to have a trapped valence character. The reasons for this important difference between low-spin (antiferromagnetic) and high-spin (ferromagnetic) clusters have been discussed in some detail in ref 6.

Although 2-Fe ferredoxins appear to exist exclusively in the low-spin form, the possibility exists that geometric or environmental influences might favor a ferromagnetic interaction between the monomers in which the high-spin, delocalized form becomes the ground state. This appears to be the case for the oxidized 4-Fe clusters, for which the interaction energies between the 2-Fe subunits is apparently sufficient to drive each subunit into a high-spin conformation. Similar behavior has been postulated for certain 3-Fe clusters, which appear spectroscopically to be composed of a high-spin, reduced 2-Fe cluster coupled to the spin of a single $Fe^{3+}$ unit.[35] An important area for future research will be to determine the circumstances favoring ferromagnetic vs. antiferromagnetic interactions in multinuclear clusters.

**Electron Transfer.** Upon reduction of all the "oxidized" clusters considered here, most of the added charge (56 to 70%) migrates to the sulfur atoms, as a result of changes in orbitals other than those which formally accept the extra electron. Both S and S* bear a negative charge, even in the oxidized complexes. These results are in accord with the extensive hydrogen bonding observed at both S and S* in iron-sulfur proteins, and with the increase in hydrogen bonding observed upon reduction of a 4-Fe "high-potential" Fe-S protein.[36]

The close proximity in energy of the filled S orbitals and empty Fe 3d orbitals (see Figures 2-4) may be important for the electron-transfer function of ferredoxins. In both 2-Fe and 4-Fe ferredoxins the iron sites are buried in the protein interior, whereas the cysteine sulfurs are more exposed to solvent. Internal electron transfer from cysteine S to Fe could be induced by the electrostatic field of a charged donor and might be the initial step in electron transfer; this would be followed by electron transfer from the donor to the more accessible hole in the S 3p band. In this context, it is of interest that the charge-transfer spectra of 2-Fe ferredoxins and their analogues begin at lower energy and have larger extinction coefficients than is the case for the 1-Fe rubredoxin. Indirect electron transfer of the type considered here should thus be a more facile process in the larger clusters.

The intimate relation between antiferromagnetic coupling and electron-transfer mechanism indicated above may also apply to other biological oxidation-reduction systems that have several metals, e.g., to cytochrome oxidase or nitrogenase. A common feature in all of these appears to be the presence of low-lying empty orbitals on the metal centers, in close proximity to filled orbitals on the ligands. A two-step electron-transfer model, such as that outlined above, could allow electrons to move over significant distances (10 Å or more) while still maintaining specificity at both the metal and the ligand sites. These characteristics are important for electron-transfer proteins and for multielectron oxido-reductases.

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# Properties of Some Protein Denaturants in *N,N*-Dimethylformamide. Enthalpic Interaction Coefficients of Urea and Substituted Urea Compounds

**M. Bloemendal and G. Somsen***

*Contribution from the Department of Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.* *Received December 3, 1984*

**Abstract:** Enthalpies of dilution of urea and several alkyl-substituted ureas dissolved in *N,N*-dimethylformamide (DMF) have been measured calorimetrically at 298.15 K. The results are analyzed in terms of the McMillan-Mayer theory in order to obtain enthalpic interaction coefficients. For urea these coefficients are extraordinarily large. When an increasing number of methyl groups is introduced in the solute molecules, the values of the interaction coefficients change gradually to values usually found for compounds of this type. This is interpreted in terms of solute-solvent association. The enthalpic pair interaction coefficients of 1,1- and 1,3-dimethylurea differ distinctly. This indicates that simple additivity models are not applicable in this case. Considering DMF as a model system for the interior of a globular protein, the results are compared with those in water and discussed with respect to the denaturation of proteins by means of urea compounds.

This paper is part of a project in which we are investigating solute-solute interactions in nonaqueous solvents by means of enthalpic interaction coefficients on the basis of the McMillan-Mayer theory.[1] In this approach, which has been discussed by several authors,[2-6] the $n$th interaction coefficient refers to the interaction of $n$ solute particles mediated by the solvent. In previous papers[6,7] we have reported results on several amides as solutes in the solvent *N,N*-dimethylformamide (DMF).

In this paper we focus our attention on urea and alkyl-substituted ureas. These compounds are of biochemical importance

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**Table I.** Melting Points of Solid Solutes

| solute | mp/°C | lit. value/°C |
|---|---|---|
| U | 134.8–135.2 | 133.6[a] |
| MeU | 100.5–101.5 | 101[b] |
| 1,1-$Me_2$U | 180.5–181.5 | 182[b] |
| 1,3-$Me_2$U | 105.9–106.1 | 106[b] |
| $Me_3$U | 74.5–75.5 | 75.5[c] |
| EtU | 91.5–92.5 | 92[c] |

[a] Reference 21. [b] Reference 22. [c] Reference 23.

because of their properties as protein denaturants. The influence of urea and its homologues on protein structure is not completely understood.[8] There is evidence of direct "binding" of the denaturant molecules to the proteins.[9,10] Alternatively, it has been proposed that changing of the water structure by the denaturant molecules plays a predominant role.[11,12] In spite of the many data available, no consensus exists as to whether the first or the second phenomenon or a combination of the two is essential for denaturation by (substituted) ureas. Whereas Ogawa et al.[12] state that "it is generally accepted that urea does not appreciably interact with either hydrophobic or hydrophylic molecules or groups", Shibata et al.[13] say that "considerable evidence exists that urea interacts strongly with peptide backbone groups". According to Paulic and Lapanje,[14] alkyl ureas are "clearly less efficient denaturants than urea". On the contrary, Herskovitz et al.[15] conclude that "the effectiveness of these types of reagents as protein denaturants increases with increasing chain length or hydrocarbon content". Feinstein and Moudrianakis[16] indicate that the mechanism of denaturation by urea and its homologues depends on the type of protein studied and that the relative denaturating ability of a series of urea compounds with respect to one particular protein might give an indication about the interactions in the native structure of that protein.

It has been put forward that *N*,*N*-dimethylformamide might be a model system for the interior of a protein.[17] Hence the interaction between urea or substituted urea molecules in DMF may give information about similar interactions within a native protein. Therefore, we report here the enthalpic interaction coefficients for urea (U), methylurea (MeU), 1,1-dimethylurea (1,1-$Me_2$U), 1,3-dimethylurea (1,3-$Me_2$U), trimethylurea ($Me_3$U), tetramethylurea ($Me_4$U), and ethylurea (EtU), all dissolved in DMF. The coefficients were calculated from calorimetrically obtained enthalpies of dilution.

## Experimental Section

**Materials.** DMF (Baker, Analyzed Reagent) was purified and dried as before.[6] Urea ("pro Analyse") was recrystallized from ethanol three times and vacuum dried at 45 °C for 24 h. 1,3-$Me_2$U (Koch, pA) was recrystallized from ethanol and dried at 50 °C and 5 mmHg for 24 h. MeU, 1,1-$Me_2$U, $Me_4$U, and EtU were purified as described by Rouw and Somsen.[18] $Me_3$U was synthesized from methyl isocyanate and dimethylamine at 0 °C according to Davis and Ebersole,[19] recrystallized from methanol + ether (v:v = 1:20), and dried under vacuum at room temperature for 24 h. The purity of liquid $Me_4$U was tested by GC analysis and Karl-Fischer titration[20] and found to be at least 99.7 mol %. The purity of the solid solutes was checked by the melting points as collected in Table I. All solutes show sharp melting points close to literature values.

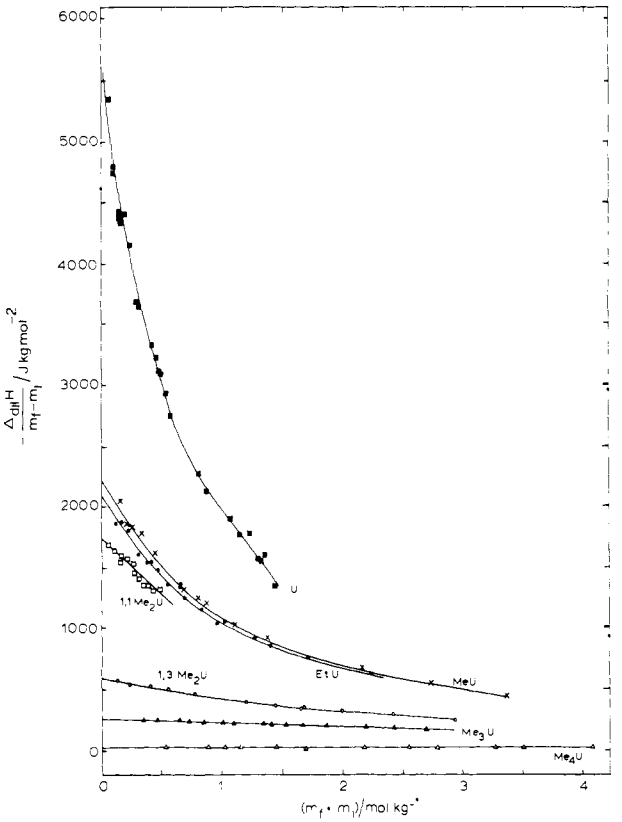


**Figure 1.** $\Delta H_{dil}/(m_f - m_i)$ as a function of $(m_f + m_i)$ for (substituted) urea compounds in DMF at 25 °C.

**Apparatus.** Enthalpies of dilution were determined with an LKB 10700-2 batch microcalorimeter system. The output signal of the calorimeter was amplified and integrated by means of a Kipp BD12 integrating recorder. Details of the experimental procedure have been described before.[6] In order to reduce the equilibration time of each measurement, the method of subsequent dilutions was used.[7,24] After the first dilution experiment, a maximal and known amount of solution in one of the compartments of the measuring cell was replaced by a known mass of pure solvent. Thus in the second experiment a solution was mixed with a highly diluted solution of the same kind. The procedure was repeated several times.

## Results

A compilation of the data in connection with the dilution experiments is given in Table II. The table presents the enthalpic change, $\Delta H$, when $n_A$ moles of solute at molality $m_{A,i}$ is mixed either with $n_B$ moles of the same solute at molality $m_{B,i}$ or with pure DMF ($n_B = 0$ mol, $m_{B,i} = 0$ mol kg$^{-1}$) to give a solution with final molality $m_f$. $\Delta H$ can be written in terms of the molar excess enthalpies at molality $m$, $H^E(m)$ as

$$\Delta H = n_A[H^E(m_f) - H^E(m_{A,i})] + n_B[H^E(m_f) - H^E(m_{B,i})] \quad (1)$$

For concentrations where the solvent activity approximates the activity of the pure solvent

$$H^E(m) = B_2^h m + B_3^h m^2 + B_4^h m^3 + \ldots \quad (2)$$

$B_2^h$, $B_3^h$, $B_4^h$, etc., are the pair, triplet, quadruplet, and higher enthalpic interaction coefficients of the solute particles.[25] These

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**Table II.** Enthalpies of Dilution of Several Solutes Dissolved in DMF at 298.15 K[a]

| $m_{A,i}$ | $n_A$ | $m_{B,i}$ | $n_B$ | $m_f$ | $\Delta H$ | $\Delta$, %[b] |
|---|---|---|---|---|---|---|
| | | | A. Urea | | | |
| 0.0357 | 0.1523 | 0.36 | 0.93 | 0.0224 | 10.72 | +4.4 |
| 0.0633 | 0.2737 | 0.70 | 1.64 | 0.0412 | 28.30 | −2.1 |
| 0.0646 | 0.2983 | 0.74 | 1.67 | 0.0430 | 30.46 | −0.7 |
| 0.0981 | 0.4164 | 0 | 0 | 0.0633 | 63.72 | −2.3 |
| 0.1002 | 0.4228 | 0 | 0 | 0.0646 | 65.41 | −3.0 |
| 0.1264 | 0.2027 | 0.92 | 3.77 | 0.0363 | 79.96 | −1.7 |
| 0.1553 | 0.1930 | 1.09 | 4.62 | 0.0360 | 100.41 | +1.2 |
| 0.1859 | 0.7220 | 2.14 | 4.78 | 0.1188 | 180.47 | +0.6 |
| 0.1966 | 0.1759 | 1.39 | 5.83 | 0.0357 | 116.65 | +0.5 |
| 0.2343 | 0.4808 | 1.69 | 6.93 | 0.0793 | 270.18 | −2.3 |
| 0.2644 | 0.8578 | 0 | 0 | 0.1553 | 310.45 | +0.9 |
| 0.2644 | 1.0596 | 0 | 0 | 0.1859 | 267.73 | +1.6 |
| 0.3505 | 1.0135 | 0 | 0 | 0.1966 | 455.31 | +1.8 |
| 0.3510 | 1.4808 | 4.18 | 8.99 | 0.2343 | 466.27 | −0.1 |
| 0.3623 | 0.7357 | 2.75 | 10.64 | 0.1264 | 532.80 | +0.7 |
| 0.3634 | 0.7882 | 2.48 | 10.67 | 0.1236 | 580.25 | +0.2 |
| 0.4661 | 2.1269 | 6.18 | 11.78 | 0.3305 | 642.59 | +0.3 |
| 0.5176 | 2.1891 | 6.38 | 13.05 | 0.3510 | 764.38 | −0.6 |
| 0.6726 | 2.8313 | 8.84 | 16.81 | 0.4661 | 1006.93 | −2.5 |
| 0.7976 | 1.6727 | 0 | 0 | 0.2768 | 1650.93 | −2.0 |
| 0.7976 | 3.0615 | 0 | 0 | 0.5176 | 1326.93 | −4.4 |
| 0.8022 | 3.3284 | 8.45 | 19.90 | 0.5149 | 1469.12 | −2.9 |
| 0.8271 | 1.4348 | 0 | 0 | 0.3935 | 1107.16 | +3.1 |
| 0.9887 | 2.2688 | 0 | 0 | 0.3623 | 2271.39 | +4.6 |
| 1.0591 | 2.3252 | 0 | 0 | 0.3634 | 2188.50 | −2.5 |
| | | | B. Methylurea | | | |
| 0.0845 | 0.3594 | 0.89 | 2.19 | 0.0540 | 22.32 | +3.3 |
| 0.1768 | 0.2828 | 1.26 | 5.24 | 0.0500 | 65.54 | −1.3 |
| 0.2074 | 0.3482 | 1.43 | 6.03 | 0.0595 | 93.63 | +1.1 |
| 0.2622 | 0.4625 | 1.84 | 7.72 | 0.0790 | 148.88 | +3.1 |
| 0.3490 | 0.4767 | 0 | 0 | 0.0846 | 202.21 | −0.6 |
| 0.4188 | 1.5672 | 0 | 0 | 0.2622 | 321.03 | −2.3 |
| 0.5033 | 0.4774 | 6.82 | 14.56 | 0.1596 | 221.18 | −0.2 |
| 0.6250 | 2.5812 | 1.30 | 1.30 | 0.5033 | 319.68 | +1.8 |
| 0.6292 | 1.0400 | 0 | 0 | 0.1768 | 586.43 | −1.1 |
| 0.6775 | 1.2181 | 4.68 | 19.35 | 0.2074 | 679.75 | +0.3 |
| 0.8541 | 3.5129 | 8.37 | 20.89 | 0.5316 | 1008.99 | +3.6 |
| 1.3140 | 5.3036 | 14.09 | 31.13 | 0.8541 | 1547.15 | +0.1 |
| 2.0518 | 8.0709 | 0.69 | 1.51 | 1.3140 | 2486.35 | +0.0 |
| 2.0671 | 4.0161 | 0 | 0 | 0.6775 | 2983.21 | −1.0 |
| | | | C. 1,1-Dimethylurea | | | |
| 0.0509 | 0.0652 | 0.26 | 1.13 | 0.0119 | 4.26 | +1.9 |
| 0.0836 | 0.1963 | 0.44 | 1.85 | 0.0300 | 17.15 | +1.8 |
| 0.0947 | 0.4062 | 1.02 | 2.44 | 0.0611 | 21.00 | −0.9 |
| 0.0992 | 0.4212 | 1.11 | 2.56 | 0.0647 | 22.94 | +1.9 |
| 0.1305 | 0.5501 | 1.23 | 2.96 | 0.0836 | 40.25 | +3.4 |
| 0.1493 | 0.6409 | 1.74 | 3.83 | 0.0992 | 48.83 | +3.3 |
| 0.1917 | 0.8001 | 2.17 | 4.32 | 0.1305 | 68.12 | −1.0 |
| 0.2185 | 0.2794 | 0 | 0 | 0.0509 | 68.52 | −0.6 |
| 0.2373 | 0.9691 | 0 | 0 | 0.1493 | 117.04 | +0.4 |
| 0.2671 | 0.6294 | 0 | 0 | 0.0947 | 149.02 | −1.2 |
| 0.2916 | 0.6599 | 0 | 0 | 0.1428 | 130.32 | +0.1 |
| 0.2916 | 1.0840 | 0 | 0 | 0.1917 | 142.58 | +2.6 |
| | | | D. 1,3-Dimethylurea | | | |
| 0.1071 | 0.2223 | 0.74 | 3.18 | 0.0353 | 9.01 | +1.0 |
| 0.1937 | 0.2927 | 1.30 | 5.71 | 0.0506 | 22.58 | −0.3 |
| 0.3076 | 0.7059 | 2.05 | 8.99 | 0.1071 | 73.52 | +2.6 |
| 0.4339 | 0.6110 | 3.12 | 12.53 | 0.1149 | 96.64 | +1.7 |
| 0.5911 | 1.1580 | 4.10 | 16.86 | 0.1937 | 210.74 | +1.8 |
| 0.8983 | 2.0040 | 0 | 0 | 0.3076 | 462.81 | −1.9 |
| 1.0745 | 3.4740 | 2.77 | 7.32 | 0.5915 | 565.92 | −0.7 |
| 1.0817 | 2.1348 | 0 | 0 | 0.3601 | 564.96 | −1.0 |
| 1.1343 | 4.5241 | 22.03 | 26.81 | 0.8743 | 363.0 | +6.4 |
| 1.2407 | 2.5517 | 0 | 0 | 0.4339 | 720.64 | +1.4 |
| 1.8122 | 6.6975 | 0.59 | 1.31 | 1.1343 | 1125.29 | −0.8 |
| 1.8266 | 3.7739 | 0 | 0 | 0.5911 | 1347.42 | +0.4 |
| | | | E. Trimethylurea | | | |
| 0.2556 | 0.5928 | 1.34 | 5.56 | 0.0924 | 24.06 | +3.2 |
| 0.2656 | 1.0429 | 0 | 0 | 0.1984 | 17.97 | +2.6 |
| 0.3985 | 1.6518 | 3.73 | 8.77 | 0.2556 | 54.31 | −0.2 |
| 0.5233 | 2.1402 | 4.93 | 11.38 | 0.3364 | 89.25 | −0.7 |
| 0.5602 | 1.0311 | 3.13 | 11.83 | 0.1855 | 89.39 | +0.6 |
| 0.6142 | 2.1816 | 0 | 0 | 0.3985 | 104.15 | −0.6 |
| 0.6625 | 2.4359 | 7.49 | 14.21 | 0.4396 | 115.13 | −2.5 |
| 0.8271 | 3.2877 | 7.46 | 17.47 | 0.5233 | 209.13 | −0.6 |
| 0.8545 | 3.0900 | 9.09 | 17.55 | 0.5602 | 190.20 | +0.3 |
| 0.9451 | 3.7306 | 8.61 | 19.74 | 0.6011 | 262.07 | −0.4 |
| 1.0139 | 3.5017 | 0 | 0 | 0.6625 | 247.49 | −1.1 |
| 1.0437 | 4.0616 | 20.66 | 21.60 | 0.8271 | 165.25 | −2.9 |
| 1.3410 | 4.4353 | 0 | 0 | 0.8545 | 413.76 | +1.8 |
| 1.4902 | 4.9089 | 0 | 0 | 0.9451 | 482.52 | −0.6 |
| 1.6599 | 5.3944 | 0 | 0 | 1.0437 | 574.84 | −0.4 |
| | | | F. Tetramethylurea | | | |
| 0.4114 | 0.7711 | 2.05 | 8.77 | 0.1267 | 3.71 | +1.3 |
| 0.6098 | 2.4312 | 6.67 | 13.04 | 0.4114 | 8.07 | +3.8 |
| 0.6843 | 2.9017 | 7.33 | 14.52 | 0.4689 | 9.86 | −1.3 |
| 0.7238 | 0.9505 | 3.49 | 14.91 | 0.1728 | 8.51 | −0.4 |
| 0.8658 | 3.3949 | 9.10 | 18.02 | 0.5785 | 16.00 | +4.4 |
| 1.0199 | 4.1785 | 10.24 | 20.88 | 0.6843 | 20.69 | −4.5 |
| 1.3169 | 4.9041 | 13.17 | 26.16 | 0.8636 | 32.68 | −1.7 |
| 1.5330 | 5.9475 | 15.04 | 29.80 | 1.0199 | 45.46 | +2.1 |
| 2.1806 | 3.2450 | 0 | 0 | 0.6098 | 73.69 | −0.4 |
| 2.1806 | 5.6768 | 0 | 0 | 1.3210 | 68.21 | +1.3 |
| 2.5516 | 3.7825 | 0 | 0 | 0.7238 | 96.57 | −0.1 |
| 2.5516 | 7.4751 | 0 | 0 | 1.5330 | 100.75 | +0.1 |
| | | | G. Ethylurea | | | |
| 0.0958 | 0.1477 | 0.50 | 2.12 | 0.0257 | 19.06 | −2.9 |
| 0.1215 | 0.2898 | 0.70 | 2.68 | 0.0470 | 40.13 | +1.4 |
| 0.1657 | 0.3151 | 0.96 | 3.65 | 0.0558 | 61.80 | +1.4 |
| 0.1896 | 0.7976 | 1.78 | 4.27 | 0.1215 | 85.89 | −3.2 |
| 0.2277 | 0.8740 | 2.64 | 5.11 | 0.1523 | 100.03 | −1.5 |
| 0.2490 | 0.9465 | 2.87 | 5.58 | 0.1657 | 120.75 | +2.0 |
| 0.2896 | 1.0642 | 0 | 0 | 0.1896 | 157.12 | +1.3 |
| 0.3318 | 1.2554 | 3.77 | 7.38 | 0.2199 | 188.29 | −1.6 |
| 0.4109 | 1.7039 | 4.64 | 9.08 | 0.2806 | 273.85 | −0.2 |
| 0.5003 | 2.0518 | 5.67 | 10.97 | 0.3418 | 372.93 | +2.3 |
| 0.5701 | 2.3420 | 6.49 | 12.43 | 0.3909 | 431.05 | −1.5 |
| 0.7756 | 2.7210 | 0 | 0 | 0.5003 | 686.27 | +1.7 |
| 0.7756 | 1.3874 | 0 | 0 | 0.2490 | 768.08 | +0.1 |
| 0.8351 | 3.3444 | 9.41 | 17.81 | 0.5701 | 795.34 | +1.0 |
| 1.3000 | 4.3478 | 0 | 0 | 0.8351 | 1275.70 | +1.2 |
| 1.3000 | 2.2682 | 0 | 0 | 0.4109 | 1527.98 | −1.1 |

[a] Units: $m_{A,i}$ and $m_f$, mol kg$^{-1}$; $m_{B,i}$, mmol kg$^{-1}$; $n_A$, mmol; $n_B$, μmol; $\Delta H$, mJ. [b] $\Delta$, % = 100[$\Delta H$(exptl) − $\Delta H$(calcd)]/$\Delta H$(exptl), where $\Delta H$(calcd) is calculated from eq 3.

enthalpic interaction coefficients are related to the cluster integrals in the McMillan–Mayer theory and to the McMillan–Mayer coefficients (see ref 6). Combination of eq 1 and 2 yields

$$\Delta H/n_A = \sum_{n>1} B_n^h[(m_f^{n-1} - m_{A,i}^{n-1}) + n_A^{-1} n_B (m_f^{n-1} - m_{B,i}^{n-1})] \quad (3)$$

We have calculated the enthalpic interaction coefficients by a least-squares analysis of the results of Table II in terms of eq 3. Only those coefficients were adopted for which the Student's $t$ test indicated a probability of more than 95% that their value was not zero. Resulting values and their standard deviation are collected in Table III.

From eq 3 it follows that

$$\Delta_{dil}H(m_{A,i} \rightarrow m_f) = \Delta H/n_A - n_A^{-1} n_B \sum_{n>1} B_n^h (m_f^{n-1} - m_{B,i}^{n-1}) \quad (4)$$

where $\Delta_{dil}H(m_{A,i} \rightarrow m_f)$ is the molar enthalpy change on diluting a solution from initial molality $m_{A,i}$ to final molality $m_f$. Since

$$\Delta_{dil}H(m_i \rightarrow m_f)/(m_f - m_i) = B_2^h + B_3^h(m_f + m_i) + B_4^h(m_f^2 + m_i^2 + m_f m_i) + \ldots \quad (5)$$

(25) H. L. Friedman, *J. Chem. Phys.*, **32**, 1351 (1960).

**Table III.** Enthalpic Interaction Coefficients of Substituted Urea Compounds in DMF[a]

| compound | $B_2^h$ | $B_3^h$ | $B_4^h$ | $B_5^h$ |
|---|---|---|---|---|
| U | −5552 (135)[b] | 7345 (540)[b] | −6845 (772)[b] | 2765 (361)[b] |
| MeU | −2200 (34) | 1612 (79) | −760 (64) | 146 (16) |
| 1,1-$Me_2$U | −1711 (23) | 888 (65) | | |
| 1,3-$Me_2$U | −595 (10) | 198 (12) | −36 (4) | |
| $Me_3$U | −252 (2) | 29 (1) | | |
| $Me_4$U | −17.3 (0.2) | 1.0 (0.1) | | |
| EtU | −2108 (42) | 1694 (142) | −1000 (180) | 263 (72) |

[a] The unit for $B_n^h$ = J $kg^{n-1}/mol^n$. [b] The numbers in parentheses are the standard deviations of the coefficients.

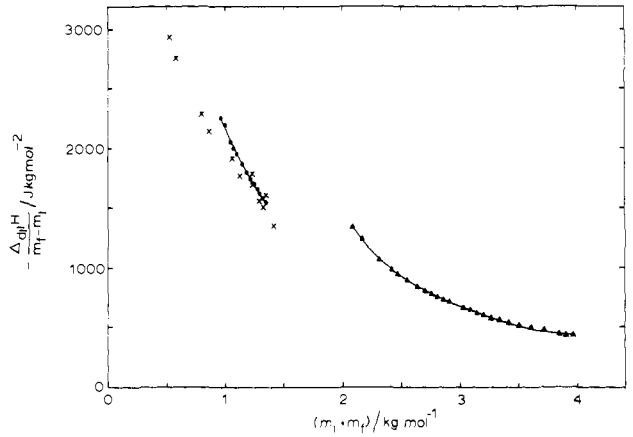


**Figure 2.** Enthalpy of dilution in relation to $(m_i + m_f)$ for urea in DMF at 25 °C: ▲, ●, data from both series of ref 26; ×, data from this paper.

and $B_4^h$ is often small as compared with $B_2^h$, we give in Figure 1 a graphical representation of the experimental results as $\Delta_{dil}H/(m_f - m_i)$ in relation to $(m_f + m_i)$, where $\Delta_{dil}H$ is calculated according to eq 4.

Enthalpies of dilution for urea in DMF have been measured earlier by Hamilton and Stokes.[26] They have performed two series of measurements. Our results can only be compared with their series at low concentration. Figure 2 shows that the agreement is reasonable. Only at very low concentrations, where our data cover a larger range, is there some deviation between their and our data.

## Discussion

**Urea Interactions in DMF.** When we compare the enthalpic interaction coefficients for urea in DMF, given in Table III, with earlier results for several types of amides in the same solvent, striking differences can be noticed. For the amides we have found values for the enthalpic pair interaction coefficient, $B_2^h$, in the range from ca. 0 J kg $mol^{-2}$ for small solute molecules as *N,N*-dimethylacetamide down to −633 J kg $mol^{-2}$ for the largest molecule measured so far, *N,N*-dipentylacetamide. In addition, the magnitude of the enthalpic triplet interaction coefficients, $B_3^h$, was generally found to be small and positive (from 0 to +105 J $kg^2$ $mol^{-3}$).[6,7] For urea in DMF the value of $B_2^h$ is much more negative than that of other molecules, while the contribution of $B_3^h$ is also substantial. Even higher enthalpic interaction coefficients (up to $B_5^h$) are indispensable in order to describe the experimental results on urea satisfactorily. This points to exceptionally strong interaction between urea molecules dissolved in DMF, in sharp contrast to the behavior of urea molecules in water, where Hamilton and Stokes[26] have found $B_2^h$ = −348 J kg $mol^{-2}$, $B_3^h$ = +10 J $kg^2$ $mol^{-3}$, and $B_4^h$ = −0.2 J $kg^3$ $mol^{-4}$. Table III demonstrates clearly that these extraordinarily large values of the interaction coefficients change gradually to normal ones when an increasing number of methyl groups is introduced in the solute molecules. Before discussing it in detail, we also would like to mention the substantial difference in enthalpic interaction coefficients for the two isomers 1,1- and 1,3-dimethylurea.

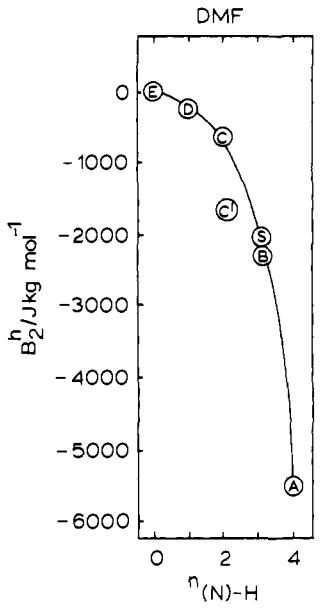


**Figure 3.** $B_2^h$ in relation with the number of (N)H groups for alkyl-substituted ureas in DMF: A, urea; B, MeU; C, 1,3-$Me_2$U; C′, 1,1-$Me_2$U; D, $Me_3$U; E, $Me_4$U; S, EtU.

The relation between our results for $B_2^h$ and the number of (N)H groups in the solute molecules is given in Figure 3. The shape of the curve can be reconciled with the ideas developed in our earlier papers.[6,7] From our study on nonsubstituted and mono-N-substituted amides, we have found that the influence of N-bonded H atoms on $B_2^h$ in DMF as solvent is considerable and leads to a decrease in $B_2^h$. The decrease is much stronger for unsubstituted amides ($NH_2$) than for mono-*N*-alkylamides (NH).[6] The very negative value for $B_2^h$ of urea (two $NH_2$ groups) in DMF fits in this picture. Hamilton and Stokes,[26] who did an extensive study on the interaction of urea molecules in different solvents, interpreted the very strong interaction in DMF as due to direct solute–solute association by hydrogen bonding. However, when the interaction between urea molecules in DMF is dominated by solute–solute hydrogen bonding, one should expect only a minor difference between the $B_2^h$ values of urea and methylurea. Experimentally a very large difference is found. Moreover it is hard to understand why the hydrogen bonding between an urea NH group and a CO group on another urea molecule should occur preferably over that with a CO group of one of the abundant solvent (DMF) molecules. For the unsubstituted and mono-N-substituted amides in DMF, we have indicated the possibility of the formation of hydrogen-bonded solute–solvent associates, where a solvent molecule is bonded to each (N)H.[6] Such an association results in extra $CH_2$ and amide groups in the interacting entity. When this type of association occurs for ureas too, it means that the replacement of a (N)$CH_3$ group by a (N)H group in the interacting entity results in a gain of one CON, one CH, and one $CH_3$ group. As was shown in previous papers[6,7] these groups give negative shifts in $B_2^h$. The increment becomes more negative when more groups are introduced. The curve in Figure 3 is in accordance with this view.

The high value for $B_3^h$ of urea in DMF may be approached along the same line. Ben-Naim has demonstrated[27] that a straightforward interpretation of interaction coefficients for more than two solute molecules is difficult because of the complexity of the potentials of average force and their complicated relation to the interaction coefficients. Moreover, higher interaction coefficients contain contributions from lower clusters.[6,27] These limitations should be kept in mind when interpreting results on $B_3^h$. Kozak et al.[28] have shown that triplet interaction coefficients can be

(26) D. Hamilton and R. H. Stokes, *J. Solution Chem.*, **1**, 223 (1972).

(27) A. Ben-Naim, *J. Chem. Phys.*, **54**, 3696 (1971).

(28) J. J. Kozak, W. S. Knight, and W. Kauzmann, *J. Chem. Phys.*, **48**, 675 (1968).

**Table IV.** Some Thermodynamical Properties for Substituted Ureas

| | $B_2^h$/J kg mol$^{-2}$ | $C_p^{o,E}$ (DMF) | $C_p^{o,E}$ ($H_2O$) | $B_{x,DMF}^h$/J kg mol$^{-2}$ |
|---|---|---|---|---|
| U | −5552[a] | 17.6[b] | −5.8[b] | −156[c] |
| MeU | −2200 | | | 104 |
| 1,1-$Me_2$U | −1711 | 6.1 | 19.2 | 283 |
| 1,3-$Me_2$U | −595 | 3.0 | 25.6 | 377 |
| $Me_4$U | −17.3 | 0.0 | 32.0 | 1187 |
| EtU | −2108 | | | 340 |

[a] This paper. [b] Reference 8. [c] Reference 34.

related to the excess effect of the formation of triplets in the system over that of the pair formation. In this sense the relatively high values of $B_3^h$ for organic solutes with large apolar parts in water and the fact that they have the same sign as $B_2^h$ have been correlated with the cooperativity of the hydrophobic interaction.[29] For urea in DMF $B_3^h$ is also relatively large, but its sign is opposite to that of $B_2^h$. This seems to indicate that the enthalpic effect due to the formation of triple clusters of solute molecules in the system is less exothermic than that due to the formation of the corresponding pairs. If so, this may be caused by steric hindrance. For a solvated urea molecule this hindrance can be relatively large, leading to increased $B_3^h$ values.

Strong (N)H–DMF association has been coined by Bonner et al.[8] on basis of heat capacities of urea compounds in DMF. In Table IV we give their values in the form of excess heat capacities relative to the intrinsic heat capacities ($C_p^{0,E}$) together with our $B_2^h$ values. The order in both sets of data is the same.

For 1,1- and 1,3-dimethylurea the marked difference between the $B_2^h$ values in DMF is also reflected in the results of Bonner et al. In water a large set of enthalpic interaction coefficients for substituted ureas has been measured by Barone et al.[30-32] They find equal values for the enthalpic pair interaction coefficients of 1,1- and 1,3-$Me_2$U, but obtained a substantial difference between the $B_2^h$ values for the isomeric diethylureas. For the two methyl isomers the triplet interaction coefficients only differ distinctly. These results indicate that simple additivity models based on random interaction of groups, which are often used to describe enthalpic interaction coefficients,[33] must be applied with great care and may give considerably deviating results. In a previous paper,[7] we have suggested that for some types of compounds (like nonsubstituted amides) the interaction coefficients are probably influenced by preferential orientations of the interacting particles. The results for the dimethylureas can be approached in a similar way. Figure 3 shows that in DMF 1,1-dimethylurea is the "deviating compound". Preferential orientations are more likely for 1,1-$Me_2$U (one $NH_2$ and one $N(CH_3)_2$ side) than for 1,3-$Me_2$U (two $HNCH_3$ sides). They may account also for the relatively small difference in $B_2^h$ between 1,1-$Me_2$U and MeU.

As DMF contains some of the main elements of the inner part of a globular protein in its native, folded state, viz. amide and hydrophobic groups, it has been considered as an appropriate model for the interior of these proteins.[8,17] Moreover, some peculiarities on contact frequencies of hydrophobic side chains in the protein interior seem to be reflected in the enthalpic interaction parameters in DMF.[17] Thus, the results on urea in DMF may imply substantial interactions of urea in the interior of a globular protein also. Bonner's and our results indicate that such interactions involve urea–urea interactions as well as interactions between urea and the CONH groups of the protein. However, for considerations about the influence of urea on the protein structure, it is not sufficient to consider the situation in a protein-like medium only. The constituent groups of a protein in its denaturated state are in an aqueous environment. Hence it is necessary to pay attention to the interaction between urea compounds and CONH groups in water also.

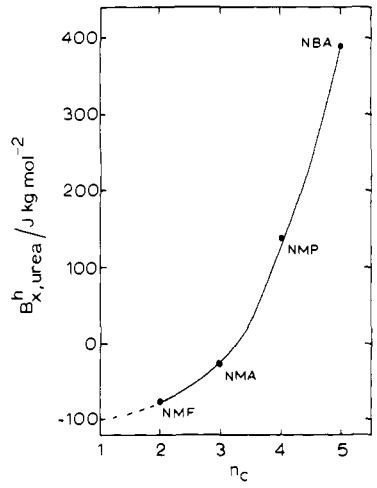


**Figure 4.** The enthalpic pair interaction coefficients between urea and *N*-alkylamides in water: NMF = *N*-methylformamide; NMA = *N*-methylacetamide; NMP = *N*-methylpropionamide; NBA = *N*-butylacetamide.

**Urea Interactions in Water.** Enthalpic pair interaction coefficients between a DMF molecule and different alkyl-substituted urea molecules in water, $B_{x,DMF}^h$, have been determined by Rouw.[34] They are given in Table IV also. Here too the difference between 1,1-$Me_2$U and 1,3-$Me_2$U is obvious, though less pronounced than in DMF. Generally the values of $B_{x,DMF}^h$ show an increase on introduction of methylene groups, undoubtedly owing to the increasing influence of hydrophobic interactions.[33,34] The values of $B_{x,DMF}^h$ for x = U is comparatively small. It follows that in water the interaction enthalpy between urea and the peptide group is much smaller than in the solvent DMF. A comparable result can be extracted from the measurements of Savage and Wood[33] on the interaction between urea and several *N*-alkylamides in water. In Figure 4 we have plotted their enthalpic pair interaction coefficients, $B_{x,urea}^h$ as a function of the number of C atoms in the amide molecule. Extrapolation to $n_c = 1$ gives $B_{x,urea}^h$ for the interaction between urea and $HCONH_2$. Also this value is relatively small ($\simeq -100$ J kg mol$^{-2}$). From results on related compounds by Okamoto, Wood, and Thompson,[35] it may be inferred that the interaction between urea and CONH groups involves a decrease in entropy due to less freedom of motion. In combination with our results this points to a small or positive Gibbs energy change and the existence of a weak urea–amide interaction only.

In the paper mentioned before,[8] Bonner presents excess partial molar heat capacities of his compounds in water too. They are given in Table IV and show a parallel trend with the values of $B_{x,DMF}^h$ from Rouw. The increase in the excess heat capacities as a result of the subsequent introduction of methyl (alkyl) groups is related by Bonner to an enhanced influence of hydrophobic interactions.

**Denaturation by Urea Compounds.** On the basis of their results both in DMF and in water, Bonner et al. conclude that there are two possible mechanisms for the denaturation of proteins by urea compounds: one by direct binding of the urea compound to the peptide CO group, in which case urea is a better denaturant than alkyl substituted ureas; the other by hydrophobic interactions between the alkyl group(s) of the urea compound and those of the protein so that substituted ureas will be the more effective denaturants. In view of this, Feinstein suggests[16] that the order of denaturating activity by substituted ureas gives information about the role of interfering groups (either hydrophobic or hy-

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drophilic) in the interior of a globular protein.

For a proper study on denaturation the influence of denaturant molecules on both the native and the denaturated state has to be considered. When DMF is a reasonable model for the interior of a protein in the native state, we may conclude from the arguments given in this paper that, disregarding steric influences, strong urea–CONH interactions may occur in the inside of a native protein, whereas in the denaturated state (aqueous environment) this interaction is of much less importance. Unfortunately no data are available on the Gibbs energy of transfer of urea from water to an amidic solvent. However the change in enthalpy of this process is clearly negative.[36] This seems to indicate that direct urea–CONH interaction would stabilize the native structure rather than the denaturated state. Therefore, it may be concluded that the disruption of the water structure by urea, leading to a reduced hydrophobic interaction,[5,37] is the dominating process in denaturation. With respect to alkyl-substituted urea compounds, it is clear that they will stabilize the denaturated state by hydrophobic interactions and show small stabilizing influences in the native state. On the other hand, they do not have the same influence on the water structure as urea. These counteracting influences of alkyl-substituted urea compounds may be the cause of the contradictory conclusions in reports on the denaturating effectiveness of these compounds. Whether alkyl substitution in urea leads to a more effective denaturating agent will be highly protein dependent, and conclusions about the hydrophobicity or hydrophilicity of proteins on basis of the relative denaturation effectiveness of (substituted) urea compounds must be taken with great care.

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## Conclusions

The enthalpy of interaction between urea molecules in DMF is exceptionally large. The pairwise and higher enthalpic interaction coefficients largely exceed any value measured before. These anomalies disappear gradually upon subsequent introduction of methyl groups in the solute molecules. Strong solute–solvent association by hydrogen bonding can account for these features. In water the enthalpies of interaction are smaller. Considering DMF as a model for the native state of a globular protein and recalling that in the denaturated state the groups of a protein are in an aqueous environment, it can be concluded that the denaturation of proteins by urea is not caused by stabilization of the denaturated state by urea–peptide binding as is often suggested. Since alkyl-substituted urea compounds have counteracting effects on the denaturation of globular proteins, conclusions on the hydrophobicity of a protein on basis of the denaturating activities of a series of substituted ureas as suggested by Feinstein[16] are cumbersome.

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**Registry No.** U, 57-13-6; MeU, 598-50-5; 1,1-$Me_2$U, 598-94-7; 1,3-$Me_2$U, 96-31-1; $Me_3$U, 632-14-4; $Me_4$U, 632-22-4; EtU, 625-52-5; DMF, 68-12-2; NMF, 123-39-7; NMA, 79-16-3; NMP, 1187-58-2; NBA, 1119-49-9.

# Stable Composite Polyelectrolyte Electrode Coatings with Morphologies That Yield Large Ion-Exchange Capacities and High Cross-Coating Charge Propagation Rates

**Donald D. Montgomery and Fred C. Anson***

*Contribution from the Arthur Amos Noyes Laboratories, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125.*
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**Abstract:** A new material for preparing polyelectrolyte coatings on electrode surfaces is described. A random ternary copolymer containing two types of hydrophilic cationic groups and hydrophobic styrene groups was mixed with a variety of conventional polycationic electrolytes to obtain coatings with exceptional properties. These include large ion-exchange capacities, remarkably high effective diffusion coefficients of incorporated counterions, and prolonged retention of multiply charged counterions. Electron microscopy revealed that the coatings spontaneously segregate into discrete hydrophilic and hydrophobic domains. The properties of these new composite coatings are especially attractive for applications in electrocatalysis.

Adsorbed polyelectrolytes are attractive as a simple means for endowing electrode surfaces with high affinities for ionic reactants that can be incorporated into the polyelectrolyte coatings[1-3] by ion exchange. Although electrodes coated with polyelectrolytes loaded with redox reactants have been exploited in a variety of applications,[3-9] the number of useful polyelectrolyte systems that are presently available is limited because all common polyelectrolytes lack one or more of the essential properties required for

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