activity or a turn of about 180° may be observed. Therefore we have again good reason for concluding that all the activities of the gravioscilloscope and the systematic variations in the ultraoptic orientation of *Melolontha* are a consequence of the continuously shifting of the GWW-pattern.

Magnetically polarized magnetite crystals yielded five times surprising results at new moon and full moon. The curves indicating the direction of the float at intervals of 5 to 2½ min showed double spikes at the moment when the sun passed a height of +60, 45 and 30° and the moon + or −60, 45 and 30°. The instrument reacts even with a torque to differences of less than 0.2° in sunheight and the beam of the moon is quite efficient after passing the earth. It is a task of future research to decide whether this reaction is connected with the cubic lattice of the magnetized magnetite crystal or with geometrical characteristics of the gravitational waves.

A detailed description of the experimental methods and results is in preparation.

1 Schneider, F., Die Fernorientierung des Maikäfers während seiner ersten Frassperiode und beim Rückflug in das alte Brutgebiet. Verh. schweiz. natf. Ges. (1957) 95–96.
2 Schneider, F., Systematische Variationen in der elektrischen, magnetischen und geographisch-ultraoptischen Orientierung des Maikäfers. Vjschr. natf. Ges. Zürich *108* (1963) 373–416.
3 Schneider, F., Devitationsrhythmen in bezug auf künstliche magnetische Felder. Mitt. schweiz. entom. Ges. *47* (1974) 1–14.
4 Schneider, F., Die Beeinflussung der ultraoptischen Orientierung des Maikäfers durch Veränderung des lokalen Massenverteilungsmusters. Revue suisse Zool. *71* (1964) 632–648.
5 Schneider, F., Gibt es sinnesphysiologisch wirksame Gravitationswellen? – Ein Problem der ultraoptischen Orientierung. Vjschr. natf. Ges. Zürich *120* (1975) 33–79.
6 Schneider, F., Die Bestimmung der Länge von «Gravitationswellen» mit sinnesphysiologischen Methoden, und weitere Beweise für ultraoptisch determiniertes Verhalten nach Ausschluss richtungsweisender magnetischer Felder. Vjschr. natf. Ges. Zürich *122* (1977) 203–232.
7 Schneider, F., Ultraoptisch induzierte circahorare Verhaltensrhythmen. Verh. schweiz. natf. Ges. (1972) 169–171.
8 Schneider, F., Der experimentelle Nachweis magnetischer, elektrischer und anderer ultraoptischer Informationen. Z. angew. Entom. *77* (1975) 225-236.
9 Schneider, F., Geometrie und Auflösungsvermögen eines tierischen Rezeptors für Gravitationswellen. Vjschr. natf. Ges. Zürich *129* (1984) 142–171.
10 Kirschvink, J. L., and Gould, J. L., Biogenic magnetite as a basis for magnetic field detection in animals. Bio Syst. *13* (1981) 181–201.

0014-4754/85/111362-03$1.50 + 0.20/0

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

F. I. Braginskaya and F. Dunn

Institute of Chemical Physics, Academy of Sciences USSR, Moscow (USSR), and Bioacoustics Research Laboratory, University of Illinois, Urbana (Illinois 61801, USA)

Key words. Ultrasonic absorption; relaxation processes; kinetic constants; polynucleotide chains; nucleic acids.

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, $-NC-\dot{C} = 0 + OH^- \rightleftarrows H_2O + -N = C-O-$, $pH_{max} = 11.7–11.8$	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.

1 Braginskaya, F.I., Sov. Phys. Acoust. *19* (1973) 286 (Engl. transl.).
2 Braginskaya, F.I., Sov. Phys. Acoust. *20* (1975) 424 (Engl. transl.).
3 Braginskaya, F.I., Proc. 3rd All Union Conference on Problems of Ultrasonic Spectroscopy, Vilnius 1976, p. 35 (in Russian).
4 Braginskaya, F.I., and El'piner, I.Ye., Biophysics *18* (1973) 16 (Engl. transl.).
5 Branginskaya, F.I., and Sadykhova, S.Kh., Biophysics *24* (1979) 337 (Engl. transl.).
6 Braginskaya, F.I., and Zaretskii, A.A., Biophysics *18* (1973) 1017 (Engl. transl.).
7 Braginskaya, F.I., Zaretskii, and Sadykhova, S.Kh., Biophysics *18* (1973) 989 (Engl. transl.).

8 Cohen, R.J., and Crothers, D.M., J. molec. Biol. *61* (1971) 525.
9 Crothers, D.M., J. molec. Biol. *9* (1964) 712.
10 Garland, F., and Christian, S.D., J. phys. Chem. *79* (1975) 1247.
11 Hawley, S.A., and Dunn, F., J. chem. Phys. *50* (1969) 3523.
12 Hemmes, P.R., Oppenheimer, L., and Jordan, F., J. Am. chem. Soc. *96* (1974) 6023.
13 Heyn, M.P., Nicola, C.U., and Schwarz, G., J. phys. Chem. *81* (1977) 1611.
14 Lang, J., and Cerf, R., J. Chim. phys. *66* (1969) 81.
15 Lang, J., Yiv, S., and Zana, R., Studia biophis. *57* (1976) 13.
16 O'Brien, W.D. Jr, and Dunn, F., J. phys. Chem. *76* (1972) 528.
17 O'Brien, W.D. Jr, Christman, C.L., and Dunn, F., J. acoust. Soc. Am. *52* (1972) 1251.
18 Porschke, D., and Eggers, F., Eur. J. Biochem. *26* (1972) 490.
19 Rhodes, L.M., and Schimmel, P.R., Biochemistry *10* (1971) 4226.
20 Sarvazyan, A.P., Buckin, V.A., and Hemmes, P., J. phys. Chem. *84* (1980) 692.
21 Sturm, J., Lang, J., and Zana, R., Biopolymers *10* (1971) 2639.
22 Yiv, S.H., Lang, J., and Zana, R., Proc. Ultrasonics Int. (1975) 276.
23 Zinov'iev, O.I., and Valaeva, S.R., in: Khim. Kinet. Kataliz, p.94. Nauka, Moscow 1979 (in Russian).

0014-4754/85/111364-03$1.50 + 0.20/0

Full Papers

Characterization of victorin C, the major host-selective toxin from *Cochliobolus victoriae*: structure of degradation products

V. Macko[1], T.J. Wolpert, W. Acklin, B. Jaun, J. Seibl, J. Meili and D. Arigoni[1]

Boyce Thompson Institute, Cornell University, Ithaca (New York 14853, USA), and Laboratorium für Organische Chemie, Eidgenössische Technische Hochschule, CH-8092 Zürich (Switzerland), 18 June 1985

Summary. Several host-selective toxins have been isolated in pure form from culture filtrates of *Cochliobolus victoriae*. Acid hydrolysis of the major toxin, victorin C (apparent mol.wt 796), produced five fragments to which structures **1**–**5** have been assigned. Spectroscopic techniques revealed that the toxin contains an additional subunit corresponding to **6**; thus all the components of victorin C are accounted for.
Key words. Host-selective toxins; *Cochliobolus victoriae; Helminthosporium victoriae;* victorin C; oats; blight of oats; unusual amino acids.

Many disease-inducing plant pathogenic fungi produce toxic metabolites which are injurious to plants at low concentrations. Those pathogen-produced substances that affect selectively only certain varieties or genotypes of a given plant species have been termed host-specific toxins or host-selective toxins. These toxins are thought to play an important role in disease, because they can induce most or all of the disease symptoms and because toxin production by the pathogen often parallels its virulence[2,3].

Among the most elusive of the selective toxins is victorin, also known as HV-toxin. Victorin was discovered as a result of an outbreak of a new disease of oats in North America in the late 1940's. The fungus *Helminthosporium victoriae* was first described by Meehan and Murphy as the causal agent of the disease called Victoria blight of oats[4]. These authors also reported that culture filtrates from the fungus contained a toxin which caused symptoms typical of Victoria blight disease on susceptible but not on resistant plants[5].

Early work by Pringle and Braun[6,7] led them to postulate that victorin was composed of a small peptide and a nitrogen containing terpenoid, victoxinine. The structure of victoxinine was later elucidated by Dorn and Arigoni[8], but despite extensive research over the years the structure of victorin itself has remained unknown. Recently two groups reported on the purification of victorin but no structural information was given[9,10]. We have independently purified several toxic components from culture filtrates of the fungus and now report on the characterization and structure of the degradation products of the major toxin, henceforth designated as victorin C.

Materials and methods

Cochliobolus victoriae Nelson (*Helminthosporium victoriae* Meehan and Murphy) isolate 033 and isolate 1 were obtained from R.P. Scheffer and H.E. Wheeler, respectively. For toxin production the fungus was grown for 14–21 days at 25 °C under constant illumination in still culture in 500-ml Erlenmeyer flasks containing 100 ml of modified liquid Fries' medium[11] supplemented with either 0.1 % yeast extract or with oat flakes[12].

Culture filtrates and fractions from each purification step were tested for toxin activity by placing excised oat leaves in 4-ml vials containing 0.5 ml of test solutions. The root growth assay was performed essentially as described by Luke and Wheeler[11] using susceptible and resistant oat varieties, Park and Rodney, respectively.

Culture fluid was separated from mycelium by filtration through cheese cloth. Extraction, concentration and initial fractionation of the toxin were accomplished in a