# Cooperativity of Solvent–Macromolecule Interactions in Aqueous Solutions of Polyethylene Glycol and Polyethylene Glycol–Urea[1]

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**Abstract:** Ultrasonic attenuation measurements have been carried out in aqueous polyethylene glycol and aqueous polyethylene glycol–urea solutions in order to investigate the cooperativity of polymer–solvent interactions. A single relaxation time is observed in aqueous polyethylene glycol solutions which can be attributed to solvent–polymer interactions involving hydrogen bonding of water to polymer, hydrogen-bonded solvent clusters around the hydrocarbon portion of the polymer, and hydrophobic interactions within the polymer. The relaxation time increases sharply with increasing molecular weight until a molecular weight of about 3400 is reached; in the molecular weight range 3400–20,000 the relaxation time remains essentially constant. This indicates an effective maximum in the size of the cooperative polymer–solvent unit occurs with a polymer of approximately molecular weight 3400. Addition of urea to the aqueous polymer solution causes a sharp decrease in the relaxation time at a urea concentration of around 2.5 *m* for polymers of molecular weight 3400–20,000. The sharpness of the change in relaxation time indicates a cooperative transition is occurring in the polymer–solvent structure. When urea is added to solutions of polymers with molecular weights below 3400, no decrease in the relaxation time occurs. Thus a minimum molecular size of the solvent–solute unit is necessary for a cooperative transition in the relaxation time to occur, namely a polymer of approximately molecular weight 3400 and its associated solvent molecules. The requirement of a minimum molecular size in order to obtain cooperative solvent–solute intreactions may be of relevance in understanding the mode of action of biological macromolecules.

Cooperative processes are frequently encountered with biological macromolecules. Protein denaturation is a particularly well-studied example of such a process[2] in which macromolecules are transformed from their ordered, native state to a disordered random-coil-like state over a narrow range in an intensive variable such as temperature or urea–guanidine concentration. The mechanism by which denaturing agents operate is not precisely known. In solution, the configuration of macromolecules is almost certainly influenced by hydrophobic interactions[3,4] which arise from the desire of nonpolar regions in the molecule to minimize their interaction with polar solvents. Denaturants may alter solvent structure so as to weaken intramolecular hydrophobic interactions. In support of this hypothesis Nozaki and Tanford[5] and Wetlaufer, *et al.*,[6] have found an increase in the solubility of amino acids and hydrocarbons in aqueous solution on increasing the amount of added urea. The increase in solubility can be attributed to changes in solvent structure. Direct experimental evidence for a significant change in water structure, probably the disruption of hydrogen-bonded aggregates, on addition of urea or guanidinium chloride has been obtained from ultrasonic attenuation measurements.[7,8] Specific binding of denaturants such as urea to macromolecules is another possible mechanism for the triggering of protein denaturation.[9] Both mechanisms are probably of importance in denaturation processes.

The simple linear polymer, polyethylene glycol, has been used as a protein model since it is highly soluble in water and possesses some basic features of proteins, namely a hydrophobic region ($CH_2$–$CH_2$) and a single hydrogen-bonding site (O) per monomer unit. Previous work[10] has shown that a single ultrasonic relaxation time is observed in aqueous polyethylene glycol solutions which can be attributed to a polymer–solvent interaction involving hydrogen bonding of water to the polymer oxygen atoms mediated by hydrophobic interactions within the polymer and by water clusters near the hydrophobic groups. The relaxation time decreases sharply[7,8] at urea and guanidine concentrations in the region of 2.5 *m*, suggesting a highly cooperative process occurs which is analogous to that found in protein denaturation. A breakdown of the local water structure and subsequent weakening of the intramolecular hydrophobic interactions is involved. In addition, the relaxation time was found to be independent of polymer chain length at molecular weights greater than about 7000. This has been ascribed to the size of the cooperative solvent–polymer structure reaching a maximum at a molecular weight of approximately 7000.

The results presented here permit an estimate of the minimum molecular size required for a cooperative transition to occur in the polymer–solvent structure. This minimum size was estimated by determining the effect of urea on the relaxation time for different molecular weight polymers. A sharp decrease in the relaxation time occurred as the urea concentration was raised for polymer molecular weights of 3400 and above, while the relaxation time is essentially inde-

(1) This work was supported by a grant from the National Institutes of Health (GM 13292).
(2) C. Tanford, *Advan. Protein Chem.*, In press.
(3) W. Kauzman, *ibid.*, **14**, 1 (1959).
(4) G. Némethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3401 (1962).
(5) Y. Nozaki and C. Tanford, *J. Biol. Chem.*, **238**, 4074 (1963).
(6) D. B. Wetlaufer, S. K. Malik, L. Stoller, and R. L. Coffin, *J. Am. Chem. Soc.*, **86**, 508 (1964).
(7) G. G. Hammes and P. R. Schimmel, *ibid.* **89**, 442 (1967).
(8) G. G. Hammes and J. C. Swann, *Biochemistry*, **6**, 1591 (1967).
(9) J. A. Gordon and W. P. Jenks, *ibid.*, **2**, 47 (1963).
(10) G. G. Hammes and T. B. Lewis, *J. Phys. Chem.*, 70, 1610 (1966).

**Table I.** Ultrasonic Parameters and Velocities for Polyethylene Glycols in Aqueous Urea Solutions at 10°

| Mol wt | Monomer mole fraction | [Monomer], $m$ | [Urea], $m$ | $10^9\tau$, sec | $10^8A/m$, sec $cm^{-1}$ $mol^{-1}$ | $10^{17}B$, $cm^{-1}$ $sec^2$ | $10^{-5}v$, cm $sec^{-1}$ |
|---|---|---|---|---|---|---|---|
| 1,000 | 0.065 | 3.9 | 0 | 3.0 | 1.1 | 49.5 | 1.56 |
| | | 4.4 | 8 | 3.1 | 1.1 | 45.8 | 1.67 |
| 1,450 | 0.065 | 3.9 | 0 | 3.9 | 1.2 | 51.3 | 1.56 |
| | | 4.4 | 8 | 4.2 | 1.0 | 47.0 | 1.66 |
| | 0.035 | 2.0 | 0 | 3.8 | 1.3 | 42.8 | 1.52 |
| | | 2.3 | 8 | 3.8 | 1.1 | 37.0 | 1.64 |
| 2,000 | 0.065 | 3.9 | 0 | 4.2 | 1.2 | 50.4 | 1.56 |
| | | 4.4 | 8 | 4.2 | 1.0 | 46.8 | 1.66 |
| | 0.035 | 2.0 | 0 | 4.4 | 1.5 | 42.8 | 1.47 |
| | | 2.1 | 2 | 4.2 | 1.2 | 39.7 | 1.57 |
| | | 2.2 | 4 | 4.3 | 1.0 | 38.3 | 1.60 |
| | | 2.3 | 8 | 4.5 | 1.0 | 38.0 | 1.65 |
| | | 2.4 | 10 | 4.2 | 1.2 | 37.6 | 1.67 |
| 3,400 | 0.035 | 2.0 | 0 | 6.1 | 1.1 | 43.2 | 1.49 |
| | | 2.1 | 2 | 6.3 | 1.2 | 39.5 | 1.58 |
| | | 2.2 | 3 | 5.3 | 1.3 | 39.0 | 1.60 |
| | | 2.2 | 4 | 5.0 | 1.4 | 38.3 | 1.59 |
| | | 2.3 | 8 | 5.1 | 1.1 | 38.0 | 1.66 |
| | | 2.4 | 10 | 5.2 | 1.3 | 37.7 | 1.67 |
| 4,500 | 0.035 | 2.0 | 0 | 6.8 | 1.0 | 45.2 | 1.51 |
| | | 2.1 | 2 | 6.2 | 1.3 | 41.7 | 1.55 |
| | | 2.1 | 2.5 | 5.8 | 1.2 | 39.5 | 1.60 |
| | | 2.2 | 3 | 5.0 | 1.0 | 39.2 | 1.60 |
| | | 2.2 | 3.5 | 4.7 | 1.1 | 38.8 | 1.62 |
| | | 2.2 | 4 | 4.1 | 1.5 | 38.8 | 1.60 |
| | | 2.2 | 6 | 4.4 | 1.2 | 38.4 | 1.63 |
| | | 2.3 | 8 | 4.0 | 1.1 | 38.0 | 1.66 |
| 7,500 | 0.035 | 2.0 | 0 | 6.1 | 1.3 | 42.8 | 1.51 |
| | | 3.2 | 8 | 4.0 | 1.3 | 37.6 | 1.65 |
| 20,000 | 0.035 | 2.0 | 0 | 6.4 | 1.3 | 41.8 | 1.54 |
| | | 2.3 | 8 | 4.0 | 1.6 | 35.7 | 1.66 |

**Table II.** Intrinsic Viscosities of Polyethylene Glycol in Water and 8 $m$ Urea at 10°

| | Molecular weight | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1000 | 1450 | 2000 | 3400 | 4500 | 7500 | 20,000[7] |
| $[\eta]_{H_2O}$, dl/g | $0.062_5$ | $0.072_3$ | $0.091_6$ | $0.12_5$ | $0.14_4$ | $0.19_3$ | 0.39 |
| $[\eta]_U$, dl/g | $0.064_5$ | $0.076_5$ | $0.095_7$ | $0.13_3$ | $0.15_7$ | $0.21_0$ | 0.45 |

pendent of urea concentration for molecular weights smaller than 3400. Thus the minimum polymer molecular weight for a cooperative structural change in the solvent–polymer system is about 3400. A more comprehensive investigation of the molecular weight dependence of the relaxation time indicates the maximum size of the cooperative unit is also about 3400 rather than 7000 as previously suggested.

## Experimental Section

Polyethylene glycol samples with sharp molecular weight distributions were generously donated by Dow Chemical Co., Midland, Mich. Aqueous urea solutions (Baker, reagent grade) were always prepared just prior to use.

The apparatus and procedures for the measurement of the ultrasonic attentuation and velocity and the intrinsic viscosity of the polymer have been described previously.[7,8,10] All experiments were performed at 10° and $\alpha/f^2$ (where $\alpha$ is the pressure amplitude attenuation coefficient and $f$ is the frequency of the ultrasonic wave) was measured for at least 12 frequencies in the range 10–165 MHz for each sample. The estimated error in $\alpha/f^2$ is ±2% while the error in the ultrasonic velocity $v$, is less than ±1%. For all samples $v$ is independent of frequency.

## Results and Treatment of Data

In all cases the ultrasonic absorption over the frequency range 10–165 MHz can be described accurately by the equation for a single relaxation process[11]

$$\alpha/f^2 = \frac{A\tau}{1 + (\omega\tau)^2} + B \qquad (1)$$

where $\omega = 2\pi f$, $\tau$ is the relaxation time and $A$ and $B$ are parameters. The values of $A$, $B$, and $\tau$ for each solution were determined using the template technique of Piercy and Subrahmanyam [2] and are given in Table I for polymers of molecular weight 1000, 1450, 2000, 3400, 4500, 7500, and 20,000 in aqueous and aqueous urea solutions at 10°. The ultrasonic velocity is also included in the table. Estimated errors in $\tau$, $A$, and $B$ are ±10, ±10, and ±2%, respectively. The intrinsic viscosity, $[\eta]$, for each molecular weight in 0 and 8 $m$ aqueous urea solutions is presented in Table II. An error of ±2% is estimated in the value of $[\eta]$.

## Discussion

The data presented here are in reasonable accord with previous work.[7,8,10] Over the necessarily restricted concentration range employed, the relaxation time is independent of polymer concentration.[7,10] A relaxation time consistent with the occurrence of a

(11) K. F. Herzfeld and T. A. Litovitz, "Absorption and Dispersion of Ultrasonic Waves," Academic Press, New York, N. Y., 1959.
(12) J. E. Piercy and S. V. Subrahmanyam, *J. Chem. Phys.*, **42**, 4011 (1965).

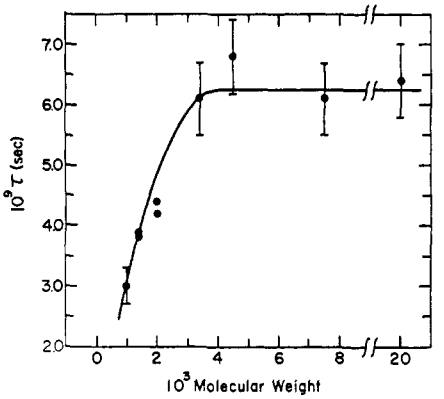



Figure 1. Relaxation time *vs.* molecular weight for polyethylene glycols in aqueous solution at 10°.

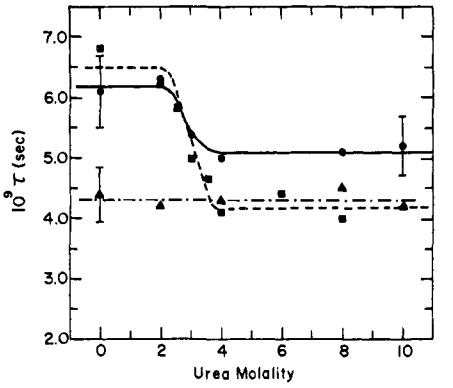



Figure 2. Relaxation time *vs.* urea molality for aqueous solutions of polyethylene glycols at 10° and a monomer mole fraction of 0.035: molecular weight 2000, ▲ -·—·-; molecular weight 3400, ●—; molecular weight 4500, ■ - - - -.

single relaxation process, was observed for all polymer molecular weights ranging from 1000 to 20,000 in urea concentrations varying between 0 and 10 *m*. This relaxation process can be regarded as a solvent–polymer interaction involving hydrogen bonding to the oxygen atom of the polymer, hydrogen-bonded solvent clusters around the $CH_2$ residues, and hydrophobic interactions within the polymer.[7,10] Thus the relaxation involves the breakdown and formation of local solvent and hydrophobic structure. As shown in Figure 1, in pure water increasing the length of the polymer chain lengthens the relaxation time until a limiting value is reached at a polymer molecular weight of about 3400. (Note that this figure is somewhat less than previously estimated;[7] since a larger range of molecular weights was employed in the present investigation, a more precise determination of the molecular weight at which a limiting value of the relaxation time is reached is possible.) For small chain lengths, the amount of intramolecular hydrophobic interactions is relatively small. Apparently the stability of the solvent–polymer structure is correspondingly decreased and the relaxation time is quite short. The fact that a limiting value is reached implies that the solvent–polymer structure involved in the relaxation process has a limiting size. At polyethylene glycol molecular weights greater than 3400 more of these structural units must exist, but each unit acts essentially in an independent manner with regard to the relaxation process.

Figure 2 shows the relaxation time as a function of urea concentration for polymers of molecular weights 2000, 3400, and 4500. For polymers of molecular weight 4500 and larger, the relaxation time decreases sharply between 2 and 4 *m* urea. The transition is sufficiently sharp that a cooperative change in the polyethylene glycol–solvent structure must be involved. This cooperative change involves a slight change in the solvent–polymer structure which leads to somewhat increased solvation and a shorter relaxation time. The slight increase in intrinsic viscosity (Table II) as the urea concentration is increased is consistent with this interpretation. For all molecular weights a slight increase in intrinsic viscosity with urea concentration occurs; no sharp changes in intrinsic viscosity are observed as the urea concentration is raised but it should be noted that the total intrinsic viscosity change is extremely small.

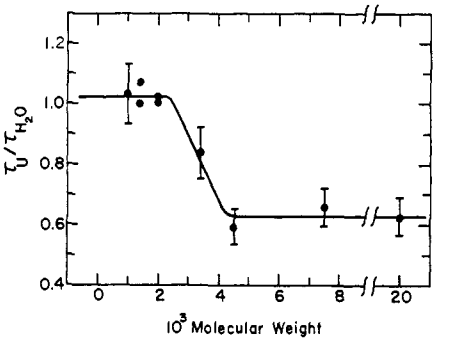



Figure 3. Ratio of relaxation times in water ($\tau_{H_2O}$) and 8 *m* urea ($\tau_U$) *vs.* molecular weight of polyethylene glycol.

At polymer molecular weights of 1000, 1450, and 2000 the relaxation time does not vary with urea concentration. The size of the polymer–solvent structure is apparently too small to give rise to a cooperative transition. In these cases the solute–solvent structure does not involve cooperative interactions and in this sense is similar to the solute–water interactions in oligoglycine solutions[13] and solutions of substituted amines,[14] where it was concluded that cooperative interactions did not occur. The polyethylene glycol of molecular weight 3400 showed a very small transition, indicating that some cooperativity exists. When a cooperative transition occurs, the urea concentration at which the change takes place does not appear to change very significantly with molecular weight. At higher urea concentrations, urea is undoubtedly involved in the solvation process (in an 8 *m* urea solution there are only seven water molecules for each urea molecule). However, urea participation is not sufficient in itself to appreciably alter the relaxation process, since for a given urea concentration the extent of urea solvation must be similar at both high and low molecular weights. The ultrasonic parameter, *B*, is reduced in 8 *m* urea relative to water; this may be interpreted as arising from a loosening of the solvent structure. For polyethylene glycol solutions, *B* always exceeds the

(13) G. G. Hammes and C. N. Pace, *J. Phys. Chem.*, 72, 2227 (1968).
(14) E. Grunwald and E. K. Ralph, III, *J. Am. Chem. Soc.*, 89, 4405 (1967).

value for the pure solvent, indicating the presence of further unrelaxed reactions and/or altered solvent structure.

The data presented here imply that a minimum molecular size of the solvent–macromolecule structure is required before cooperativity is displayed. The requirement of a minimum molecular size has been demonstrated in the case of the helix–coil transition in polypeptides,[15] but not for solute–solvent interactions. The change in cooperativity with molecular weight can be seen in Figure 3, in which the ratio of the relaxation time in 8 *m* urea to that in pure water is plotted against molecular weight. The minimum size of the cooperative unit for polyethylene glycol involves a polymer of about molecular weight 3400; this is also the approximate maximum size of the effective cooperative unit. Thus the size of the cooperative structural unit appears rather precisely fixed and is surprisingly large: approximately 75 monomer units are involved in this unit and if only the first layer of water molecules is of importance, roughly 500–1000 solvent molecules. The fact that a minimum molecular size of solvated macromolecule is required before cooperativity can be exhibited may be of considerable relevance to biological systems.

(15) M. Goodman, M. Langsam, and I. F. Rosen, *Biopolymers*, **4**, 305 (1966), and references therein.

# *Communications to the Editor*

## An Unusually Low *exo*:*endo* Rate Ratio in the Solvolysis of the 2,7,7-Trimethyl-2-norbornyl *p*-Nitrobenzoates. Evidence for Steric Effects as a Major Factor in the *exo*:*endo* Rate and Product Ratios of Norbornyl Derivatives

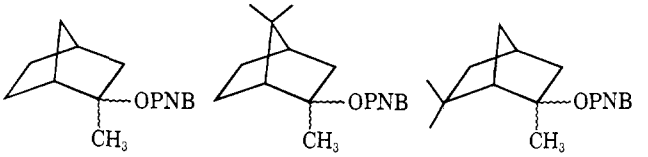
*Sir:*

The *exo*:*endo* rate ratio at 25° decreases from 885 for 2-methyl-2-norbornyl *p*-nitrobenzoate to 6.1 for the related 2,7,7-trimethyl-2-norbornyl esters (for solvolyses in 80% aqueous acetone). This major decrease in the *exo*:*endo* rate ratio, as compared to the parent compound, arises primarily from a major increase in the rate of solvolysis of the *endo* isomer, 2,7,7-trimethyl-*endo*-norbornyl *p*-nitrobenzoate, attributable to major steric interactions between the 2-*exo*-methyl and the *syn*-7-methyl group, relieved during ionization. This result supports the conclusion that steric effects can be very large in the rigid norbornyl system and can play a major role in the observed *exo*:*endo* rate ratio.[1]

As was recently pointed out, the evidence from a wide variety of approaches is becoming overwhelming in favor of the conclusion that tertiary norbornyl cations, such as 2-methylnorbornyl, are classical in structure.[2,3] Yet this classical system exhibits an *exo*:*endo* rate ratio of 885, easily comparable to the titrimetric ratio of 350 (1600 if we allow for internal return) exhibited by 2-norbornyl brosylate.[4] Since neither $\sigma$ participation nor torsional effects[5] apparently make a significant contribution to this high *exo*:*endo* rate ratio in the tertiary derivative,[3] we appear to be left with steric hindrance to ionization as the major contributor.

Steric effects in acyclic and alicyclic systems are usually relatively small,[6] unless exceedingly bulky groups are introduced.[7] Consequently, there is a natural reluctance to attribute such a large *exo*:*endo* rate ratio simply to the operation of steric effects in such a simple, relatively uncluttered system. However, it is apparent that the conformational mobility of acyclic and alicyclic systems provides a mechanism for minimization of steric effects, whereas such an escape mechanism is absent in the rigid, three-dimensional norbornane structure.[8] Consequently, it might be anticipated that this system should be capable of exhibiting huge steric effects, even with relatively small substituents. Accordingly, we undertook to examine the *exo*:*endo* rate ratio in 2-methylnorbornyl containing *gem*-dimethyl substituents in appropriate positions.

In this communication we report that the presence of *gem*-dimethyls in the 7 position (II) decreases the *exo*:*endo* rate ratio to 6.1 from the 885 value observed in the parent compound (I). On the other hand, the presence of *gem*-dimethyls in the 6 position (III) increases the *exo*:*endo* rate ratio to 3,630,000![9] Thus the *exo*:*endo* ratio changes by a factor of 600,000 merely by a shift of the methyl substituents from the 7 to the 6 positions—a truly remarkable steric effect.




885 I 6.1 II 3,630,000 III

Apocamphor, treated with a threefold excess of methylmagnesium iodide, gave an *exo*:*endo* alcohol mixture of 97:3, with 20% of recovered ketone. Retreatment of the crude product gave 95% of the tertiary alcohol (with the same *exo*:*endo* ratio as before) with 5% of residual ketone. Purification by chromatography over alumina afforded pure 2,7,7-trimethyl-*exo*-

(1) H. C. Brown, F. J. Chloupek, and M.-H. Rei, *J. Am. Chem. Soc.*, **86**, 1248 (1964).
(2) H. L. Goering and K. Humski, *ibid.*, **90**, 6213 (1968).
(3) See H. C. Brown and M.-H. Rei, *ibid.*, **90**, 6216 (1968), for pertinent references.
(4) S. Winstein and D. Trifan, *ibid.*, **74**, 1147, 1154 (1952).
(5) P. von R. Schleyer, *ibid.*, **89**, 701 (1967).
(6) H. C. Brown and R. S. Fletcher, *ibid.*, **71**, 1845 (1949).
(7) P. D. Bartlett and M. Stiles, *ibid.*, **77**, 2806 (1955).
(8) H. C. Brown and J. Muzzio, *ibid.*, **88**, 2811 (1966).
(9) S. Ikegami, D. L. Vander Jagt, and H. C. Brown, *ibid.*, **90**, 7124 (1968).