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The paper is dedicated to Professor Maciej Wiewiórowski
Review

The decisive role of the water structure in changes of conformation of nucleic acids*

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This review summarizes data on the structure and properties of water under normal conditions, at high salt concentration and under high pressure. We correlate the observed conformational transitions in nucleic acids with changes in water structure and activity, and suggest a mechanism of conformational transitions of nucleic acid involving these changes. We conclude that the Z-DNA form is induced only at low water activity caused by high salt concentrations and/or high pressure.

Water molecules influence specific interactions in all biological systems and yet it is still extremely difficult to understand and describe their effect in terms of precise atomic models. In recent years, there has been a tremendous increase in the number of new X-ray solved crystal structures of protein–nucleic acid complexes and other biomolecules. Many of those structures feature water molecules buried at the intermolecular interface, and allow to assign them with a reasonable confidence to the coordination space of hydrogen bond donors and acceptors. Thus, hydrogen bonding networks between proteins and

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Abbreviations: CD, circular dichroism; VRT, vibration-rotation tunneling.

nucleic acids often include water molecules, which have not been anticipated from our previous understanding of the molecular basis for macromolecular recognition, restricted to formation of direct contacts between functional groups. The main purpose of this article is to correlate the effects of salt and pressure induced changes in water structure with changes in conformation of nucleic acids. We were strongly motivated to do this by the results of our own studies in this field of research. We would like to put forward the question of how water interacts with a solute, which is more appropriate than the question of how macromolecules interact with water. For this purpose in this paper we review the results of various studies on water structure under normal and high pressure in the presence of electrolytes and on interaction of water with nucleic acids under these conditions.

PROPERTIES AND STRUCTURE OF WATER

Water has physical properties very different from those of many other liquids. The peculiarity of these properties is often ascribed to the unique structure of water, which is believed to consist of the three dimensional arrangements of hydrogen-bonded single H_2O molecules. The versality of water interactions has exerted a profound influence on the function, specificity, and evolution of biomolecules. There are many compounds of similar electronic structure to that of H_2O, but water is unique as a liquid because of its great ability to form clusters of highly hydrogen-bonded regions surrounded by non-hydrogen bonded molecules [1]. The complexity of liquid water structure is due in particular to a combination of its small molecular size and distinct polar charge distribution. Water molecule can be modeled as four charges located along the four arms of a tetrahedron, this allows each water molecule in the liquid state to participate in strong polar interactions (electrostatic charge-dipole or hydrogen bonding) with a high degree of spatial directionality [2, 3]. The tendency of water to form the most stable energetically hydrogen-bonded networks at normal pressure leads to appearance of various polymorphs of ice characterized by a different local arrangement of H_2O molecules with its four neighbours [4]. Recent calculations have supported the view that water occurs in quasi-planar cyclic forms with each monomer acting as a single donor and single acceptor, with free hydrogens oriented above or below the ring, which constitute the lowest energy structures for the trimer, tetramer and pentamer forms [5, 6]. Rotational constants and VRT spectroscopic data indicated the occurence of a cyclic, quasi-planar minimum energy structure with the O-O (interoxygen) separation of 2.78-2.76 Å. This is in compliance with the *ab initio* calculations, which gave the O-O distance of 2.74 Å [7, 8]. Very similar results have been obtained using the resonant ion-dip infrared spectroscopy. In the infrared spectra, the O-H stretch in the region of 3000-4000 cm^{-1} shows a strong dependence on the water cluster size. Hydrogen bonds polarize water molecules, and the induced polarities become maximized in the cyclic ring structure. Within the limits of geometrical restraints, the smaller the ring, the greater the polarization and the greater the cooperativity [9]. Using the available data on the structure of ice and water dimers in the gas phase, it has been shown that it is possible to provide a model of the water structure involving isomerization between clusters which reproduces the anomalous heat capacity of liquid water from 0 to 100°C. The clusters are polycyclic, cubic octamers which can dissociate into two cyclic water tetramers: $8H_2O \leftrightarrow 2(H_2O)_4$. The calculated interoxygen distances of octamers are of 2.83 Å. If the van der Waals radius of 1.51 Å is assigned to the oxygen atoms at the corners of the cube, then its edge has

2.83 + 2(1.51) = 5.85 Å and its volume is 16.6 cm^3/mol of H_2O. For the tetrameric form the O-O distance is 2.89 Å and the volume 17.9 cm^3/mol of H_2O. Here the H atom increment is based on the 4 cm^3 difference in molecular volume between cyclobutane (77.8 cm^3/mol) and cyclobutene (73.7 cm^3/mol). Both these clusters have molecular volumes that are too small to account for the observed molar volume of liquid water. This, however, immediately suggests that, if liquid water is an assembly of those units, there has to be strong H-bonding between clusters accounting for the larger molar volume of liquid water [10]. Such H-bonding would prevent efficient space filling, suggested by the cubic and half cube shapes of these simple clusters, without adding too much to the cohesion of liquid water. The pure liquid octamer should have a total energy of vaporization 10.6 kcal/mol of water monomer made of contributions from H-bonding (8.4 kcal/mol), dipole interaction (0.4 kcal/mol), van der Waals interaction (1.2 kcal/mol) and RT (0.6 kcal/mol at 300 K). The calculated energy of vaporization is in agreement with the measured value of 10.5 kcal/mol. These calculations, although semiquantitative, strongly support the model [10]. Application of neutron diffraction to water/heavy water mixtures enabled to obtain an approximate picture of the atomic structure of water. This approach made possible direct determination of radial distribution functions of individual pairs, which for water are represented by $g_{HH}(r)$, $g_{OH}(r)$ and $g_{OO}(r)$. The first two define the hydrogen bonding network of the liquid [11].

It is interesting to know to what extent the structure of water is modified by the presence of inorganic ions. For example, in aqueous solution of lithium chloride Li^+ ions have a coordination number which varies from about 6 at a concentration of 4 M to about 3 at 10 M, but Cl^- ion always possesses 6 water molecules in the first hydration shell over a wide range of concentrations, and a much weaker hydration structure [11]. As one can expect, both ions modify the water network to a significant degree. It has been shown that the number of hydrogen bonds is significantly reduced in 10 M solution relative to that in 1 mM LiCl and intramolecular water structure is not influenced by addition of LiCl. However, at 10 M LiCl concentration, there is a significant decrease in correlation between the nearest non-hydrogen bonded neighbouring molecules [11].

HIGH PRESSURE

High pressure is still a mysterious technique in biology. When applied to the naupliar stages (larvae) of the marine copepod *Tigriopus californicus* high pressure converts into females some individuals that would have become males [12].

At -22°C and 210 MPa (megapascals), water remains in the liquid state due to the fact that transition from the liquid to the ice I phase is accompanied by an increase in volume, which is counteracted by pressure. The melting point of the ice rises with increasing pressure and is 20°C at 880 MPa and 30°C at 1036 MPa [13]. This means that self-organization of water is efficiently promoted by pressure. The change in volume of water on ionization into H_3O^+ and OH^- ions is -22.2 cm^3/mol at 25°C. The dissociation volume of ammonia is even higher than that of water. Protonation of amine groups is promoted by pressure, as is the transition of NH_3^+-glycine and NH_3^+-alanine to the zwitterionic form (7 to 8 cm^3/mol) [13]. However, proton transfer from NH_3^+ to water is associated with a positive change in the reaction volume. Since the length of covalent bonds is limited by the Born repulsion, therefore for the practical purposes of various experiments in liquid water (pressure < 12 kbar) these bonds are considered rather incompressible (by 4% at 100 MPa and 22°C, and by 15% at 600 MPa and

22°C). Under high pressure the bond angles behave similarly to the bond length, so that a molecular covalent architecture may be considered as unchangeable within the range of pressures at which water remains liquid at ambient temperature [14, 15]. The interaction of water with ionic charges provides one of the most important reasons for the volume decrease. Due to the high electric field of an ion, water molecules are oriented around it and cause a local collapse of the bulk water structure. Since the water molecules are more firmly packed around the ion than in bulk water, the volume of the system decreases. This process, called electrostriction, is a result of ion-water interactions and it mainly depends on the charge and the radius of the ion and the changes in the local dielectric constant around the ion. The electrostriction results in compression of adjacent dipoles at pressures equivalent to a few thousand bars. Reactions in which reactants and products differ in the number of ionizable groups are the most strongly influenced by pressure. The application of pressure shifts the equilibrium towards the state that occupies a smaller volume and accelerates processes for which the transition state has smaller volume than the ground state. This simply means that pressure favours processes that are accompanied by negative volume changes. Non-covalent interactions constitute the main target for the modulation of molecular characteristics through changes in pressure. On the other hand, formation of coulombic interactions following dehydration of charged atoms is accompanied by positive changes in volume (ΔV) and is not favoured by pressure [16]. Hydrophobic reactions have a positive ΔV, whereas stacking of aromatic rings leads to a negative ΔV but hydrogen bonds are pressure insensitive [17]. However, for complex biological macromolecules it is difficult to interpret ΔV on the molecular level, because too many interactions are being disrupted and formed simultaneously.

THE EFFECTS OF HIGH PRESSURE ON WATER STRUCTURE

Application of pressure leads at first to a decrease in the melting temperature at 207 MPa and 251 K, until the triple point: water-ice I-ice III is achieved and a positive slope for $T_m(p)$ of ice III and ice IV is observed [4, 16–18]. At the resonant pressure of 25 GPa, ice VII has a body centred cubic lattice of oxygen atoms with some disorder in the positions of the protons. At 2.6 GPa and 293 K, the observed interoxygen distance in phase VII of D_2O is 2.9 Å, whereas the O–H distance is about 0.95 Å. However, the distance O–O falls to 2.56 Å at 25 GPa [15]. The changes in the density of water on increasing the pressure from 1 bar to 10 kbar was calculated from the simulations to be 25.3%, but compressibility was estimated to be 1.8×10^{-2} kbar^{-1}. The latter value is smaller than the experimental value of 4.5×10^{-2} kbar^{-1}, most probably because of the non-linear response of water to applied pressures greater than 4 kbar [19]. Alkali halides at high concentrations significantly affect the phase diagram of water. At low concentration and low pressure, NaCl acts as the structure breaker distorting the hydrogen bond network of pure water. At higher concentrations and higher pressure, the structure of neat water is already distorted [18, 20]. Using neutron diffraction to compare the effects of high pressure and high salt concentrations on the hydrogen-bonded network of water, it has been found that the ions induce a change in the water structure equivalent to that evoked by high pressure. In addition, this effect is ion specific. Ion concentrations of a few moles per litre are equivalent to pressure of a thousand atmospheres. These changes may be understood in terms of partial molar volume of the ions, relative to those of water molecules [21]. Under ambient conditions, the intermolecular HH-pair correlation function for pure water has two main peaks at 2.4 Å and 3.8 Å

with a minimum between them at 3 Å. Two peaks are the result of the strong orientation correlations between neighbouring water molecules in the hydrogen bonded water. However, in NaCl solutions and at high pressure, the peaks disappear almost completely. These findings strongly suggest that the orientation correlations become more disordered as pressure is applied to pure water [21]. The same arguments are valid to explain the lowering of the melting point of water as a function of pressure. Assuming that the density of water changes by 0.0012 molecules per $Å^3$ per 1 kbar pressure, the equivalent pressure is 1.4 kbar for 4 M sodium chloride, 0.3 kbar for 2 M sodium sulphate and 0.8 kbar for 2 M ammonium sulphate. The differences in equivalent pressures can be understood by considering the partial molar volumes of the respective salts. Both ammonium and chloride ions have partial molar volumes close to that of water (18 cm^3/mol) and these values do not change substantially with salt concentration. On the other hand, both sodium and sulphate ions have significantly smaller partial molar volumes than water, which suggests that these ions have substantial electrostrictive effects on water structure and introduce changes in the common tetrahedral coordination of water molecules [20, 21]. The short range spatial arrangement of the water molecules is weakly affected by temperatures ranging from 298 K to 423 K and pressures up to 1.9 kbar. Water molecules show strong orientation with the O–H bond on one molecule pointing towards the oxygen of another, although from the broadening of the peaks of the HH function one can argue that the angular spread of preferred orientations increases with temperature. Hydrogen bonds are still present at 423 K in a significant number. At 573 K and 95 kbar the average separation of the pair correlations for OO, OH and HH atoms on neighbouring molecules is increased and the number of hydrogen (H) atoms around a central oxygen (O) up to a distance about 2.4 Å is decreased significantly compared to water at ambient conditions. Since the peak at 4.5 Å in $g_{OO}(r)$ function disappears, it has been suggested that on such conditions the tetrahedral coordination of water is lost. However, increasing of the pressure up to 2.8 kbar at 573 K gives the same density as at 423 K and leads to an increase in the number of nearest neighbour water molecules (about 8) with a better definition of the average OO nearest neighbour distance than at the lowest density. These results show that as the pressure is increased, the hydrogen bonds are easily formed, but the local structure of water molecules is no longer in the perfect tetrahedral arrangement. It was also observed that pressure is ineffective in changing the longer range structure at elevated temperatures [22].

B→Z CONFORMATIONAL TRANSITIONS IN DEOXYRIBONUCLEIC ACIDS

More than thirty years ago it was noticed that CD spectra of *Escherichia coli* DNA differed over a wide range of humidity. When the relative humidity reached 84%, there was a very strong CD minimum at 280 nm due to n-π^* transitions, very characteristic of the Z-DNA form [23]. The crystal structures for Z-DNA, A-DNA and B-DNA were solved with resolutions around 1.2 Å, 1 Å and 2Å, respectively, and therefore positions of water molecules were well determined. They are hydrogen bonded to the oxygen and nitrogen atoms of the nucleic acid bases and to the phosphate groups. The equilibrium between the right handed B-form and the left handed Z-form of DNA, is determined by three factors: chemical structure of the polynucleotide (sequence and modification), environmental conditions (solvent, salts, pH, temperature) and the degree of topological stress (supercoiling, cruciform formation). The intrinsic free energy difference between the Z- and B-forms is close to 2 kJ/mol for poly(dG-dC), only 1 kJ/mol for

poly(dG-dm^{5}C) and larger than 5 kJ/mol for poly(dA-dT) pairs. The transition enthalpy ΔH for the B- to Z-DNA transition per base pair mole amounts to 2, 4 and 3.1 kcal for poly d(G-C), poly d(A-T) and poly d(A-C) poly d(G-T), respectively [23]. It has been suggested that the regular Z-type helices of DNA require a minimum of four consecutive G–C base pairs for left handed helix stabilization. However, the structure of methylated or brominated nucleotide CGTACG at the C5 position does not possess the four consecutive CG base pairs, nevertheless it occurs as Z-DNA, probably due to the effect of stabilization offered by either substituent. At the d(CG) steps, the deoxyribose of the cytidine nucleoside is situated in such a way that its O4′ atom sits directly over the six membered ring of the guanine base residue [24]. Specific location of the two O4′ lone pair-electrons provide stabilization through an inter-cytidine O4′···H6···C6 hydrogen bond [25], probably due to $n_{04} \rightarrow \sigma^{**}_{C6H}$ hyperconjugative effect and an $n \rightarrow \sigma^{**}$ interaction with guanidinium system of the stacked guanine base [24]. It has been suggested that stabilization of Z-DNA upon modification stems from the destabilization of B-DNA through distorting hydration in the major groove and favourable hydrophobic interactions of the C5 substituents on the convex surface of Z-DNA. Electronically, the methyl group is a σ donor which will raise the pK of C6 rendering thus N3 ring nitrogen a stronger donor. The most important difference between various forms of DNA seems to be in the conformation of the sugar ring, which is related to the distance between the phosphate groups at both ends of each nucleotide and to the displacement of the base pairs relative to the helical axis. Different sugar puckers give rise to the wide phosphate-phosphate average separation of 6.6 Å in B-DNA, 5.3 Å in A-DNA and 5.1 Å, in Z-DNA. If so, B-DNA occurs under conditions of high water activity and A- and Z-DNA at low water activity. This means that hydration of A- and Z-DNA, where adjacent phosphate groups are bridged by water molecules, is more economical and efficient than that of B-DNA, where each phosphate group is separately hydrated. It is known that binding of water molecules to the charged phosphate oxygen atoms is energetically more favourable than binding to base and sugar atoms. If the water activity (effective concentration) is high, the number of water molecules available for hydration is high, and the phosphate groups will be fully and individually hydrated. If salt or an organic polar solvent is added, water activity is reduced and its molecules are moved out from the DNA and, consequently, hydration becomes more restricted or economical. This means that the economy of hydration is achieved by bridging adjacent phosphate groups though water molecules, followed by a change in conformation of the sugar residues that results in the phosphate groups moving closer to one another. Besides the structure dependent hydration of DNA, there are sequence dependent hydration schemes such as 'the spine of hydration' in the minor groove. It has also been found that upon a continuous increase of ionic strength, the well established B→Z conformational change is followed by another transition, namely from Z- to a B-like structure. The CD spectra exibited by the high salt conformation of the B-like and the B-DNA are almost superimposable. Interestingly, apart from a few water molecules coordinated to phosphate groups and bases, the DNA minor groove is essentially dry [23]. To a certain extent, this lack of hydration can be attributed to the stacking of terminal base pairs from two neighbouring molecules into the minor groove. The extensive hydration of this groove in RNA is directly related to the presence of the ribose 2′-OH groups, which are engaged in the total number of 25 hydrogen bonding interactions. Each of these 2′ oxygens is coordinated, on average, to two water molecules, except for the two terminal cytidines from one strand. Importantly, in the majority of base pairs, water molecules link the 2′ hydroxyl groups with

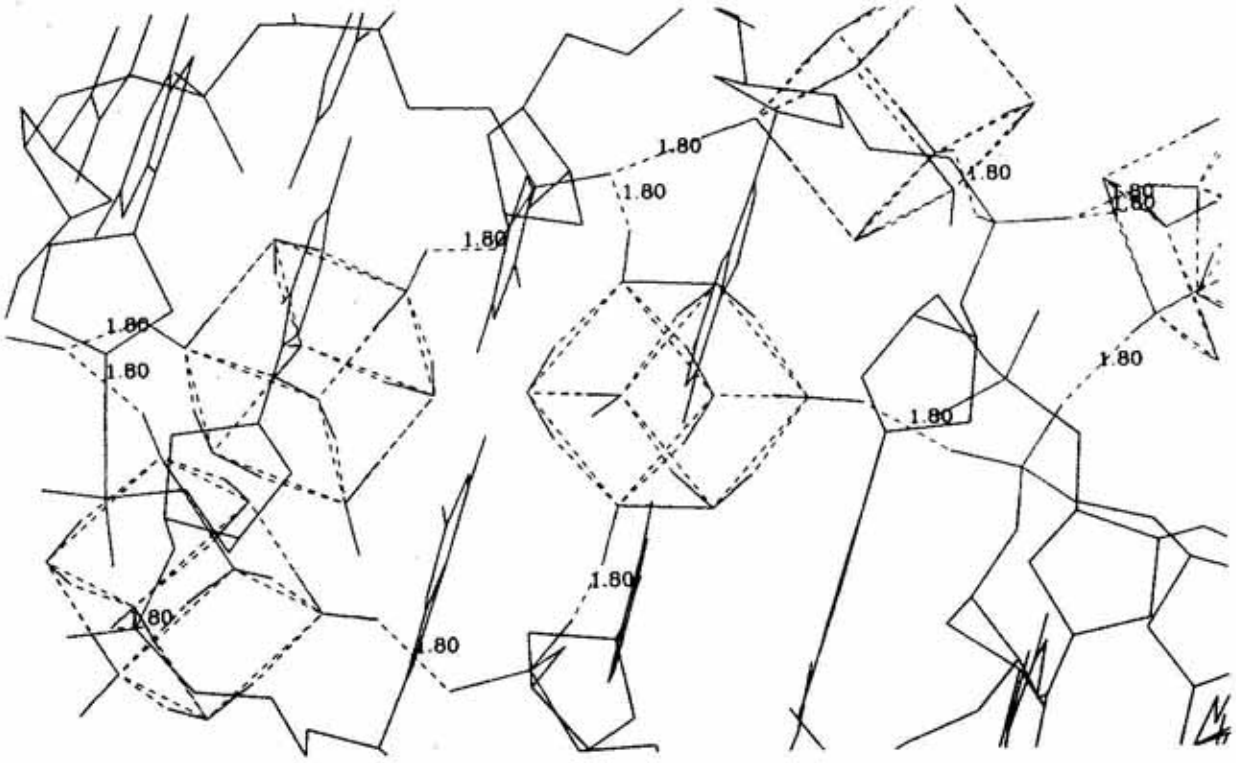
the O2′ oxygen of cytidines and the N3 nitrogen of guanines [24].

MECHANISM OF THE HIGH PRESSURE INDUCED CHANGES IN DNA CONFORMATION

In the discussion of the mechanism one should keep in mind that pH of water is shifted by 0.3 unit as pressure is raised up from 0.1 to 100 MPa [27].To understand the effects of high pressure on DNA conformation, we have studied poly(dG-dC) with circular dichroism spectroscopy. The CD spectra of the samples were measured after releasing of high pressure. It has been shown that, at the pressure of 6 kbar, poly(dG-dC) changes its conformation from B-DNA to Z-DNA as traced by the appearance of a negative Cotton effect at 295 nm [27]. This Z-DNA diagnostic band has not been observed at 10 kbar. Similarity between the CD spectra of poly(dG-dC) exposed to a pressure of 10 kbar, and that at ambient pressure (e.g. B-form), suggested that at very high pressure (10 kbar) a transition to B-like DNA may occur. As earlier discussed, equivalence of pressure and salt effects on the structure of water seems to be in agreement with the data obtained for the B⇔Z⇔B* DNA transitions of two oligodeoxynucleotides, $d(CG)_8$ and d(ATATATCGCGCGCGCGCG), induced by a high magnesium chloride concentration. It has been shown that the B→Z transition in DNA in solution is sensitive to many experimental parameters, such as temperature, salt species and concentrations, and polyamines as well as high pressure [27, 28]. The pressure-induced changes in the CD spectrum of poly(dG-dC) attributed to B→Z DNA conformational transition proved fully reversible at normal pressure. Therefore one can conclude that dehydration is the driving force of the B→Z DNA conformational transition. To understand the data indicative of the B→Z transition in DNA at high pressure one should consider them in terms of the nucleic acid hydration shell, the density, compressibility and structure of which are different from those of bulk water. The compressibility of bulk water approaches the compressibility of water in the hydration shell of DNA only under hydrostatic pressure greater than 1 kbar. The density of water in the DNA hydration shell is by about 25% higher than that of bulk water. From neutron diffraction studies it is known that the characteristic tetrahedral structure of water becomes modified in concentrated ionic solutions [22]. The ions induce changes in the structure of water equivalent to that caused by application of high pressure [11]. For ionic solutions orientations correlations of water (HH correlation functions of pure water) become more disordered when pressure is applied [21]. The formation of cubes (octamers) of water is accompanied by reduction of its volume by 1.3 cm^3/mol. Thus, octameric water structure can accommodate the oxygens of phosphates of the polynucleotide chain folds into the Z-DNA. To prove this we present our results on docking (Barciszewski, J., unpublished) of octameric water, formed under high pressure, as proposed by Benson & Siebert [10] to the crystal structure of the Z-DNA of $(CG)_3$ (Fig. 1). The highly symmetric octameric water cluster interacts with the phosphate backbone of Z-DNA. The distances between the phosphate oxygens determined by X-ray crystallography exactly fit the size of the water cube. The water molecules are precisely localized between the two phosphate groups: 1–3, 3, 5 etc., on each DNA strand (Fig. 1) and stabilize the Z-DNA form. The octamers of water are easily accommodated in the minor groove by interstrand bridging which stabilizes the Z-DNA form. The cubes of water make contacts not only with phosphates along and across the helix, but also with other cubes, and form a network (Fig. 1). It is clear that reversible changes in water structure from tetrameric to octameric are the driving force for the conformational switches of DNA.

HIGH PRESSURE MEDIATED CHANGES IN RNA CONFORMATION

The effects of high pressure on RNA duplexes: $r(GC)_6$ and $r(AU)_6$ were studied by means of circular dichroism spectroscopy [29]. The measurements were done at the ambient pressure, after release of the high pressure. The CD spectra of oligoribonucleotides in normal conditions showed maxima at 260 nm, but at the pressure of 6 kbar the CD maximum was shifted to higher wavelengths. A new band above 300 nm also appeared, indicative of aggregation of RNA. At the pressure of 10 kbar, aggregates were also visible but the maximum of the main peak was shifted by 5 nm and showed a higher intensity. Thus, apart from the observed aggregates, the high pressure induced some small changes in the conformation of RNA [29]. It is known that the salt concentrations suitable to induce Z-DNA formation, are not sufficient to drive A-RNA into the Z-RNA form. For this purpose, dehydration agents, such as concentrated solutions of $MgCl_2$ or $NaClO_4$, have to be used. Indeed, the CD spectrum of poly r(G-C) in 6 M $NaClO_4$ at 41°C has been identified as due to the Z-RNA form [29]. Treatment of the oligoribonucleotides $r(GC)_6$ or $r(AU)_6$ with 5 M sodium chloride alone did not affect their A-RNA conformation. However, exposure of these oligoribonucleotides to 5 M NaCl and high pressure simultaneously induced conformational changes manifesting themselves in the CD spectra by appearance of two new positive peaks around 240 nm and 295 nm, atributable to the Z-RNA form [29]. The transition of A→Z in RNA depends on dehydrating properties of the solvent [30]. Water molecules are involved in strong interactions



Barciszewski Fig. 1

Figure 1. The Z-DNA conformation of the (CG) containing oligodeoxynucleotide with the water docked in octahedral form (A).

The cubes of water [10] which are formed under pressure are located exactly between the two phosphate groups of DNA chains and bind them through hydrogen bonds, length of which is shown in ångstroms. Such interaction induces formation of the Z-DNA form, which is furthermore stabilized by the conjugate effect between O4′ of the deoxyribose of cytosine with H6 of the same nucleoside, and the NH_2-group of guanosine at the 3′ side [24]. The Z-DNA coordinates were taken from Brookhaven Data Bank.

with the 2'-OH groups of RNA and therefore synergetic effects, e.g. high pressure and high salt, are needed to effect a decrease in water activity, and thus in its structure and in conformation of RNA. An oligoribonucleotide with the purine-purine-pyrimidine-pyrimidine alternating sequence does not form Z-RNA at high pressure alone, and only but slight changes in CD spectrum of r(GGCCGGUUAAUU) r(AAUUAACCGGCC) duplex were observed. The CD spectra of DNA–RNA heteroduplex $d(GC)_3(AT)_3$ $r(AU)_3(GC)_3$ were not very sensitive either to salt or to high pressure [31]. For DNA:RNA heteroduplexes there was no A→Z RNA conformational transition observed though nucleotide sequence is made of alternating purine-pyrimidine bases. It is possible that each of the two strands is hydrated differently due to the presence of 2'OH group on the RNA part. Therefore, each strand may have a different geometry so that, the double-stranded molecule is not able to attain the Z-type conformation stabilized by a regular water network under a high pressure. CD spectra suggest that, at high pressure conformation of heteroduplexes is neither in the A nor B form. In the presence of 5 M sodium chloride, high pressure induced a shift of the Cotton band to longer wavelengths suggesting rather an A type conformation. This seems to be in good agreement with earlier observations that at lower humidity heteroduplexes tend to attain the canonical A-RNA form [31].

Recently we have shown that tRNA can be specifically aminoacylated upon high pressure treatment in the absence of an aminoacyl-tRNA synthetase (AARS) and adenosine

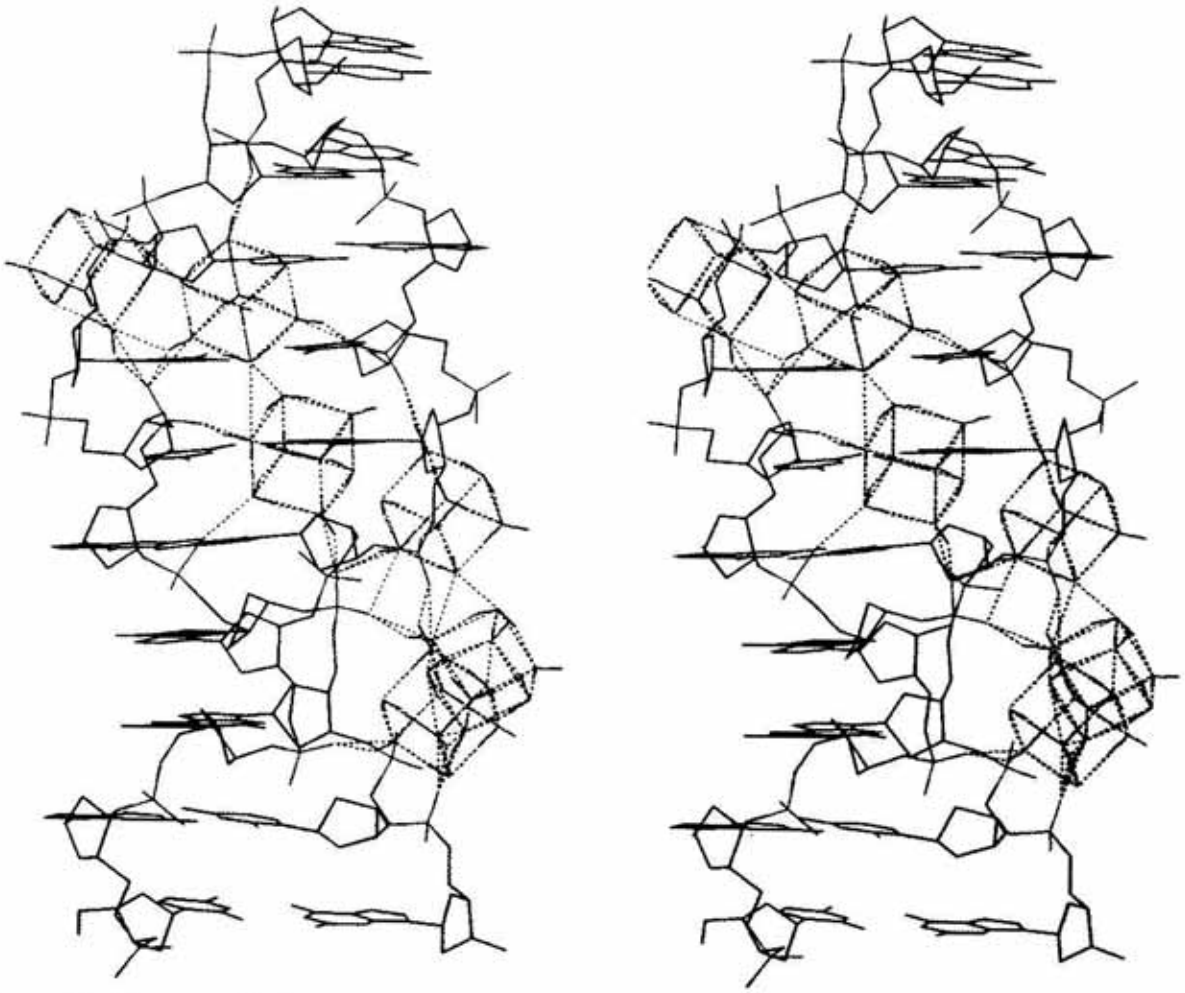


Figure 1B. The same in the stereo presentation.

triphosphate. The CD spectra of tRNA measured before and after exposure to the high pressure were only slightly different than, but similar to, those induced by AARS [32] Therefore we used an amino acid as an "internal probe" for tRNA changes. Once we got tRNA aminoacylated at high pressure we speculated that functional tRNA conformation, formed by means of the energy transmitted to the system under high pressure might be similar to that induced by AARS [32, 33]. In the newly acquired conformation, tRNA would have higher free energy, sufficient to induce acylation of the molecule by dissipation of energy into the charging pathway. At the high pressure we have obtained $tRNA^{Phe}$ and $tRNA^{Met}$ specifically charged with phenylalanine and methionine, respectively [32, 33]. It is known from organic chemistry that esterification of acids with alcohols in the absence of a catalyst is an extremely slow process. However, higher yields of esters can be obtained at 10 kbar. The most interesting question here is the specificity of tRNA charging. We showed formation of cognate Phe-$tRNA^{Phe}$ and Met-$tRNA^{Met}$ in the presence of non-cognate serine. In spite of many potential acylation sites present in native tRNAs, only the terminal 3' adenosine was charged, but not other nucleosides, e.g. nucleoside X, 3-amino-3-propyluridine in position 47 of *E. coli* $tRNA^{Phe}$. The high pressure charged tRNA was fully active in protein biosynthesis assays *in vitro* [33–37]. The mechanism of high pressure aminoacylation of tRNA is an intriguing question. From the previous discussion it is clear that effects of high pressure depend on the hydration rather than on intrinsic properties of nucleic acids. Since DNA susceptibility to changes in conformation is much higher than that of RNA, it is obvious that 2'-OH groups are involved in the hydrogen bonded water network. Pressuring of tRNA leads to changes in its conformation and to its aminoacylation. Thus, an amino acid molecule has to be fixed in a "pocket" formed within the amino-acid stem close to the 3' end of the tRNA molecule. These observations support the hypothesis that recognition of amino acids by RNA might have been developed in the evolutionary sense in conjunction with an operational RNA code present in the tRNA acceptor stem. They support also the notion that genetic coding originates from direct templating on RNA surface of amino acids at the least polar ones.

CONCLUSIONS

High pressure is a physical parameter which only recently began to be applied in biological studies and biotechnology. It is an especially "clean" and efficient agent used to perturb the equilibria and kinetic processes, and permitting to obtain information used subsequently in establishing the relationship between structure and function of macromolecules.

The presented analysis of pressure effects on nucleic acids suggests that lowering of the molecular volume of water under the high pressure leads to formation of water octamers which finally induce the B→Z change in DNA conformation. The same concerns RNA although the A→Z transition in RNA is more difficult to achieve due to the presence of the strongly hydrated 2'OH group. Full reversibility of pressure induced conformational transitions in nucleic acids after pressure release clearly supports the involvement and specific role of the structure of water in conformational changes of biological molecules.

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