$ and 1–3 M aqueous solutions of guanidinium thiocyanate. From these data, free energies of transfer of amino acid side chains and peptide-bond units from water to this potent protein denaturant were calculated. The results demonstrate that aqueous solutions of guanidinium thiocyanate are more effective than corresponding concentrations of urea, guanidinium chloride, ethanol, or dioxane in decreasing the free energies of transfer of hydrophobic amino acid side chains and the peptide bond from water to these solvents. The results account for the previously demonstrated fact that guanidinium thiocyanate is approximately twice as effective a protein denaturant as is guanidinium chloride.

We have previously shown that the denaturing effectiveness of salts of guanidinium, carbamoylguanidinium, and guanylguanidinium cations toward several proteins increases according to the Hofmeister anion series: $Cl^- < Br^- < I^- < CNS^-$ (Castellino and Barker, 1968). Further, when the anion is held constant, the denaturing effectiveness of the cation increases according to the series: guanidinium < carbamoylguanidinium < guanylguanidinium (Castellino and Barker, 1968). In another article, we have demonstrated that this same anion and cation series occurs in increasing the solubility of a model hydrophobic compound, benzoyl-L-tyrosine ethyl ester, and a model peptide compound, acetyltetraglycine ethyl ester (Castellino and Barker, 1969). In many previous studies it has been shown that guanidinium chloride is a more effective denaturant than compounds such as urea, dioxane, ethylene glycol, etc., and this difference in denaturing effectiveness has been explained in terms of the free energies of transfer, calculated from solubility measurements, of constituent parts of the protein from water to these solvents (Nozaki and Tanford, 1963, 1970, 1971; Tanford, 1968, 1969). Since protein denaturation is accompanied by a transfer of the side chains which exist in the interior of the native protein molecule, *i.e.*, hydrophobic residues and peptide bonds, to the denaturing solvent, it would be expected that solvents which interact more favorably with these newly exposed residues would promote denaturation more readily. This has been shown to be the case in a semiquantitative sense with the above model compounds.

The purpose of this manuscript is to describe studies on the solubility of various amino acids and a model for the peptide bond in aqueous GdmCNS[1] solutions in order to quantitatively account for the increased denaturing potency of this

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[1] Abbreviations used are: GdmCNS, guanidinium thiocyanate; GdmCl, guanidinium chloride.

compound compared to denaturatants such as GdmCl and urea.

## Materials and Methods

*Amino Acids.* All unlabeled L-amino acids were purchased from Calbiochem and all radioactive [$^{14}$C]amino acids were purchased from the New England Nuclear Corp. Prior to use all radioactive amino acids were diluted by dissolving a given amount of unlabeled amino acid in a minimum amount of water at 80°. Solutions of lysine monohydrochloride, arginine hydrochloride and histidine hydrochloride were neutralized with a small amount of 0.01 N NaOH. The corresponding [$^{14}$C]amino acid (100 $\mu$l) was added to each solution. After thorough mixing and cooling, 95% ethanol was added to each solution in order to crystallize the amino acids. In the case of proline, 1-butanol was used in place of ethanol. Crystals were collected and dried in a vacuum desiccator for 24 hr. All amino acids were recrystallized to constant specific activity and were labeled to the extent of 900–1600 dpm/mg.

Tetraglycine ethyl ester was prepared according to the method of Fisher (1904) and recrystallized four times from ethanol. Acetylation with acetic [1-$^{14}$C]anhydride (New England Nuclear Corp.) was performed as previously described (Castellino and Barker, 1969).

*Other Reagents.* Scintillation fluid was prepared by adding 8.0 g of diphenyloxazole (Packard Chemical Co.) and 0.2 g of 1,4-bis[2-(5-phenyloxozolyl)]benzene (Packard Chemical Co.) to 2 l. of toluene followed by addition of 1 l. of Triton X-100 (Packard Chemical Co.).

Guanidinium thiocyanate was purchased from Eastman Organic Chemicals.

All other reagents were of the best available quality.

*Solubility Measurements.* Weighed quantities of each amino acid were placed in soft glass test tubes and measured volumes of the desired solvents were added. The tubes were flushed with nitrogen and flame sealed. Six tubes for each amino acid in each solvent were prepared, three which undersaturate and three which oversaturate the solvent. The tubes were immersed in a rocking water bath maintained at 27.0 ± 0.3° for 24 hr when water was the solvent and for 72 hr when aqueous GdmCNS was the solvent. These conditions were determined to ensure equilibrium. At the end of this period, the water bath was stopped and the tubes allowed to sit for 10 min. The tubes were then broken and a small measured amount of each supernatant was obtained and placed in a scintillation vial. Enough solvent was added such that there was 1 ml in each vial. Scintillation mixture was added and the radioactivity determined with a scintillation counter. The amount of solvent quenching was determined by running appropriate controls.

Solutions containing tryptophan were analyzed by absorbtion at 280 nm.

## Results

The method by which solubilities were determined is illustrated in Figure 1 for histidine, phenylalanine, and leucine in water. There is a sharp break in each curve indicating the purity of the amino acids used. Data such as this were obtained for each amino acid in each solvent and the amount of solute in a saturated solution was obtained from the break in the curves. Solubilities obtained in this study for each amino acid in water and 1, 2, and 3 M GdmCNS are presented in Table I. The solubilities of our amino acids in water appear to be in line with solubilities obtained from other laboratories, a notable exception being glycine. Most values for glycine range around the value of 25 g/100 g of solvent. Our value is considerably higher than this. However, this value is reproducible and is obtained with pure glycine. Part, if not all, of this discrepancy is probably due to the higher temperature which we have employed compared to the other studies. Since solubility of these amino acids is endothermic in nature higher solubilities would be expected and dependent upon the exact amino

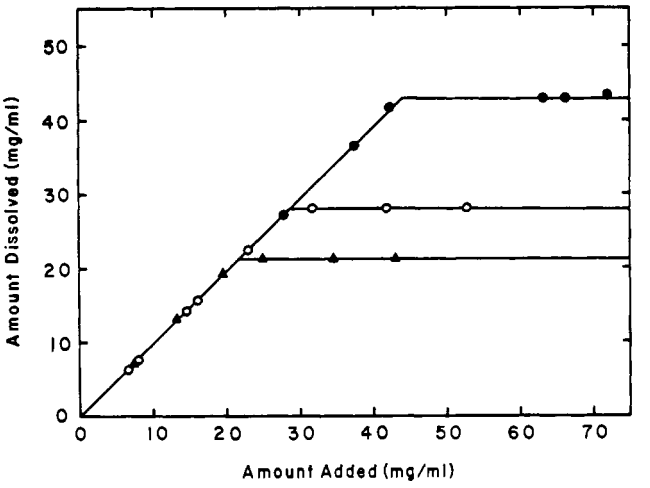


FIGURE 1: Solubilities of histidine (●), phenylalanine (○), and leucine (▲) in water at 27° as a function of the amount of solute added. The amount added was weighed and the amount in solution was determined by radioactivity measurements.

TABLE I: Solubilities of Amino Acids at 27°.

| Solute | Solubilities at GdmCNS Concn (M) of | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| | mg/ml of solution | | | |
| Alanine | 151.0 | 144.0 | 152.0 | 156.0 |
| Arginine | 533.0 | 556.0 | 568.0 | 568.0 |
| Asparagine | 29.5 | 47.8 | 55.3 | 65.0 |
| Aspartic acid | 4.85 | 7.20 | 7.98 | 8.22 |
| Cystine | 0.118 | 0.156 | 0.219 | 0.492 |
| Glutamic acid | 8.26 | 13.4 | 14.7 | 15.5 |
| Glutamine | 40.0 | 58.6 | 69.6 | 69.9 |
| Glycine | 236.0 | 230.0 | 221.0 | 217.0 |
| Histidine | 43.3 | 68.9 | 82.6 | 91.8 |
| Isoleucine | 35.6 | 47.3 | 57.0 | 65.5 |
| Leucine | 21.0 | 29.2 | 38.9 | 43.4 |
| Lysine | 584.0 | 630.0 | 632.0 | 590.0 |
| Methionine | 52.2 | 89.0 | 114.0 | 128.0 |
| Phenylalanine | 27.8 | 56.0 | 77.7 | 99.5 |
| Proline | 766.0 | 772.0 | 780.0 | 798.0 |
| Serine | 376.0 | 398.0 | 361.0 | 377.0 |
| Threonine | 97.3 | 108.0 | 111.0 | 113.0 |
| Tryptophan[a] | 36.5 | 111.0 | 188.3 | 277.8 |
| Tyrosine | 0.485 | 0.705 | 1.03 | 1.76 |
| Valine | 58.8 | 71.3 | 73.8 | 81.5 |
| $AcGly_4OEt$ | 1.14 | 51.3 | 10.9 | 19.3 |

[a] Optical density values are listed here. Determination of values was made at 280 nm.

TABLE II: Some Comparisons between Solubilities in Water by Different Methods or from Different Laboratories.

| Solute | Solubility (g/100 g of Solvent) | | | | |
|---|---|---|---|---|---|
| | Nozaki and Tanford (1970) | Cohn *et al.* (1934) | Dunn *et al.* (1933) | Dalton and Schmidt (1935) | This Study[a] |
| Alanine | 16.67 | | | 16.7 | 17.9 |
| Asparagine | 2.51 | 2.50 | | 2.63 | 2.60 |
| Glycine | 25.09 | 25.0 | 25.3 | 25.0 | 30.0 |
| Leucine | 2.16 | 2.29 | 2.19 | 2.43 | 2.37 |
| Phenylalanine | 2.79 | | | 2.97 | 2.92 |
| Tyrosine | 0.0475 | | 0.0479 | 0.0453 | 0.0499 |

[a] Our studies were performed at 27° whereas all others are at 25°.

acid in question. Table II lists some comparisons in solubilities of amino acids in water from different laboratories. We have converted our solubility values in Table I which are expressed in mg/ml to g/100 g of solvent for ease in comparison. It can be noted in Table I that the solubility of tryptophan is listed as the optical density of a saturated solution in each solvent. We did not convert these values to absolute solubilities since our subsequent calculations did not require this. However, it is necessary to correct for the effect of GdmCNS solutions on the absorbance of a known quantity of tryptophan. We have made these corrections by preparing a tryptophan solution with a measured optical density in water and diluting this solution with equal volumes of water and 2, 4, and 6 M GdmCNS. This procedure allowed us to evaluate the effect of 1, 2, and 3 M GdmCNS on the absorbance of tryptophan. We found that the correction factors are 1.011, 1.020, and 1.032, which should be multiplied by the measured optical density obtained in 1, 2, and 3 M GdmCNS, respectively.

TABLE III: Free Energies of Transfer ($\Delta F_t'$)[a] from Water to Aqueous Guanidinium Thiocyanate Solutions at 27°.

| Solute | $\Delta F_t'$ at GdmCNS Concn (M) of | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Alanine | +29 | −5 | −19 |
| Arginine | −25 | −38 | −38 |
| Asparagine | −288 | −375 | −471 |
| Aspartic acid | −236 | −297 | −315 |
| Cystine | −166 | −369 | −851 |
| Glutamic acid | −286 | −344 | −375 |
| Glutamine | −228 | −333 | −333 |
| Glycine | +15 | +39 | +50 |
| Histidine | −277 | −385 | −488 |
| Isoleucine | −170 | −284 | −364 |
| Leucine | −226 | −445 | −533 |
| Lysine | −45 | −47 | −6 |
| Methionine | −318 | −466 | −535 |
| Phenylalanine | −417 | −613 | −760 |
| Proline | −5 | −11 | −25 |
| Serine | −34 | +25 | −2 |
| Threonine | −60 | −79 | −89 |
| Tryptophan | −663 | −979 | −1210 |
| Tyrosine | −223 | −449 | −768 |
| Valine | −115 | −135 | −195 |
| $AcGly_4OEt$ | −880 | −1340 | −1680 |

[a] These values were calculated by eq 1, neglecting the last term. They are expressed in cal/mole.

TABLE IV: Side-Chain Contributions ($\Delta f_t'$)[a] to Free Energy of Transfer from Water to Aqueous Guanidinium Thiocyanate Solutions at 27°.

| Solute | $\Delta f_t$ at GdmCNS Concn (M) of | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Alanine | +14 | −44 | −69 |
| Arginine | −40 | −77 | −88 |
| Asparagine | −303 | −414 | −521 |
| Aspartic acid | −251 | −336 | −365 |
| Cystine | −181 | −408 | −901 |
| Glutamic acid | −301 | −383 | −425 |
| Glutamine | −243 | −372 | −383 |
| Histidine | −292 | −424 | −498 |
| Isoleucine | −185 | −323 | −414 |
| Leucine | −211 | −406 | −483 |
| Lysine | −60 | −86 | −56 |
| Methionine | −333 | −505 | −585 |
| Phenylalanine | −432 | −652 | −810 |
| Proline | −20 | −50 | −75 |
| Serine | −49 | −14 | −52 |
| Threonine | −75 | −118 | −139 |
| Tryptophan | −678 | −994 | −1225 |
| Tyrosine | −238 | −488 | −818 |
| Peptide bond[b] | −220 | −335 | −420 |

[a] Values are expressed in cal/mole. [b] Obtained by dividing the $\Delta F_t'$ of $AcGly_4OEt$ by 4.

Free energies of transfer, $\Delta F_t$, of the amino acids from water to aqueous solutions of GdmCNS were calculated from

$$-\Delta F_t = RT \ln \frac{S_G}{S_W} + RT \ln \frac{\gamma_G}{\gamma_w} \quad (1)$$

where $S_G$ and $S_w$ are the molarities of saturated solutions of each amino acid in the desired GdmCNS solution and water, respectively. The last term on the right represents the effect of solute–solute self-interactions on the chemical potential. Since activity coefficients have not been determined for amino acids in GdmCNS we must ignore this term in our calculations. This same procedure has been previously employed for solubilities in GdmCl (Nozaki and Tanford, 1970) and necessarily leads to approximate $\Delta F_t$ values. We will designate these values as $\Delta F_t'$ in order to maintain the same symbolism as did Nozaki and Tanford (1963, 1970). These can easily be corrected in the future if activity coefficients are measured.

Table IV lists free energies of transfer ($\Delta f_t'$) of amino acid side chains from water to aqueous GdmCNS and are obtained by subtracting the $\Delta F_t'$ of glycine at each GdmCNS concentration from the $\Delta F_t'$ of any amino acid at the same GdmCNS concentration. These calculations are based upon the additivity of the component free energies to yield the total

TABLE V: Terms for Conversion of Free Energies of Transfer from One Concentration Scale to Another.

| M of GdmCNS | Mole Fraction ($X_G$) | Density ($\rho$) | Constant[a] for Conversion of $\Delta F_t'$ from M to Mole Fraction | Constant[a] for Conversion of $\Delta F_t'$ from Mole Fraction to $m$ |
|---|---|---|---|---|
| 1.0 | 0.0196 | 1.021 | −14 | 26 |
| 2.0 | 0.0423 | 1.052 | −25 | 55 |
| 3.0 | 0.0698 | 1.075 | −46 | 89 |

[a] Values expressed in cal/mole.

free energy of the compound. The $\Delta F_t'$ for an amino acid can therefore be assumed to be made up of the $\Delta f_t'$ of glycine + the $\Delta f_t'$ of the side chain.

Although we have calculated $\Delta F_t'$ and $\Delta f_t'$ values on the molarity scale, it has also been common practice in the past to calculate these values on the molality and mole fraction scale. This can be easily done according to

$$\Delta F_t'(\text{mole fraction}) = \Delta F_t'(\text{molarity}) - RT \ln [(1 + 2.303 X_G/\rho)] \quad (2)$$

$$\Delta F_t'(\text{molality}) = \Delta F_t'(\text{mole fraction}) + RT \ln (1 + 2.303 X_G) \quad (3)$$

where $X_G$ is the mole fraction of the GdmCNS solution and $\rho$ is the corresponding density. Of course, the last term in the equation is constant and is evaluated for each solvent composition in Table V.

## Discussion

The study has been undertaken with the intent of explaining the basis of the much more potent denaturing effectiveness which occurs upon changing the $Cl^-$ in GdmCl to $CNS^-$ in GdmCNS. Toward this end, we have chosen to study the solubility of amino acids in these solvents since this approach has been effectively utilized in explaining the greater denaturing potency of GdmCl over such classical denaturants as urea, dioxane, ethanol, etc. (Nozaki and Tanford, 1970, 1971). The rationale behind this approach is simply explained. For the unfolding of the protein molecule, which accompanies denaturation, to be thermodynamically favorable, the residues which come into contact with solvent in the unfolded state, for the first time, must exhibit favorable interactions with the solvent. Therefore, determining $\Delta f_t'$ of amino acid side chains in various solvents represents a good approach to studying interactions of amino acid side chains with various denaturing solvents. In studying protein denaturation by means of model compounds we are assuming that the principle of the additivity of the free energies of transfer is valid. This concept essentially states that in the unfolded state, the free energy of transfer of the protein can be expressed as the sum of the free energies of transfer of the component amino acid side chains and peptide bonds. The limitations to this approach have been discussed in detail previously (Nozaki and Tanford, 1970; Tanford, 1969) and no purpose would be served by repeating them here since these same limitations apply to this study.

TABLE VI: A Comparison of $\Delta f_t'$ Calculated for 2 M Solutions of Various Denaturants.

| Solute | $\Delta f_t'$[a] at 2 M Concn: Urea[b] | GdmCl[c] | Ethanol[d] | Dioxane[e] | Gdm-CNS |
|---|---|---|---|---|---|
| | | | cal/mole | | |
| Peptide bond | −40 | −135 | 25 | 55 | −310 |
| Alanine | 0 | −20 | −20 | | −19 |
| Asparagine | −135 | −320 | 75 | −25 | −389 |
| Glutamine | −80 | −215 | 55 | −10 | −347 |
| Histidine | −100 | −285 | | −115 | −399 |
| Leucine | −110 | −210 | −120 | −165 | −381 |
| Methionine | −115 | −245 | | | −480 |
| Phenylalanine | −180 | −355 | −170 | −375 | −627 |
| Threonine | −40 | −90 | | | −93 |
| Tyrosine | −225 | −385 | −215 | | −463 |
| Tryptophan | −270 | −630 | −300 | −765 | −969 |

[a] All $\Delta f_t'$ values are expressed in mole fraction scale. [b] Nozaki and Tanford (1963). [c] Nozaki and Tanford (1970). [d] Nozaki and Tanford (1971). Refers to 3.4 M ethanol. [e] Nozaki and Tanford (1971). Refers to 2.3 M dioxane.

In order to compare $\Delta f_t'$ values for GdmCNS with those of urea, GdmCl, ethanol, or dioxane, we have compiled our values in GdmCNS at approximately the same solvent concentration along with those obtained by Tanford's group with the other solvents. This comparison is given in Table VI. From this comparison it is evident that GdmCNS should be a more potent denaturant than urea, GdmHCl, etc., since it allows more favorable $\Delta f_t'$ values for hydrophobic and peptide bond residues to occur. Since these are usually in the interior of the protein molecule and become exposed upon denaturation, more favorable interactions between these residues and the solvent predict greater denaturing potency for the solvent. Also, from this table, it is easy to see why compounds such as dioxane or ethanol are not potent denaturants although they solubilize hydrophobic side chains. These compounds do not allow favorable transfer of the peptide bond to the solvent media and thus cannot be potent denaturants.

In order to further elucidate the reasons for the greater denaturing potency of GdmCNS a useful comparison is made in Table VII. From this table it can be seen that GdmCNS is greater than twice as effective as GdmCl in allowing more favorable interactions with the peptide bond and this is probably the chief reason for GdmCNS being twice as effective a protein denaturant as is GdmCl. GdmCNS also allows 1.5–2.0 times more favorable interactions than GdmCl with hydrophobic residues such as tryptophan, phenylalanine, tyrosine, etc., and this of course also contributes to its greater denaturing effectiveness. However, since there are many more peptide bonds in a protein than there are residues of highly hydrophobic amino acids, the basis of the greater denaturing potency of GdmCNS over GdmCl must lie in peptide-bond considerations. We have also previously found that GdmCNS is approximately a four- to fivefold more effective denaturant than urea (Castellino and Barker, 1968) and this can also be explained chiefly on the basis of greater effectiveness in sol-

TABLE VII: Comparisons between GdmCNS, GdmCl, and Urea.

| Moiety Transfered[a] | Ratio of $\Delta f_t'$[b] in 2 M GdmCNS/ 2 M Urea | 2 M GdmCNS/ 2 M GdmCl |
|---|---|---|
| Peptide bond | 7.75 | 2.30 |
| Tryptophan | 3.59 | 1.54 |
| Phenylalanine | 3.48 | 1.77 |
| Tyrosine | 2.06 | 1.20 |
| Leucine | 3.46 | 1.81 |
| Methionine | 4.17 | 1.96 |
| Histidine | 3.99 | 1.40 |
| Alanine | | 1.00 |
| Threonine | 2.32 | 1.00 |

[a] Amino acids are listed in decreasing hydrophobicity (Nozaki and Tanford, 1971). [b] Mole fraction scale.

ubilizing the peptide bond. Solubilization of hydrophobic residues plays an important role here as well as for GdmCl.

The final point to consider is the mechanism of the increased effectiveness of $CNS^-$ over $Cl^-$ in the solubility of $AcGly_4OEt$ as well as hydrophobic residues. The effect on $AcGly_4OEt$ can be readily explained by assuming the peptide group is polarizable and can interact with cations and anions. Since $CNS^-$ exhibits stronger interactions than $Cl^-$ with positively charged groups such as anion-exchange resins, it is likely that this same effect can exist on the polarized peptide moieties. This would result in a more favorable interaction of the peptide bond with $CNS^-$ and would result in a lower $\Delta f_t'$ of the peptide group than that of $Cl^-$, as is observed. With regard to hydrophobic groups the order of effectiveness of the anion is more ambiguous. This differential anion effectiveness on solubilities can either be due to a direct effect of the anion on the amino acid or can be due to an indirect effect mediated through alterations in the structure of water. No useful mechanism can be forwarded at this point.

Finally, it should be noted that we are studying denaturing solvents which produce randomly coiled proteins since in this case solubility studies are easily interpretable. Certainly there are other very effective denaturants such as long-chain detergent molecules which produce denaturation but do not produce random coils. Since in these cases all amino acid side chains are not in contact with solvent molecules in the denatured state, solubility studies would have a very different meaning than what is described in this manuscript and in earlier manuscripts from Tanford's laboratory. It is likely that compounds of this class produce denaturation by having binding sites on the denatured form of the protein which are inaccessible on the native protein, thus leading to excess binding energies accompanying denaturation. While this mechanism is also extended to the effect of guanidine salts and urea on peptide-bond solubility, it should be clear that guanidine salts and urea produce other effects which ultimately lead to randomly coiled protein molecules.

## References


Castellino, F. J., and Barker, R. (1968), *Biochemistry 7*, 4135.

Castellino, F. J., and Barker, R. (1969), *Biochemistry 8*, 3439.

Cohn, E. J., McMeekin, T. L., Edsall, J. T., and Weare, J. H. (1934), *J. Amer. Chem. Soc. 56*, 2270.

Dalton, J. B., and Schmidt, C. L. A. (1935), *J. Biol. Chem. 109*, 241.

Dunn, M. S., Ross, F. J., and Read, L. S. (1933), *J. Biol. Chem. 103*, 579.

Fischer, E. (1904), *Chem. Ber. 37*, 2486.

Nozaki, Y., and Tanford, C. (1963), *J. Biol. Chem. 238*, 4074.

Nozaki, Y., and Tanford, C. (1970), *J. Biol. Chem. 245*, 1648.

Nozaki, Y., and Tanford, C. (1971), *J. Biol. Chem. 246*, 2211.

Tanford, C. (1968), *Advan. Protein Chem. 23*, 121.

Tanford, C. (1969), *Advan. Protein Chem. 24*, 1.