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Features and principles of ultrasonic spectroscopy of aqueous solutions of nucleic acids and their derivatives

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The development of the principles of ultrasonic spectroscopy of biological macromolecules, such as nucleic acids, was initiated because of the necessity of studying the acoustic properties of biological media at the molecular level, and because of the opportunity to investigate chemical and physical reactions with relaxation times comparable to the period of the ultrasonic waves, viz., 10^{-9} to 10^{-7} s. The physical mechanisms of absorption considered significant relative to aqueous solutions of nucleic acids and their derivatives are: viscoelastic relaxation[11, 17], rotational isomerism[2, 12, 18], proton exchange reactions[15, 22, 23], breakdown-formation of hydrogen bonds[7], hydration equilibria[16, 20], and base stacking[10, 13, 18]. These investigations generally showed that the frequency dependencies of the ultrasonic absorption coefficients of these solutions exhibit broad distributions of relaxation processes in the range 1–100 MHz. This finding imposed limitations on the unequivocal interpretation of the ultrasonic absorption mechanisms in biomacromolecular solutions and accounts for the predominantly qualitative nature of analyses of the spectroscopic data. Nevertheless, analysis of the experimental material does permit us to establish principles and describe the main features of these preparations, in the megahertz frequency range. An effective approach, which reveals the predominant molecular mechanisms contributing to ultrasonic absorption in DNA, RNA nucleoside and nucleotide solutions, involves changing the physicochemical conditions of the sample during the acoustic measurements and enables the usual correlation between the absorption coefficient and particular intramolecular alterations to be estimated.

Only for purine nucleoside solutions (adenosine, guanosine) can the observed relaxation processes be described by single characteristic times, viz., 4.6×10^{-9} to 5×10^{-9} s associated with the reversible transition among two rotational forms[2, 12, 19] and 14.6×10^{-9} s for self-association of N^6, N^6-dimethyladenosine in water (at 25 °C). The kinetic constants of the forward and reverse reactions and the equilibrium constants of syn-anti transition

Possible ultrasonic absorption mechanisms and predominant relaxation processes in aqueous solutions of nucleic acids and their derivatives

Biopolymer	Ultrasonic absorption mechanism	Relaxation processes 10^{-7} to 10^{-8}
Purine nucleosides (adenosine, guanosine)	1) Intramolecular transition at glycosidic bond	Syn-anti transition equilibrium
	2) Self-association of bases	Base stacking relaxation
Nucleotides (5′-AMP, 5′-ADP, 5′-ATP)	Proton exchange between base nitrogen, as proton acceptor, and phosphate secondary groups, as proton donor, $pH_{max} = 5.1–5.5$	Relaxation of protolytic equilibrium reaction
Nucleic acids (DNA, RNA, polynucleotides)	1) pH < 5: proton transfer between adenine and cytosine bases and primary phosphate groups, N-Pl exchange, $pH_{max} = 3$	Intramolecular
	2) pH > 9: hydrolytic proton exchange, $-NC-\overset{}{C}=0 + OH^- \rightleftarrows H_2O + -N=C-0-$, $pH_{max} = 11.7–11.8$	Relaxation of protolytic reactions between charged groups of macromolecules
	3) pH = 6.7–7.5: breakdown-formation of hydrogen bonds and/or hydration interactions free water-bound water at polynucleotide chains	Relaxation of fast processes occuring in secondary structure transitions and/or biopolymer-solvent equilibria

glycosidic bonds in the adenosine molecule were first calculated using methods of ultrasonic spectroscopy[2].

The composition of the adenine nucleoside molecule with addition of phosphate groups results in the ultrasonic absorption mechanism in which proton transfer occurs between the secondary acid phosphate, as donor, and the protonated nitrogen, as acceptor, in aqueous solutions of 5′-AMP, 5′-ADP, 5′-ATP, according to the scheme[4,6,15,22,23] $R\text{-}PO_4H^- + = N\text{-}_1 \rightleftarrows R\text{-}PO_4^{-2} + -N_1H^+ -$.

The demonstrated participation of phosphate groups permitted the use of kinetic curves, of ultrasonic absorption decrease during the nonenzymatic hydrolysis of ADP and ATP, to calculate the apparent ('effective') rate constants of these reactions as 0.029 and 0.043 min^{-1}, respectively[6]. Ultrasonic spectroscopic methods can be used not only for determining the kinetic parameters of hydrolytic reactions in monomolecular solutions, but also for pseudomonomolecular reactions of RNA hydrolysis[1], for which the parameters are significant for comprehensive understanding of the biological functioning of these molecules.

Proton-transfer equilibrium reactions may yield excess ultrasonic absorption, of DNA and RNA solutions, only at pH extremes[5,14,21]. Absorption in the acid pH range below 5 is caused by proton exchange between the base nitrogen and the primary phosphate group of the nucleic acid N-Pl[14,21]. In the alkaline pH range, the hydrolytic proton-transfer reactions of the lactam groups of the bases guanine and thymine (uracil) are responsible for absorption maxima at pH 11.7–11.8[5]. The ultrasonic absorption in DNA and RNA solutions at neutral pH is probably not related to protolytic reactions, but may be caused by the participation of the breakdown-formation of hydrogen bonds and/or the hydration equilibria, with characteristic relaxation time of 10^{-6} to 10^{-8} s[7]. Such processes may characterize the fast stage in DNA heat denaturation; breakdown of the Watson-Crick structure when hydrogen bonds become broken, but the polynucleotide chains remain folded. The other stages in the helix-coil transition of DNA denaturation do not cause relaxation absorption at ultrasonic frequencies since the times required for conformational transitions are several orders of magnitude longer than ultrasonic measurements allow to be determined conveniently[8,9].

Perturbation of the solute-water hydration equilibrium by the ultrasonic wave is considered to provide an explanation of the possible mechanisms of ultrasonic relaxation in nucleic acid and synthetic polynucleotide solutions at physiological pH. It is believed that because of the acoustic wave propagation in the macromolecular solution, redistribution of water molecules, weakly bonded to polynucleotide chains, takes place, i.e. perturbation of the bound water-free water molecule equilibrium. Such relaxation frequencies are in the range of observation of nearly all investigations of nucleic acid and other biomacromolecular solutions. The significance of ultrasonic spectroscopy at present is mainly that of obtaining information on processes associated with the interactions of molecular groups and bonds with the solvents. That is, ultrasonic absorption in dilute biomacromolecular solutions is due to solute effects.

The table lists the possible predominant ultrasonic absorption mechanisms and relaxation processes, with characteristic times of 10^{-7} to 10^{-8} s, studied during the past 10–15 years.

The usefulness of ultrasonic spectroscopy is that it bridges the gap from methods for determining time constants by the traditional kinetic methods, greater than 10^{-5} s, to the optical spectroscopic methods, less than 10^{-10} s. Many studies have been carried out dealing with the ultrasonic absorption of nucleic acids and their derivatives and much detail has emerged. Prospects for future significance depend, perhaps, upon the development of theories describing the physical mechanisms of ultrasonic energy absorption associated with solvent-biopolymer interactions.

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