Effect of Denaturing Agents of the Urea-Guanidinium Class on the Solubility of Acetyltetraglycine Ethyl Ester and Related Compounds*

Dwight R. Robinson and William P. Jencks

From the Graduate Department of Biochemistry, Brandeis University, Waltham 54, Massachusetts

(Received for publication, January 17, 1963)

It has been demonstrated by several investigators, most convincingly by studies of the effect of urea on the solubility of various compounds, that aqueous urea solutions are better solvents than pure water for many solutes containing hydrophobic groups (1-5). It has further been suggested that this property of aqueous urea solutions is responsible for their denaturing activity toward proteins and deoxyribonucleic acid (1-5). There is evidence supporting this hypothesis for the denaturation of DNA and some evidence suggesting that this property may contribute to the denaturing activity of compounds of the urea-guanidinium class toward certain proteins (5, 6). On the other hand, the absence of a positive correlation between the hydrophobic character and the denaturing effectiveness of denaturing agents toward bovine serum albumin and ovalbumin indicates that other properties of these reagents must contribute to their denaturing activity (6-9). The effects of substances of the urea-guanidinium class on the solubility of model compounds, which are reported in this communication, demonstrate that this class of denaturing agents can promote protein denaturation by causing a decrease in the activity coefficient of the peptide group. This effect is not due to an increase in the solvent power of the solutions toward hydrophobic solutes; i.e. it is not a “hydrophobic” effect in the sense that this term is ordinarily used.

N-Acetyltetraglycine ethyl ester, a model compound composed largely of amide groups, was prepared from tetraglycine ethyl ester hydrochloride (10) and acetic anhydride in 40% aqueous pyridine at 0°. The product crystallized from the reaction mixture; after one recrystallization from water it had a melting point of 263-264° (with decomposition).

\[ \text{C}_9\text{H}_{12}\text{O}_4\text{N}_4 \]

Calculated: C 45.56, H 6.37, N 17.71
Found: C 45.30, H 6.31, N 17.67

Infrared spectrum (KBr): 3270, 3070, 1753, 1670, 1635, 1558, 1203, 1027 cm⁻¹ (strong). The solubility of this compound in aqueous solutions of purified reagents was determined by the biuret method (11) after shaking for 24 to 48 hours at 25°. Identical results were obtained with samples which had been incubated at a higher temperature and then shaken at 25°, indicating that equilibrium had been reached. The solubilities of toluene and carbobenzyoxycarbonylglycineamide were measured spectrophotometrically at 257, 262, and 275 μg.

The solubility of acetyltetraglycine ethyl ester (S/S₀) is increased in the presence of urea or guanidinium chloride (Fig. 1).

In 8 M urea and 7 M guanidinium chloride the solubility is increased 3.35 and 7.7 times, respectively, compared to that in water. The effects of 3 M solutions of a number of compounds on the solubility of acetyltetraglycine ethyl ester are compared in Table I. The effects of a more limited series of compounds on the solubilities of toluene, benzyl alcohol, and carbobenzyoxycarbonylglycineamide are shown in Table II. The solubilizing action of urea, guanidinium chloride, formamide, and acetamide acetyltetraglycine ethyl ester is not due to a “hydrophobic” effect, in the sense that this term is ordinarily used, for the following reasons.

1. Increasing the hydrophobic character of the denaturing agent by the addition of alkyl substituents causes a decrease or a reversal of the solubilizing action of these compounds. In contrast, alkyl substitution causes an increase in the solubilizing effect of these compounds toward toluene and benzyl alcohol which may be ascribed to a normal “hydrophobic” effect.

2. The known hydrophobic compounds, ethanol, dioxane, and tetrahydrofuran, have only very small effects on the solubility of acetyltetraglycine ethyl ester.

3. Solvent effects on the solubility of carbobenzyoxycarbonylglycineamide are more complex because this molecule contains a large hydrophobic group as well as a short peptide chain; the observed effects on the solubility of this compound reflect the properties of both portions of the molecule. Urea and guanidinium chloride cause a much larger increase in the solubility of this compound than in the solubility of toluene and benzyl alcohol, models for
the hydrophobic portion of the molecule. This suggests that these reagents exert their effect primarily upon the amide chain of the molecule and that it is not a simple "hydrophobic" effect. On the other hand, alkyl-substituted reagents increase the solubility of carbobenzoxydiglycinamide, toluene, and benzyl alcohol, but not acetyltetraglycine ethyl ester. This suggests that these compounds exert their effects largely on the hydrophobic portion of the molecule.

These results clearly show that the order of effectiveness of denaturing agents in increasing the solubility of acetyltetraglycine ethyl ester is in the opposite direction from the order of their increasing hydrophobic character. When both the hydrophobic and amide groups are combined in the same molecule (in carbobenzoxydiglycinamide and also, presumably, in a protein) both types of effect may be seen. An increase in solubility reflects a decrease in activity coefficient (a "stabilization") of a molecule. Since the peptide groups of a protein are more exposed to the solvent in the denatured than in the native state, compounds which decrease the activity coefficient of the peptide group will favor denaturation of the protein. Thus, the addition of urea, guanidinium chloride, or formamide to a protein would be expected to favor the denatured state of the protein by causing a decrease in the activity coefficient (a "stabilization") of the amide groups. This conclusion, of course, does not preclude the possibility that the hydrophobic character of these and other reagents may also promote denaturation by decreasing the activity coefficient of hydrophobic groups in certain proteins. It is of interest that the order of effectiveness of the reagents reported here in increasing the solubility of acetyltetraglycine ethyl ester is very similar to their order of effectiveness in denaturing bovine serum albumin, as measured by changes in optical rotation (5, 7). The increased solubility of asparagine in aqueous urea solutions, mentioned by Whitney and Tanford (3), may reflect an effect on the amide group of this molecule similar to that reported here for acetyltetraglycine ethyl ester.

The nature of the change in the interaction of amide groups with solvent in the presence of denaturing agents of the urea-guanidinium class is still undetermined. Monofunctional hydrogen bonding between the denaturing agent and the amide cannot account for the results in view of (a) the low activity of ethylammonium ion and acetic acid, both of which are much stronger hydrogen donors than urea, (b) the activity of guanidinium, which cannot act as a proton acceptor, and (c) the opposite effects of guanidinium and tetramethylguanidinium ions on acetyltetraglycine ethyl ester, in spite of the very similar acidity of these two compounds (12). Furthermore, monofunctional hydrogen bonds between solute molecules are known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydrogen bonding between solute molecules is known to have little or no stability in water because of the strong hydrogen-bonding ability of water itself (13). Polysubstitution by functional hydro
The Effect of Replacement of the Carboxamide Group by Hydrogen in the Glutamine or Asparagine Residue of Oxytocin on Its Biological Activity*

VINCENT DU VIGNEAUD, GEORGE S. DENNING, JR., STEFANIA DRABAREK, and W. Y. CHAN

From the Department of Biochemistry, Cornell University Medical College, New York 21, New York

(Received for publication, January 29, 1963)

In studying the significance of the chemical functional groups of oxytocin to its biological activity, a systematic approach has been under way for several years in this laboratory in which a given chemical functional group is replaced by hydrogen through synthesis of the desired analogues. For example, the phenolic group of oxytocin (Fig. 1) has been replaced with hydrogen by starting with phenylalanine in place of tyrosine and thus obtaining deoxy-oxytocin (1), and the amino group in oxytocin has been replaced with hydrogen by starting with N-carbobenzoxy-X-benzylcysteine and thus obtaining deamino-oxytocin (24). In certain bioassays, the deamino-oxytocin was found to be more potent than oxytocin itself. On the other hand, the deoxy-oxytocin has possessed the characteristic activities of oxytocin, but was considerably less potent than oxytocin, as also found by Aschner and coworkers (5-7). The deamino-deoxy-oxytocin has also been synthesized and found to possess the same potency as deoxy-oxytocin (8).

As an extension of this type of approach we have now synthesized two analogues in which the carboxamide group in either the 4- or 5-position is replaced by hydrogen, namely, 4-decarboxamido-oxytocin and 5-decarboxamido-oxytocin. Both of these analogues were made by the stepwise synthesis that was used previously for oxytocin (9) and involves the appropriate p-nitrophenyl esters. For the 4-decarboxamido-oxytocin, L-α-aminobutyric acid was used in place of L-glutamine, and for the 5-decarboxamido-oxytocin, L-alanine was used in place of L-asparagine. In the isolation of the analogues, countercurrent distribution in the solvent system, butanol-propanol-0.05% acetic acid (2:1:3) was used. The 4-decarboxamido-oxytocin possessed a distribution coefficient (K) of 0.53, compared with a value of 0.37 for oxytocin in this system. A sample of each analogue was hydrolyzed in 6 N HCl at 100° for 17 hours and analyzed for amino acids in the Beckman/Spinco amino acid analyzer.

| TABLE I

Elementary analyses and rotations

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>H</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotation (± 0.05 in 1 N acetic acid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Decarboxamido-oxytocin</td>
<td>52.2</td>
<td>6.90</td>
<td>15.6</td>
</tr>
<tr>
<td>5-Decarboxamido-oxytocin</td>
<td>52.3</td>
<td>6.94</td>
<td>15.6</td>
</tr>
<tr>
<td>Calculated value</td>
<td>52.3</td>
<td>6.79</td>
<td>16.0</td>
</tr>
</tbody>
</table>

| TABLE II

Biological activities of 4-decarboxamido-oxytocin, 5-decarboxamido-oxytocin, and oxytocin

<table>
<thead>
<tr>
<th></th>
<th>Mean activities and standard errors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (μg)</td>
<td>Oxycotin</td>
</tr>
<tr>
<td></td>
<td>4-Decarboxamido-oxytocin</td>
</tr>
<tr>
<td>μg/mg</td>
<td>507 ± 15</td>
</tr>
</tbody>
</table>

A striking difference was found in the activities of the two...
Effect of Denaturing Agents of the Urea-Guanidinium Class on the Solubility of Acetyltetraglycine Ethyl Ester and Related Compounds
Dwight R. Robinson and William P. Jencks


Access the most updated version of this article at http://www.jbc.org/content/238/4/PC1558.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/238/4/PC1558.citation.full.html#ref-list-1