Kosmotropes and Chaotropes

Definitions of kosmotropes and chaotropes

The terms 'kosmotrope' (order-maker) and 'chaotrope' (disorder-maker) originally denoted solutes that stabilized, or destabilized respectively, proteins and membranes; thus chaotropes unfold proteins, destabilize hydrophobic aggregates and increase the solubility of hydrophobes whereas kosmotropes stabilize proteins and hydrophobic aggregates in solution and reduce the solubility of hydrophobes. Later these terms referred to the apparently correlating property of increasing, or decreasing respectively, the structuring of water (a recent review of structure-making and structure-breaking ions has been presented [1567]). Although useful, the terminology may sometimes be misleading as such properties may vary dependent on the circumstances, method of determination or the solvation shell(s) investigated. For example a solute may not always act in the same way at different concentrations or in the presence of macromolecules or gels [276]. Also some solutes with less well-defined properties (for example, urea) are sometimes classified as kosmotropes [276] and sometimes as chaotropes [283]. An alternative term used for kosmotrope is 'compensatory solute' as they have been found to compensate for the deleterious effects of high salt contents (which destroy the natural hydrogen bonded network of water) in osmotically stressed cells, but again behavior as a kosmotrope in one system does not mean that a solute may behave as a 'compensatory solute' in another or even that it will stabilize the structuring of water in a third. Both the extent and strength of hydrogen bonding may be changed independently by the solute but either of these may be, and has been, used as measures of order making. It is, however, the effects on the extent of quality hydrogen bonding that is of overriding importance as true kosmotropes shift the local equilibrium

$$\text{less dense water (for example, ES)} \rightleftharpoons \text{more dense water (for example, CS)}$$

to the left and chaotropes shift it to the right. The ordering effects of kosmotropes may be confused by their diffusional rotation, which creates more extensive disorganized junction zones of greater disorder with the surrounding bulk water than less hydrated chaotropes. It seems clear that most kosmotropes do not cause a large scale net structuring in water [595].

Temperature and pressure both have effects on the kosmotropic/chaotropic status with the effects disappearing at high temperatures, particularly at high concentrations [1170]. For example, at very high pressures (0.6 GPa) Na^+ ions change from being weak kosmotropes into weak chaotropes as their links to water molecules are preferably broken [1170]. [**Back to Top** ⬆]

Ionic kosmotropes and chaotropes

Ionic kosmotropes[a] should be treated differently from non-ionic kosmotropes, due mainly to the directed and polarized arrangements of the surrounding water molecules. They are best not described as 'structure-makers' or structure-breakers' in terms of their effects on the properties of water outside their immediate solvation shells [1389]. Generally, ionic behavior parallels the Hofmeister series. Large singly charged ions, with low charge density (for example, SCN^-, $H_2PO_4^-$, HSO_4^-, HCO_3^-, I^-, Cl^-, NO_3^-, NH_4^+, Cs^+, K^+, $(NH_2)_3C^+$ (guanidinium) and $(CH_3)_4N^+$ (tetramethylammonium) ions; exhibiting weaker interactions with water than water with

itself and thus interfering little in the hydrogen bonding of the surrounding water), are chaotropes whereas small or multiply-charged ions, with high charge density, are kosmotropes (for example, SO_4^{2-}, HPO_4^{2-}, Mg^{2+}, Ca^{2+}, Li^+, Na^+, H^+, OH^- and HPO_4^{2-}, exhibiting stronger interactions with water molecules than water with itself and therefore capable of breaking water-water hydrogen bonds).[b] Kosmotropes remain hydrated near the water surface, while the chaotropes lose their hydration sheath [1663]. The radii of singly charged chaotropic ions are greater than 1.06 Å for cations and greater than 1.78 Å for anions [284]. Thus the hydrogen bonding between water molecules is more broken in the immediate vicinity of ionic kosmotropes than ionic chaotropes [2046]. Reinforcing this conclusion, a Raman spectroscopic study of the hydrogen-bonded structure of water around the halide ions F^-, Cl^-, Br^- and I^- indicates that the total extent of aqueous hydrogen bonding increases with increasing ionic size [685] and an IR study in $HDO:D_2O$ showed slow hydrogen bond reorientation around these halide ions getting slower with respect to increasing size [895]. It is not unreasonable that a solute may strengthen some of the hydrogen bonds surrounding it (structure making; for example, kosmotropic cations will strengthen the hydrogen bonds donated by the inner shell water molecules) whilst at the same time breaking some other hydrogen bonds (structure breaker; for example, kosmotropic cations will weaken the hydrogen bonds accepted by the inner shell water molecules) [274], so adding to the confusion in nomenclature. Kosmotropic ions such as Na^+ reduce the average diffusion of water by slightly less than that expected if the hydrated water was not diffusionally active [2001].

Much local organization around a solute is entropically compensated by reduced organization between the water molecules further away [2087], such that K^+ and Cl^- (and Ar) have almost identical hydration entropies [1495]. Other factors being equal, water molecules are held more strongly by molecules with a net charge than by molecules with no net charge; as shown by the difference between zwitterionic and cationic amino acids [532].

Rather surprisingly, chaootropic ions such as K^+ slightly the average diffusion of water at moderate to high concentrations (0.25 - 2 M) [2001], showing that they have a net destructive effect on the structuring in water at these concentrations. Weakly hydrated ions (chaotropes, K^+, Rb^+, Cs^+, Br^-, I^-, guanidinium$^+$) may be 'pushed' on to weakly hydrated surfaces by strong water-water interactions with the transition from strong ionic hydration to weak ionic hydration occurring where the strength of the ion-water hydration approximately equals the strength of water-water interactions in bulk solution (with Na^+ being borderline on the strong side and Cl^- being borderline on the weak side) [284]. Strongly hydrated surfaces, where the water molecules are prevented from forming ES-like expanded water clusters, favors strongly hydrated kosmotropic ions and effectively repels weakly hydrated chaotropic ions [1726]. Neutron diffraction studies on two important chaotropes (guanidinium and thiocyanate ions) show their very poor hydration, supporting the suggestion that they preferentially interact with the protein rather than the water [488]. In contract to the kosmotropes, there is little significant difference between the properties of ionic and nonionic chaotropes due to the low charge density of the former.

Optimum stabilization of biological macromolecule by salt requires a mixture of a kosmotropic anion with a chaotropic cation. As ionic kosmotropes primarily achieve their increased structuring solely within their hydration shell, they partition into the more dense (CS) water where they can obtain this hydration water more readily, whereas the ionic chaotropes, by avoiding interference with water's hydrogen-bonded network, tend to clathrate formation within the less dense (ES) environment. Thus there is agreement with the defining characteristic of an ionic chaotrope in that it partitions selectively into low-density water whereas a kosmotrope partitions selectively into high-density water [276]. The stabilizing of structured low-density water (by ionic chaotropes) in turn stabilizes the hydration shell around lower molecular weight hydrophobes, as seen in the promoted

association of polyene antibiotics by ionic kosmotropes and the stabilization of their solution by ionic chaotropes [1030]. [**Back to Top** ⬩]

Non-ionic kosmotropes and chaotropes

Non-ionic kosmotropes are very soluble well-hydrated molecules with little tendency to aggregate, having no net charge and strongly hydrogen bonding to water, that stabilize the structure of macromolecules in solution. They are preferentially solubilized within the bulk of the solution and excluded from the solvation layers of macromolecular surfaces. Consequentially, they decrease the water diffusion around the proteins and the exchange rate of backbone amide protons [621]. This leads to the dehydration of such surfaces and ensures that they are less flexible and therefore more thermally stable but less enzymatically active.[c] Kosmotropes reduce the volume of water available to hydrate the larger surface exposed by denatured proteins, so tending to prevent the denaturation process (the 'excluded volume' effect).These kosmotropes may be divided into two groups which act somewhat differently: (1) polyhydroxy compounds fit in well with the hydrogen bonding arrangements, but stabilize any cluster 'flickering' via hydrogen bond rearrangement, as their hydroxyl groups are similarly separated to water-water separations (see sugar hydration), and (2) zwitterions where the balance between hydrophilicity, hydrophobicity, anionic and cationic characteristics ensures good solubility but only weak net interactions to surrounding water.

Chaotropes break down the hydrogen-bonded network of water, so allowing macromolecules more structural freedom and encouraging protein extension and denaturation. Kosmotropes are stabilizing solutes which increase the order of water (such as polyhydric alcohols [307], trehalose, trimethylamine *N*-oxide, glycine betaine, ectoine, proline and various other zwitterions) whereas chaotropes create weaker hydrogen bonding, decreasing the order of water, increasing its surface tension (but see anomaly) and destabilizing macromolecular structures (such as guanidinium chloride and urea at high concentrations). Urea (H_2NCONH_2) can only strongly bind one water molecule using two hydrogen bonds, donating from a nitrogen atom and accepting onto the urea oxygen atom [1130]. Otherwise it can hydrogen bond to itself (see (b) opposite) or water with close to equal ease, and hydrogen bonds to water slightly weaker than water to itself but seems to increase the water-water interactions [1233]. Different studies describe urea as either a poor chaotrope or poor kosmotrope over much of its concentration range [1171, 1233]. Its conformation is not well-defined in water [1309] and it can fit into a tetrahedral water network showing little preference between ES-like and CS-like water clusters [1171], perhaps behaving rather like a water dimer [1130], although this is not shown in a molecular dynamics study [1233]. Other work has shown that urea weakens both hydrogen bonding and hydrophobic interactions [283]. The idiosyncratic behavior of urea may well be due to its concentration-dependent oligomerization; cyclic hydrogen-bonding dimers and oligomers behaving differently from monomers [364].
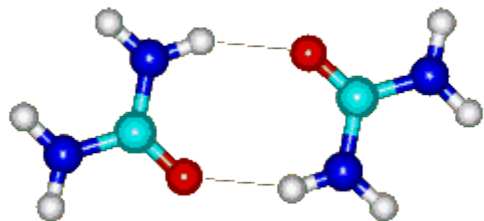
Thus, when urea molecules are less than optimally hydrated (about 6 - 8 moles water per mole urea) urea hydrogen bonds to itself (see opposite) and the protein (significantly involving the peptide links [528]) in the absence of sufficient water, so becoming more hydrophobic and hence more able to interact with further sites on the protein, leading to localized dehydration-led denaturation (i.e. urea is preferred over water for binding to the protein backbone so leading to protein denaturation [1711]).

Guanidinium [1777] is a planar ion that may form weak hydrogen bonds around its edge but may establish strongly-held hydrogen-bonded ion pairs to protein carboxylate groups, similar to commonly found quaternary structural arginine-carboxylate 'salt' links. Also, guanidinium possesses rather hydrophobic surfaces that may interact with similar protein surfaces to enable protein denaturation [571]. The hydrophobic surfaces allow the formation of contact guanidinium-guanidinium ion pairing [2034] with the repulsion of the like, if somewhat dispersed, positive charges being compensated by the increased entropy and enthalpy of the released water molecules from the surfaces on forming the stacked pair. Both denaturants (urea and guanidinium) may cause protein swelling and destructuring by sliding between hydrophobic sites and consequently dragging in hydrogen-bound water to complete the denaturation. The hydration of the counterion is important to the action of guanidinium with chloride ions, being only weakly hydrated, allowing the water molecules to easily rearrange around the surface and allowing the denaturation process outlined above whereas sulfate ions hold on to their water molecules very strongly preventing the water rearrangement and hence preventing the guanidinium-protein interactions and consequent denaturation [1747].

Glucose clearly acts as a kosmotrope, enhancing both hydrogen bonding and hydrophobic interactions [283]. Rather unexpectedly, whereas L-amino acids and D-glucose prefer a less dense (ES) environment, D-amino acids and L-glucose prefer more dense (CS) water [574]; a consequence of which may be that condensed polymers of the former (rather than their optical isomers) may have formed in primordial clays so setting the trend for the molecular evolution [374]. Although the arrangement of oxygen atoms within water clusters may be symmetrical, the energetic preference for particular hydrogen-bonding arrangements ensures the necessary chirality. ⋀

Kosmotropes
a Trimethylamine *N*-oxide
b Proline
c Ectoine; R varying
d α,α-Trehalose
e Glycine betaine
f 3-Dimethylsulfoniopropionate
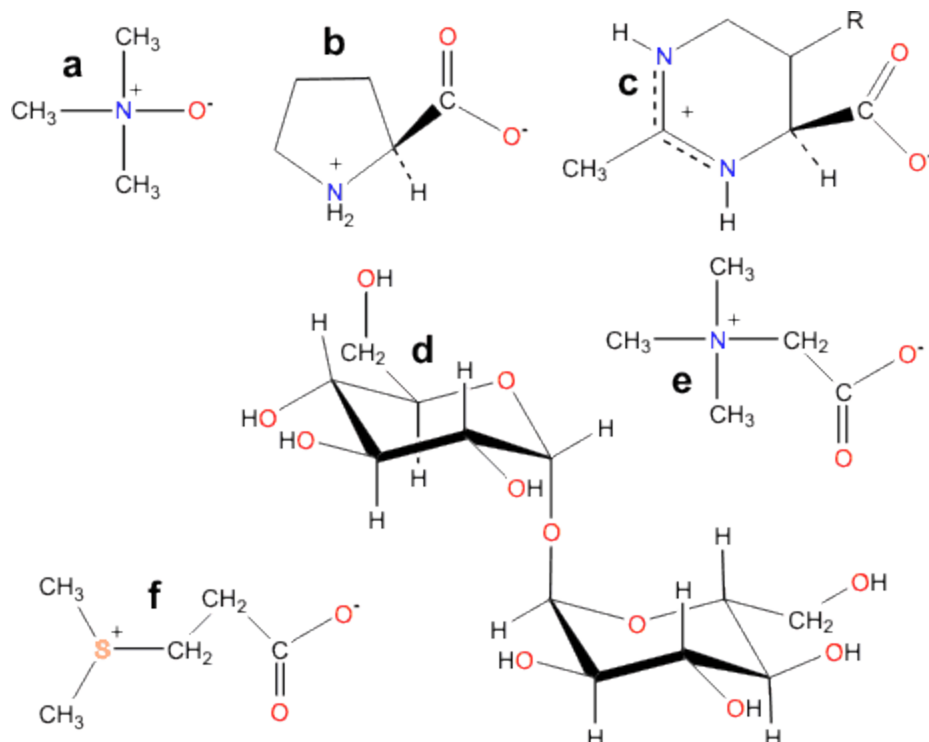
Generally the kosmotropic/chaotropic nature of a solute is determined from the physical bulk properties of water, often at necessarily high concentration. The change in the degree of structuring may be found, for example, using NMR ([307]) orvibrational spectroscopy. Protein-stabilizing solutes (kosmotropes) increase the extent of hydrogen bonding (reducing the proton and ^{17}O spin-lattice relaxation times) whereas the NMR chemical shift may increase (showing weaker bonding for example, the zwitterionic kosmotrope, trimethylamine N-oxide[f]) or decrease (showing stronger bonding for example, the polyhydroxy kosmotrope, trehalose). Trehalose shows both a reduction in chemical shift and relaxation time, as to a lesser extent does the protein stabilizer $(NH_4)_2SO_4$, whereas NaCl only shows a reduction in chemical shift and the protein destabilizer KSCN shows an increase in relaxation time and a reduction in chemical shift (note these NMR parameters are both time and structurally averaged values, where the weighting of the averaging is unclear) [281]. Vibrational spectroscopy may make use of the near-IR wavelength near 5200 cm$^-$^{1} ($v_2 + v_3$ combination), which shifts towards longer wavelength (smaller wavenumber) when hydrogen bonds are stronger [282]. It should be noted however that ranking of kosmotropic/chaotropic character by different measures shows inconsistencies. [**Back to Top** ⋀]

α,α-Trehalose

One of the most important kosmotropes is the non-reducing sugar α,α-trehalose (structure given above and described in [1675]). It should perhaps be noted that trehalose has a much more static structure than the reducing sugars, due to its lack of mutarotation, or the other common non-reducing disaccharide, sucrose, due to its lack of a furan ring. Both inelastic neutron scattering [543] and ^{17}O-NMR show that a rotational restriction exists in trehalose-bound water, indicating that trehalose is definitely a net water-structurer. These stronger interactions[d] (between the trehalose and water compared to water-water interactions) increase the bioprotective effectiveness of trehalose [887] by reducing the (re-)activity of the water molecules, creating a rigid shell around biomolecules and so reducing thermal stress [1319]. This effect is increased by the introduction of greater static hydration with trehalose-trehalose interactions [1920]. Trehalose interferes with the tetrahedral network of water, structuring out to at least the third solvation layer,[e] in such a way as to reduce the amount of freezable water [285]; terahertz spectroscopy indicating 27% more

hydration water (~190 mols/mol) than lactose and almost four times the hydration water than glucose [1427]. This is consistent with an increase in the local structuring, due in part to maximizing the number of singly hydrogen-bonded water molecules (to trehalose) without any (trehalose) intramolecular hydrogen bonds or oriented water molecules held by two (trehalose) hydrogen bonds. There is also reducedtetrahedrality amongst the associated water molecules [660]. Neutron diffraction studies indicate that the hydrogen bonds to water are somewhat shorter (trehalose-O-**H**···**O**H$_2$ ~0.15 nm and trehalose-**O**···**H**OH ~0.15 nm) than those between water molecules but with an average of ~0.9 (trehalose-**O**···**H**OH) and ~0.15 (trehalose-O-**H**···**O**H$_2$) hydrogen bonds per hydroxyl group respectively [1422]. At lower water contents, essentially all the water is associated with single hydrogen bonds to trehalose or first shell water with consequential reduction in the ability to form four hydrogen bonds per water molecule but retaining an expanded structure. Trehalose is particularly effective at stabilizing macromolecules as it has a large hydrated volume (2.5 times that of sucrose, indicating that there is less water for the same volume; that is, low-density water is present [279]). Trehalose can also protect membranes (and whole organisms [1426]) in anhydrobiosis by hydrogen bonding directly to the phosphate groups in the phospholipids at low water content, so spreading their head groups and reducing the membrane's tendency to undergo phase transitions and thus leak during rehydration [1201]. Thus trehalose exhibits different mechanisms of action with proteins from those with membranes [308]. As further protective action trehalose crystals, formed at low water content, can lose (and regain) up to two molecules of water per molecule, without changing their crystal structure or volume [327, 1486]. [**Back to Top** ▲]

Footnotes
^{a} Perhaps ionic kosmotropes should be called antichaotropes to distinguish them from non-ionic kosmotropes. [Back]

Ionic Chaotropes
convex shell

Ionic Kosmotropes
puckered shell
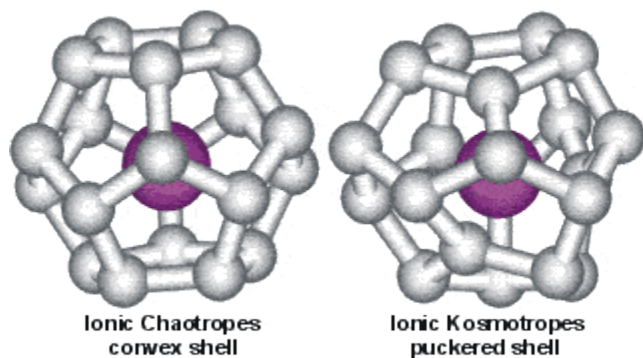
The water dodecahedra shown only as connected O-atoms of water, surrounding the central ion..

^{b} Opposite is shown a cartoon illustrating the main difference between ionic chaotropes and kosmotropes. Water molecules surrounding ionic chaotropes tend towards a convex dodecahedral (clathrate) arrangement, whereas those surrounding ionic kosmotropes tend towards a puckered arrangement with a number of water molecules lying close to the ion (see elsewhere for more details), causing bent and broken hydrogen bonding further out. The dodecahedral water cluster may be thought of as constantly fluctuating (over a period of a few ps or so, see [895]), interconverting between convex and puckered forms with the preferred clustering governed by the chaotropic-kosmotropic balance. The strength of the ion-water links explains why the ionic kosmotropes remain hydrated near the interface, while the ionic chaotropes lose their hydration sheath [1663]. [Back]

^c A few adventitious and deleterious enzymatic and non-enzymatic reactions may occur during lengthy dehydration, which may be reduced by the presence of specific amphiphilic molecules that concentrate at the interfaces [321]. [Back
]

^d This 'stronger' character of the system is sometimes referred to as 'less fragile' [232]. [Back]

^e This description has been disputed by recent neutron diffraction experiments combined with empirical potential structure refinement simulations, that found trehalose solvation induces more minor modifications of the water structure. [1647].

^f Trimethylamine N-oxide (TMAO) exemplifies the difficulty in describing molecules as structure makers or breakers as molecules that can form very strong links to water may weaken water-water links further away; whether experimental evidence finds structure-making or -breaking depends on which of these molecules of water are examined. For example, infrared spectroscopy shows that TMAO forms strong hydrogen bonds with water [1893]. Vibrational sum frequency spectroscopy shows that the methyl groups point away from hydrophobic surfaces indicating a more hydrophilic
nature of methyl groups attached to this strongly electron-withdrawing quaternary nitrogen atom and helping to explain TMAOs depletion at such interfaces compared to the bulk solution [1913]. However, fluorescence studies using model hydrophobic compounds indicate that TMAO reduces hydrophobic interactions in aqueos solutions [2040]. [Back]

Source:http://www1.lsbu.ac.uk/water/kosmos.html

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