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Role of Co-Solute in Biomolecular Stability: Glucose, Urea and the Water Structure

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Abstract. We have studied the action of urea and glucose on the stability of DNA and micelles. We measured the melting temperature of aqueous solutions of DNA with urea or glucose as a co-solute; we have also measured the changes in the critical micelle concentrations (cmc) of Sodium Dodecyl Sulfate and Triton X-100 by addition of urea and glucose. Our experimental results show that glucose increases the melting temperature of DNA and decreases the cmc, while urea acts in the contrary direction. The effects of urea and glucose on the stability of DNA and micelles can be explained by the weakening and enhancement of hydrophobic interactions, respectively. These effects on hydrophobic interactions are discussed in this paper.

Key words: DNA, glucose, hydrophobic interactions, micelles, micellization, water structure, urea

1. Introduction

Biomolecular stability depends of weak but numerous interactions. Consequently, the constitution of the medium is highly relevant to maintain the native structures. Hydrogen bonds and hydrophobic interactions are representative of such weak interaction that strongly depend on the nature of solvent and other solutes present.

Hydrogen bonds have been extensively studied. The hydrophobic interaction, in spite of it high relevance, appears in several aspects obscure. Its interaction distance, temperature dependence, modification by the presence of different solutes, and other properties are subject of controversies. Even its relative importance compared with van der Waals interaction has been questioned. The first modern description of the effect was by Kauzmann [1], who presented the phenomenon as a consequence of hydrophobic hydration. The hydrophobic hydration has been questioned [2], in spite of its experimental confirmation [3] and support from simulation [4].

Globular proteins are believed to be stabilized by, among other forces, hydrophobic interaction of non-polar residues that, in the native folding state, appear mostly inside the protein. Addition of co-solutes may affect the protein stability by indirect effect through the solvent. The effect of co-solutes can be interpreted by

the *preferential hydration* described by Gekko and Timashef [5]. Under this scope the stabilization or destabilization effect of co-solute depends on the ability of such solute to be excluded or incorporated in the hydration layer of protein. In one or other case the system will be a three (co-soluteþwater medium, protein, hydration water) or two (co-solute – water medium, protein) phases system. Thermodynamics forces will act the sense of minimize the presence of a third phase.

Hydrophobic forces will also be affected by the presence of co-solute by changing on the water propertied. As solute may act directly with the biomolecule, other effects, as competition for hydrogen bonds, may be present.

Specific hydration and hydrophobic interactions are compatible concepts, but to split its contributions is not an easy task.

The purpose of this paper is to analyze the effect of urea and glucose as denaturant and protector respectively on biomolecular stability. An experimental approach on DNA and micelle formation is used to gain insight into the basic mechanism of such effects.

As the main force in forming micelles is originated in the hydrophobic effect, we have measured the critical micelle concentration (c.m.c) of sodium dodecyl sulfate (SDS) and polyoxyethylene ter-octylphenyl ether (Triton X100) in water in the presence of urea or glucose. We have also measured the melting temperature of DNA samples in the presence of various amounts of glucose and urea.

2. Experimental

DNA preparation. Using calf thymus sodium salt of DNA (Sigma, D-1501), we made stock solution of 4 mg of DNA in 10 ml buffer (0.15 m NaCl plus 0.015 m sodium citrate -SSC 1X). For the measurements, the DNA concentration was 20 μg/ml. Neutral urea (Carlo Erba and Merck MW = 60,056) and glucose (Mallickrodt MW = 180,16) co-solutes were of analytical grade. Freshly double distilled water of neutral pH and conductivity less than 10^{-6} S/cm and free from CO_2 was used. DNA solutions were prepared at temperatures below 10 °C and stored at 4 °C until used. Melting temperatures for DNA were obtained by UV absorbance measurements at 260 nm and were carried out with a Metrolab 2500 double beam spectrophotometer with a thermostatic chamber attachment. The sample was placed in the spectrophotometer chamber at a temperature of 10 °C; the temperature was adjusted by circulating water/glycerol mixture. Temperature was controlled with a Lauda cryostat TUK 30D, with a precision of 0.04 °C. The sample temperature was measured with a digital Cole Parmer thermocouple thermometer 8110-15 with a precision of 0.1 °C. All DNA measurement solutions contained SSC 0.1X. The native state of DNA of each sample was ascertained by recording its UV spectra between 230 to 320 nm at 25 °C.

We prepared ionic micelles with sodium dodecyl sulfate (SDS – Mallickrodt) and no-ionic micelles with polyoxyethylene ter-octylphenyl ether [Cn H2n+1 C6 H4 (O C2 H4)n OH *(n = 9–10)*] (Triton X-100 – Rohm and Haas) in solutions of

urea and glucose in doubly distilled water. Critical micelle concentrations (cmc) were obtained by measuring conductivity (for SDS) or extinction at 278 and 285 nm (for Triton X-100) of solutions at constant co-solute and different concentration of surfactant. The change in slope of the plot of the measured property against concentration was taken as determinant of the cmc. Conductivity was measured with a WTW 8120 conductimeter and absorption with above-mentioned spectrophotometer.

3. Results

Formation of micelles. Surfactants in water form micelles at some critical concentration (c.m.c). The cmc is sensitive to temperature and medium composition and gives a clear indication of the stability of micelles. These aggregates, in equilibrium with monomers, are stabilized mainly by hydrophobic interactions between the apolar region of the surfactants.

The polar region (ionic or non-ionic) usually plays an adverse role for micelle stability. For the ionic micelles the degree of ionization regulates the repulsive electrostatic forces between the polar heads. Clearly there are no hydrogen bonds involved within the surfactant and therefore, any effect on micelle stability should involve any other mechanism those breaking or weakening hydrogen bonds. This makes micelles an interesting model to study the effect of co-solutes on the hydrophobic effect.

Figure 1 shows the deviation of cmc from pure water solutions for SDS and Triton X-100 as a function of the concentration of glucose and urea. We see that in both kinds of micelles, ionic SDS and non-ionic Triton X-100 micelles, urea increases the cmc, destabilization effect, while glucose lowers the cmc, showing a stabilization effect. Triton micelles are more sensitive than SDS ones to the action of co-solute; this is clearer for the urea solutions in which the effect appears above 2 mol/l urea. The results are consistent with those obtained by Schick [7], within the range of their measurements.

We also studied the action of glucose and urea on the stability of DNA. Figure 2 shows the melting temperature of DNA samples against the concentration of glucose or urea present in the bathing medium. Again, we see that the glucose contributes to stability while urea in concentrations above 2 mol/l decreases stability.

4. Discussion

Although we can try to search for direct actions of co-solute on the micelles there is a little room for speculations. On the basis of the coherent results for both kind of micelles we can conclude that the co-solute effect is done through altering the hydrophobic effect

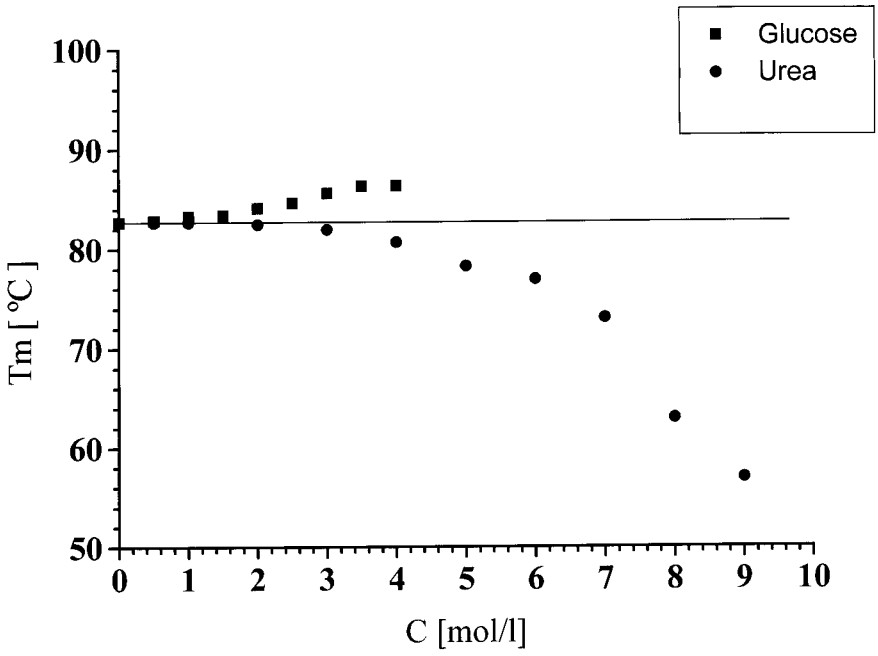

Figure 1. Normalized dependence of the critical micelle concentration (cmc) of aqueous solutions of sodium dodecyl sulfate (SDS) and Triton X-100 in function of the concentration of the co-solute (glucose or urea), at 25 °C. The over imposed circles indicate the true limit of micelle formation.

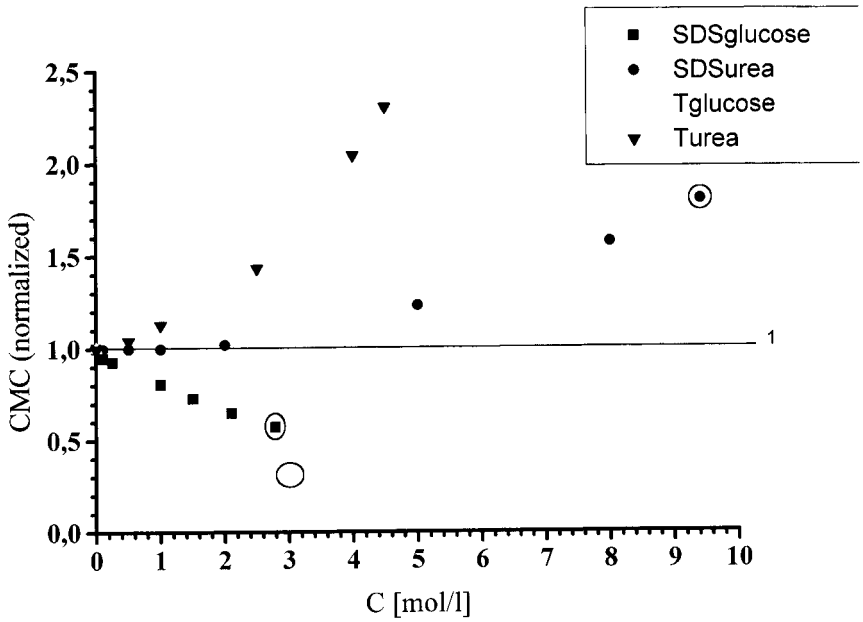

Figure 2. Melting temperature of the DNA in function of the concentration of the co-solute (glucose or urea).

Interpretation of the data of critical micelle concentration changes produced by urea and glucose seems to be clear enough to conclude that the main effect of co-solutes is to alter the hydrophobic interaction through modification on water structure.

On one hand there are only two forces acting on micelles: the electrostatic forces between head groups and hydrophobic interaction between apolar tails. The former is mostly repulsive, although in ionic micelles some counter-ions may act as a bridge between head groups helping stability. Also for non-ionic micelles the polar heads that having their electric dipole moments oriented in the same direction repelled each other, may be linked by hydrogen bonds through water molecules. In both cases the polar heads produce a repulsive force, although tempered by the action of the medium. In ionic surfactants the solvent permittivity produce a degree of ionization for which the net effect of electrostatic force is repulsive acting then in the direction of destroy the micelles, higher concentration of counter-ions stabilize the micelles [6–8]. Counter-ions also stabilize the non-ionic micelles by screening dipole-dipole repulsion [9, 10].

For the action of urea and glucose seems that the observed effect cannot be explained with the electrostatic forces. Although recent experiments [11] suggest a direct action of urea on ionic micelles, inducing the increasing in cmc by removing part of surface ions, this will not suffice to account for all experimental results. These results cannot be extrapolated to non-ionic micelles suggesting that may be just other factor but not the only one. For the non-ionic micelles we may consider some replacement of water bridges between polar heads by urea in such a way that does not allow the bridging. Glucose may act in the same way but, as the experiments show, the effect is contrary. The allowed geometry of hydrogen bonds for glucose and urea are quite different, partly due to the difference in size, in such a way that the regular water network cannot be sustained at high concentrations of urea.

There is a number of experimental and computational evidence that glucose enhance the water structure [12–18]. This enhancement will make more favorable the four hydrogen bonds structure in water.

As hydrophobic interaction is the most relevant force to form the micelles, the other possible effects we have mentioned cannot be considered as determinant of the changes in stability. So we conclude that the main effect of urea and glucose is to weak and enhance respectively the hydrophobic interaction. This fact points to the correlation between to enhance or to distort the regular water structure and hydrophobic interactions.

On the interpretation of the experiments of melting temperature of DNA we have to bear in mind that DNA exhibit also hydrogen bonds as key forces to maintain its structure. Possible competence between co-solute and DNA for hydrogen bonds may decreases the melting point. So we may expect that both glucose and urea would produce the same effect, since both compounds are capable of forming hydrogen bonds. The observed effect is that the action on melting temperature is

opposite for urea and glucose. A similar destabilizing effect of urea on RNA has been reported by Shelton et al. [19].

Analyzing the relative strength of hydrogen bonds formed by urea and glucose with water [12] we found that seems to be that the glucose HB are stronger than those of urea, i.e. if this will be the main effect we should found an opposite result as the experiments. Owning the importance of hydrophobic interaction on base stacking we may conclude that also here urea debilitate the hydrophobic interaction while glucose enhance it.

Some of these results can also be explained through the preferential hydration hypothesis. However we must carefully consider when the preferential hydration stabilize or destabilize a protein. Assuming that the co-solute do not come into the macromolecular hydration layer of the *unfolded* macromolecule, it will contribute to the stabilization of the folded state, following the Thermodynamics tendency to decrease the pure water phase. Since it is unlikely that urea molecules remain close to the hydrophobic regions the expectation, under the preferential hydration framework, will be indeed a stabilizing effect, against what we observed. Following the same reasoning we see that for glucose preferential hydration may be, alone or in conjunction with the indirect effect on the hydrophobic interaction, one of the stabilizing mechanisms.

5. Conclusions

The results show that a system in which the hydrophobic interaction dominates its stability is sensitive to the effect of urea. The hydration of urea produces a disordering on the water structure and, indirectly, weaken the hydrophobic interaction. Glucose, in contrary, acts as stabilizer of hydrophobic interaction by enhancing the regular water structure.

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