LIQUID WATER AND THE ORIGIN OF LIFE*

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Abstract. Liquid water has many peculiarities which confer special properties. The most important among them probably are the ability to establish hydrogen bonds, a high polarity and a high dielectric constant. In the presence of liquid water, large organic molecules have to manage the conflict between hydrophobic groups and hydrophilic groups. This duality can generate interesting prebiotic situations such as the stereoselective aggregation of short peptide sequences of alternating hydrophobic – hydrophilic residues into thermostable β-sheets endowed with chemical acrivity.

Liquid water is also a powerful hydrolytic agent. As such, it allows pathways which would have few chances to occur in an organic solvent.

Introduction

It is generally thought that life on Earth emerged in liquid water. For this reason, water is considered as one of the prerequisites for life to appear and evolve on a planet. The reasons put forward to support this statement are borrowed to contemporary living systems. As solvent, water allows the organization of biopolymers in three-dimensional structures and it acts as chemical partner of most biochemical pathways. The present paper presents some experimental results illustrating the many-faceted character of water and its possible role in chemical evolution.

Liquid Water, an Exceptional Solvent

According to its molecular weight, water should be a gas under standard terrestrial conditions by comparison with CO_2, SO_2, H_2S, etc. Its liquid state is due to its ability to form hydrogen bonds. This is not restricted to water molecules since alcohols exhibit a similar behavior. However, the polymeric network of water molecules via H-bonds is so tight that the boiling point of water is raised from 40 C (temperature inferred from the boiling point of the smallest alcohols) to 100 C (Figure 1). Hydrogen bonds are formed between water molecules and organic molecules providing that the latter contain –OH, –NH, –SH groups in addition to carbon and hydrogen. As a consequence of this affinity many CHONS organic molecules are soluble in water. Hydrocarbons cannot form hydrogen bonds with water. These molecules are not soluble in water and escape water a much as possible

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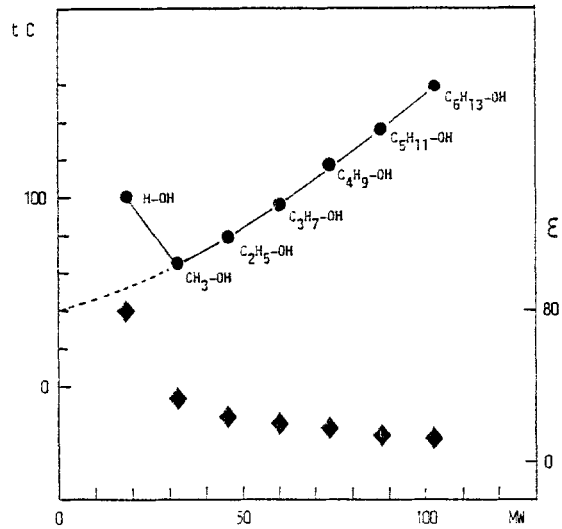
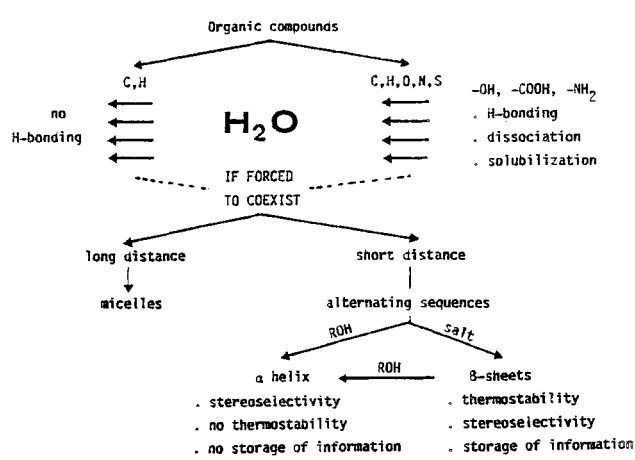


Fig. 1. Boiling point (●) and dielectric constant (♦) of water compared to a series of alcohol homologs.

(hydrophobes).

In addition to the H-bonding capability, water exhibits a large dipole moment (1.85 debye) as compared to alcohols (< 1.70 debye). This large dipole moment favors the dissociation of ionizable groups such as $-NH_2$ and -COOH leading to ionic groups which can form additional H-bonds with water molecules, thus improving the solubilization.

Water is also an outstanding dielectric (Figure 1). When oppositely charged groups are formed, their recombination is unfavored because the attraction force for reassociation is proportional to $1/\epsilon$.



Scheme 1. Liquid water drives the conformation of organic molecules.

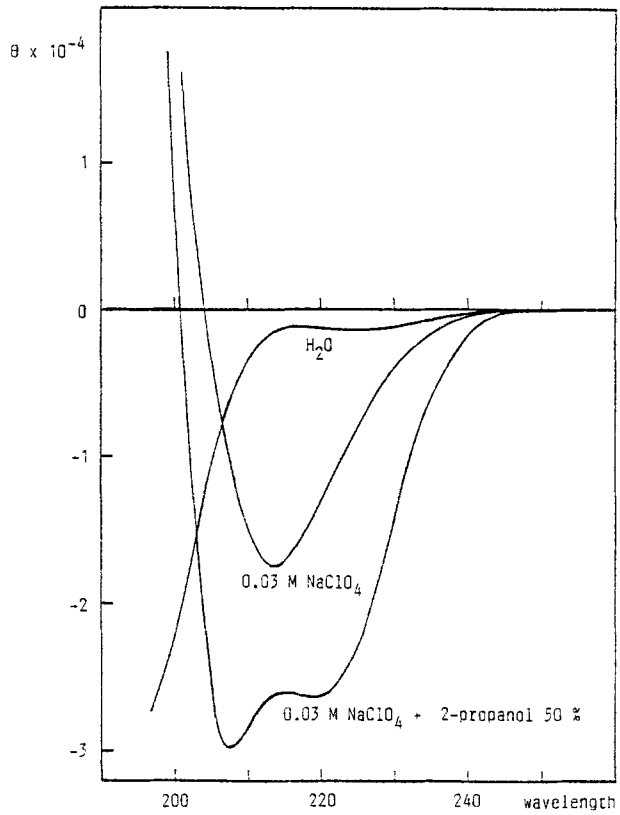


Fig. 2. Circular dichroism spectra of poly(Leu-Lys) in the random coil conformation obtained in pure water (H_2O), as the β-sheet structure obtained in the presence of salt (0.03 M $NaClO_4$) and in the α-helical form obtained when alcohol was added to the previous solution (0.03 M $NaClO_4$+ 2-propanol 50%).

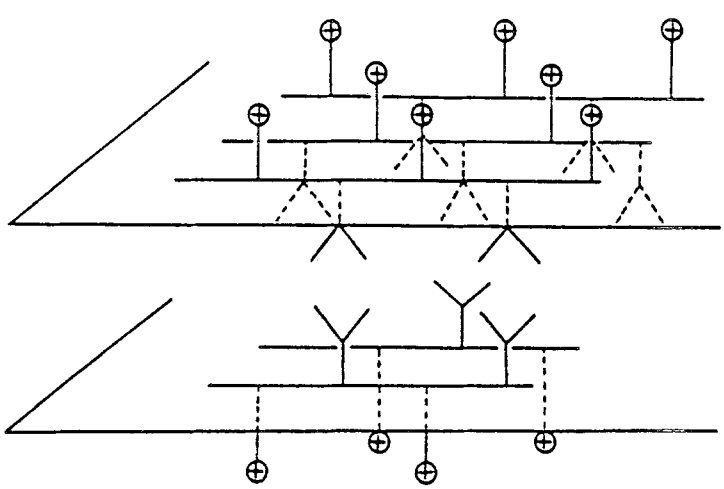


Fig. 3. Schematic representation of a bilayer composed of alternating polypeptide chains with charged hydrophilic (+) and hydrophobic (Y) residues.

Liquid Water Determines the Conformation of Organic Molecules

Abiotic organic molecules, produced in primitive terrestrial atmospheres or in space, can be divided into two families: hydrocarbons and CHONS containing molecules. When brought into the presence of liquid water, hydrocarbons try to escape the water molecules while CHONS have some affinity for water, especially those which bear ionizable groups. When these two species are forced to coexist within the same molecules, the duality generates interesting topologies. When they are separated by long distances, such as in fatty acids or phospholipids, micelles, vesicles or liposomes are formed due to clustering of the hydrophobic groups. Over short distances, hydrophobic and ionizable groups generate chain conformations which depend strongly on the sequence as illustrated by synthetic polypeptides (Scheme 1).

Strictly alternating homochiral poly (L-leucyl-L-lysyl), hereafter referred to as poly(Leu-Lys), is soluble in water. At neutral pH, the lysyl side-chain amines are ionized as NH_3^+ groups. Due to charge repulsion, the chain cannot adopt a regular conformation (Figure 2). Addition of salt to this solution, for instance 0.1 M NaCl, produces a screening of the charges and allows the polypeptide to adopt a β-sheet structure illustrated in Figure 3 (Brack and Orgel, 1975). Because of the alternating sequence, all hydrophobic residues are confined to one side of each strand. The chains aggregate into asymmetrical bilayers with a hydrophobic interior and a hydrophilic exterior because of hydrophobic side-chain clustering.

Due to bilayer formation, strictly alternating hydrophobic-hydrophilic sequences are thermostable. Non-alternating sequences form α-helices which are thermolabile. Heating samples in which α-and β-structures coexist increases the amount of β-structure with a loss of α-helix. Alternating sequences are also more resistant to chemical degradation than a-helical sequences (Brack and Spach, 1981a). To get a β-sheet and therefore a high resistance to degradation, the hydrophobic amino

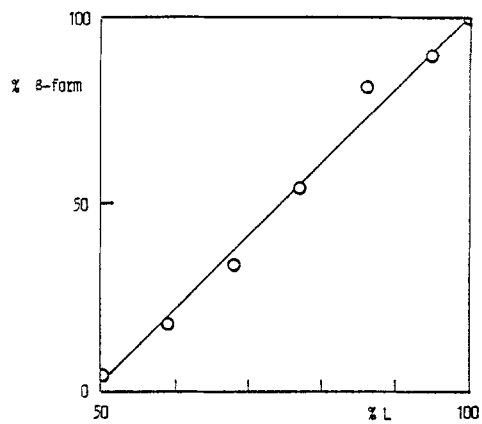


Fig. 4. Variation of the percentage of β-sheet structure in a heterochiral, alternating poly (leucyl-lysyl) as a function of the proportion of L-enantiomers in the polymer.

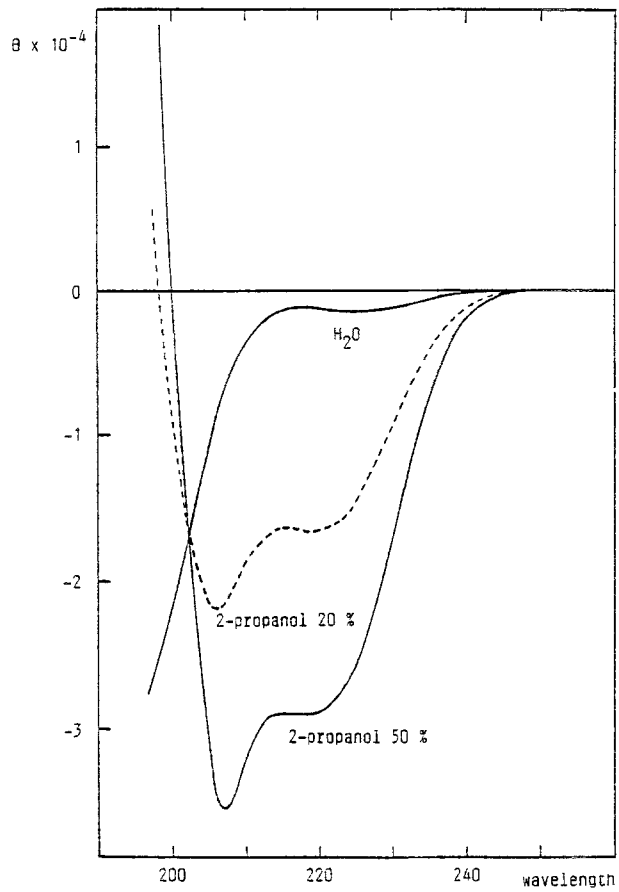


Fig. 5. Circular dichroism spectrum of poly (Leu-Lys) in the random coil conformation obtained in pure water (H_2O), and in the α-helical form obtained in two alcohol/water mixtures.

acids must display their property to a marked degree. For instance, poly (αAbu-Lys) associating L-α amino butyric acid with L-lysine, does not form β-sheets and was found to be 15 times more sensitive to mild hydrolysis than poly (Leu-Lys) (Brack, 1987).

Aggregation of alternating sequences to form β-sheets is possible only with homochiral (all-L or all-D) polypeptides. For instance, racemic alternating poly (D,L-Leu-D,L-Lys) is largely unable to adopt the β-structure and remains mostly unstructured (Brack and Spach, 1979a). When increasing amounts of L-residues are introduced into the racemic alternating polypeptide, the proportion of β-sheets increases and there is a good relationship between the percentage of β-form and the amount of L-residues in the polymer (Figure 4). The molecules can be described as a mixture of β-sheets and disordered segments. Those segments containing six or more homochiral residues aggregate into stable nuclei of optically pure β-sheets surrounded by the more fragile heterochiral disordered segments (Spach and Brack,

1979; Brack and Spach, 1981b). The samples were subjected to mild hydrolysis. The kinetic measurements showed two rate constants in agreement with the existence of two conformational species. After partial hydrolysis, the remaining polymeric fraction was enriched in the dominant enantiomer (Brack and Spach, 1979b). The enantiomeric excess (absolute value of %L-%D) had increased from 54 to 68%. An identical effect has been observed with homochiral helices since they are more stable than the disordered segments. Bonner *et al.*, (1980) raised the enantiomeric excess from 45 to 55% by partial hydrolysis of heterochiral polyleucine.

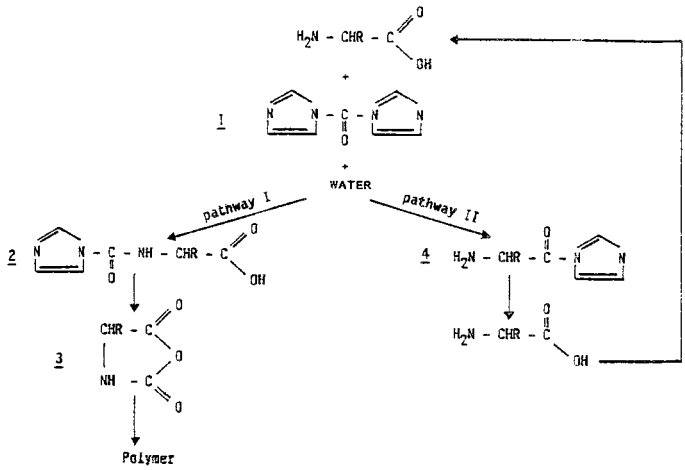
It is hard to see what factor other than hydrophobic interaction between residues confined to one side of a β-sheet could account for the general tendency to form β-sheets. When the properties of water are modified by addition of increasing amounts of alcohol, the repulsion between charges and the stress on the hydrophobic groups are progressively released. Under these conditions, an α-helix is obtained for poly(Leu-Lys) (Figure 5) instead of the β-bilayer described above. Even more interesting, when the alcohol is added to an aqueous solution of alternating poly-(Leu-Lys) already transconformed into the β-structure by addition of salt, a β to α transition is obtained (Figure 2), probably by relaxation of the water constraint on the hydrophobic groups (Brack and Spach, 1981c).

Poly (Leu-Lys) has an additional power. It strongly accelerates the hydrolysis of oligoribonucleotides (Barbier and Brack, 1988; Barbier and Brack, 1992). The polypeptide binds to the oligonucleotides and undergoes a coil to β-sheet transition. The β-sheet geometry plays a determinant role in the observed activity since basic polypeptides which do not adopt this conformation, such as poly (D,L-Leu-D,L-Lys) are inefficient. Even a β-forming basic decapeptide is large enough to exibit an efficient hydrolytic activity (Brack and Barbier, 1990).

Liquid Water Can Drive Chemical Reactions

Water molecules are good nucleophiles which compete efficiently with other nucleophiles. Water is feared by organic chemists because of its ability to spontaneously hydrolyze energy rich chemical bonds. However, under certain conditions, hydrolysis favours unexpected pathways.

Activation of amino acids by N,N'-carbonyldiimidazole (CDI) in aqueous solution illustrates this point. In organic solvents, CDI is known to activate N-protected amino acids R-COOH to give the corresponding imidazolide R-CO-Im (Anderson and Paul, 1958; Paul and Anderson 1960). Added to free amino acids in organic solvents, CDI led to aminoacylimidazolides (Gottikh *et al.*, 1970; Weber and Lacey, 1974). In the presence of water, aminoacylimidazolides are quite stable between pH 3 and pH 9, with nevertheless some hydrolysis (pathway II in Scheme 2). They polymerize to afford oligopeptides but also substantial amounts of diketopiperazine, a cyclic dipeptide which is a dead end (Weber and Lacey 1974). The formation of imidazolides directly in water seems unlikely. Only 6% of the expected acetylimidazolide was measured *in situ* by IR spectroscopy when CDI was added to



Scheme 2. Liquid water allows the polymerization of amino acids via N-carboxyanhydrides.

acetic acid in D_2O solution and the imidazolide absorption band could not be detected when CDI was added to free amino acids (Brack, 1987). Under those conditions, most organic chemists would consider as hopeless the use CDI for the polymerization of amino acids in water. Ehler and Orgel (1976) nevertheless did the experiment and obtained oligopeptides *via* the intermediary formation of N-imidazoyl carbonyl amino acid *2*. By continuous extraction of the reaction mixture with chloroform, we were able to isolate a compound presenting the IR absorption bands characteristic of N-carboxyanhydrides *3*. N-carboxyanhydrides are good candidates for the selective polymerization of proteinaceous amino acids in water (Brack, 1982; Brack, 1987). L-leucine, treated with CDI, afforded optically pure oligo-L-leucines in 70% yield with an average molecular weight of 8. Oligomers up to the 11-mer were identified with glutamic acid. No γ-linked Glu-Glu, cyclo (Glu-Glu) or PyroGlu-Glu could be detected. The preformed Glu-Glu dipeptide and γ-aminobutyric acid did not condense significantly under the same conditions although they form N-imidazoylcarbonyl derivatives. Ring-closure of *2* is possible with 5-membered rings (α-amino acids) and to a lesser extent with 6-membered rings (β-amino acids); it is not effective with larger rings (γ-amino acids, γ-carboxylic group of glutamic acid, dipeptides etc.). α-aminoisobutyric acid did not polymerize. N-derivatization does not occur because of the steric hindrance of the disubstituted α carbon atom. A mixture of amino acids close to that found in the Murchison meteorite was treated with CDI in water. The condensate was found to be enriched in proteinaceous amino acids (Brack, 1987).

Conclusion

Hydrolysis is not always the sterile cul-de-sac which threatens the organic chemists. When the chemistry has two possible pathways, hydrolysis can forbid the main pathway, thus allowing other reactions which would have few chances to occur in an organic solvent.

Thanks to the physical properties of liquid water illustrated in scheme I, a small piece of information, i.e. alternation of hydrophobic and ionizable hydrophilic residues can be amplified *via* stereoselective aggregation into thermostable β-sheets endowed with chemical activity.

The early histories of Mars and Earth clearly show some similarities. The existence of large valley networks and different channels strongly suggests that liquid water was once stable on the surface of Mars. If organic molecules were brought to Mars by comet or meteorite impact, then an aqueous organic chemistry might have existed on Mars until liquid water disappeared about 3.5 billion years ago. The search for organic molecules and fossilized primitive life below the Martian surface is a fascinating prospect for Mars exploration.

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