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High pressure fourier transform infrared spectroscopy of poly(dA)poly(dT), poly(dA) and poly(dT)

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Abstract

The effect of hydrostatic pressure upon the DNA duplex, poly(dA)poly(dT), and its component single strands, poly(dA) and poly(dT) has been studied by fourier-transform infrared spectroscopy (FT-IR). The spectral data indicate that at 28°C and pressures up to 12 kbar (1200 MPa) all three polymers retain the B conformation. Pressure causes the band at 967 cm^{-1}, arising from water–deoxyribose interactions, to shift to higher frequencies, a result consistent with increased hydration at elevated pressures. A larger pressure-induced frequency shift in this band is observed in the single stranded polymers than in the double stranded molecule, suggesting that the effect of pressure on the hydration of single strands may be greater than upon a double stranded complex. A pressure-dependent hypochromicity in the bands attributed to base stacking indicates that pressure facilitates the base stacking in the three polymers, in agreement with previous assessments of the importance of stacking in the stabilization of DNA secondary structure at ambient and high pressures.

Keywords: DNA; Pressure; Fourier transform infrared spectroscopy; Structure

1. Introduction

Elucidation of the mechanistic details of the formation and denaturation of nucleic acid helices is of fundamental and practical importance. Recently, we investigated the effect of hydrostatic pressure upon the kinetics of these processes for DNA duplexes and triplexes as a means of probing the role of water–DNA interactions in the stabilization of these complexes [1,2]. Our results show that duplex and triplex molecules exhibit similar trends, i.e. hyperbaric pressure causes the rate of helix formation to increase and the rate of denaturation to decrease. Measurements of the effect of pressure on the helix–coil equilibrium have shown that the helix–coil transition temperature (T_m), and thus the stability of DNA increases with pressure [1–14]. In our interpreta-

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tion of these results, we have proposed that high pressure increases base stacking and decreases the extent of charge neutralization by cations. The loss of base stacking is expected to contribute a positive volume change to the overall process while an increase in the linear charge density of DNA due to decreased counter-ion interaction should contribute a negative molar volume change. However, whatever the origin of the volume changes accompanying DNA denaturation, the net effect is small, the total volume change of a helix–coil transition being on the order of $+3$ ml/mol.

Formation of the activated states leading to either a helix or a denatured coil also involves changes in base stacking and ionic interactions and when the molar volume of the activated state differs from that of the initial state, then the kinetics will be sensitive to pressure. The comparatively large effect of pressure on the rate constant for helix denaturation may be due to the formation of a partially open state prior to complete strand separation. In this state the base stacking is presumably disrupted; however, the extent of disruption is insufficient to allow water in between the bases. Formation of such a partially open state would be consistent with the large positive activation energy we observe for helix denaturation.

To assist us in the interpretation of our kinetics and equilibrium data in terms of structural changes, we have undertaken an investigation of the influence of hydrostatic pressure on the infrared spectra of double and single stranded DNA. Because many of the chemical groups that give rise to the infrared absorption bands of nucleic acids at atmospheric pressure have been assigned [15–18] infrared spectroscopy is a powerful tool for studying conformational changes of nucleic acids in vivo and in vitro. In contrast to other spectroscopic regions, there are two experimental methods available to study vibrational transitions, infrared absorption spectroscopy and Raman spectroscopy. The origin of the signal differs for these two techniques and they yield similar, although not identical data.

We present here the first report of the effect of hydrostatic pressure upon the fourier transform infrared (FT-IR) spectrum of DNA. The present study was undertaken to probe the conformational changes that may occur in the double and single stranded forms at high pressure. The system we have chosen is poly(dA)poly(dT) and its component single strands, poly(dA) and poly(dT). The physical properties of the homopolymer poly(dA)poly(dT) have been studied intensively because of its unusual structure and distinctive physical properties [11,19–25]. In addition, our kinetics studies thus far have focused on the interactions between homopurine and homopyrimidine oligonucleotides. For practical reasons we could not employ the same oligonucleotides we used in the kinetics experiments. The other homopurine–homopyrimidine polymer, poly(dG)poly(dC) can form non-duplex structures and poly(dG) can self-associate thus making this system unacceptable for our present purposes. Therefore, although none of our kinetics studies have focused on the poly(dA)poly(dT) system, it appeared to be a reasonable compromise in terms of practical considerations and in terms of the ability to use the findings in the interpretation of the kinetics results.

2. Experimental procedures

2.1. Materials

We purchased the lyophilized synthetic polymers, poly(dA), poly(dT) and poly(dA)poly(dT), from Pharmacia Biotech and used them without further purification. All other chemicals were reagent grade or better. The buffer solution consisted of 20 mM Tris–HCl, pH 8.8, 20 mM NaCl and 0.1 mM EDTA. The concentrations of sample solutions are based on the information proved by the supplier.

2.2. Fourier transform infrared spectroscopy at high pressure

Small amounts of the DNA solution (concentration $\sim 10\%$ w/v) and a small amount of powdered α-quartz were placed in a 0.37-mm diameter hole in a 0.23-mm thick stainless steel gasket mounted on a diamond anvil cell similar to

the original design of Mao and Bell [26]. FT-IR spectra at various pressures between ambient and approximately 12 kbar (1200 MPa) were acquired with a Digilab FTS-60 fourier-transform spectrometer equipped with a liquid nitrogen-cooled mercury–cadmium–telluride detector. For each spectrum, 512 scans were recorded at a spectral resolution of 4 cm^{-1}. The pressure within the diamond anvil cell was determined by monitoring the infrared absorption band of α-quartz at 695 cm^{-1} as described by Wong et al. [27]. Data analysis was performed with software developed at the University of Ottawa.

3. Results

The conformation adopted by DNA in solution is dependent on parameters such as the ionic strength and base pair sequence. The range of different conformations available to DNA is quite broad and can involve changes as profound as reversal of the helical sense; however, there are many more subtle structural changes that occur as well. Among the important determinants of DNA conformational polymorphism are the orientation of the deoxyribose sugar and base with respect to the helix axis and the relative orientation of these two groups with respect to each other [22]. The two double stranded conformations that occur under what can be broadly defined as standard conditions are called the A and B conformations. These two conformations exhibit characteristic differences in the disposition of the sugar and base. The A family of DNA structure occur under conditions of low water activity and the sugar/glycosidic bond is described as being in the C3′ endo/anti conformation (an N-type sugar). The B DNA structures exhibit C2′ endo/anti geometry (an S-type sugar).

The distinctive absorption of IR radiation by N- and S-type sugars is a useful marker to characterize sugar geometries and backbone conformations of nucleic acids [28–32]. These bands are general and independent of base composition or sequence. Spectra of structures containing N-type sugars present a strong absorption band in the vicinity of 865 cm^{-1} while absorption at approximately 840 cm^{-1} is observed for sugars in the S

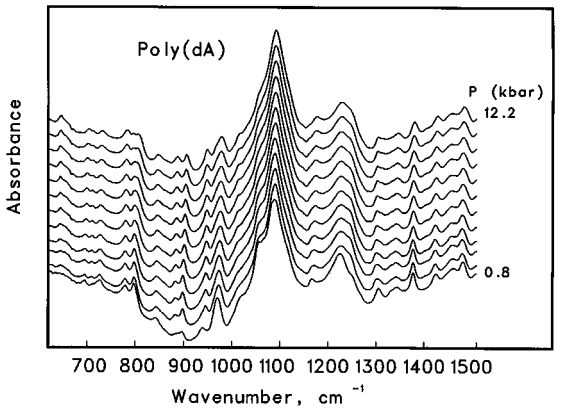

Fig. 1. Fourier-transform infrared spectra of poly(dA) at 28°C. The pressure of the spectra are 0.8, 1.0, 2.0, 3.0, 3.5, 4.5, 5.2, 6.5, 9.0, 10.5, 11.0, and 12.2 kbar.

conformation [33]. The IR spectra of the three polymers in the 800–900 cm^{-1} region (Figs. 1–3) exhibit strong absorption at 840 cm^{-1} at pressures up to 12 kbar, indicating that the sugars remain in the S conformation.

3.1. Deoxyribose geometry

In the spectral region between 900 and 1000 cm^{-1} (Figs. 1–3) all three polymers absorb at approximately 967 cm^{-1} reflecting standard B-form conformation deoxyribose intra-ring vibrations [17,34]. The position of this band is sensitive

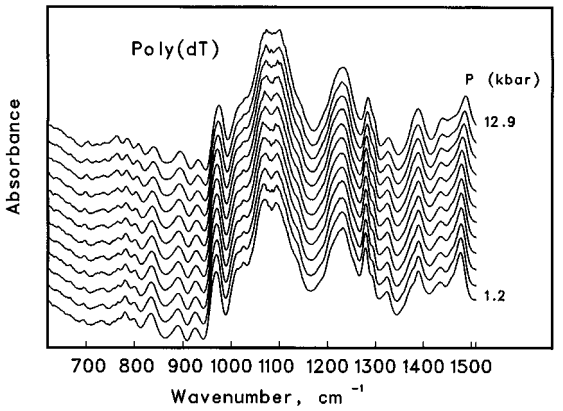

Fig. 2. Fourier-transform infrared spectra of poly(dT) at 28°C. The spectra were recorded at the following pressures: 1.2, 1.5, 2.7, 3.4, 4.5, 5.3, 6.0, 6.8, 8.5, 10.0, 10.9 and 12.9 kbar.

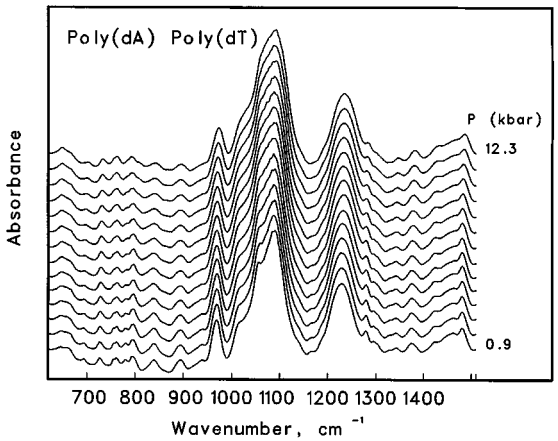

Fig. 3. Fourier-transform infrared spectra of poly(dA)poly(dT) at 28°C. The spectra were recorded at 0.9, 1.5, 1.8, 2.2, 3.2, 3.8, 5.5, 6.1, 7.7, 8.2, 8.5, 10.3, 10.7 and 12.3 kbar.

to the extent of hydration of deoxyribose. As shown in Fig. 4, the band at 967 cm^{-1} shifts to higher frequencies for all three polymers. The two single stranded polymers exhibit a somewhat greater shift than the double stranded polymer. This suggests that the poly(dA) and poly(dT) may experience a larger pressure-induced change in hydration than the duplex, poly(dA)·poly(dT). Further evidence that the deoxyribose sugars maintain their B conformation at high pressure is provided by the absence of absorption at 1188 cm^{-1} (Figs. 1–3). This strong, characteristic transition arises from vibrations of deoxyribose in DNA in the A conformation and is not observed in any of the spectra reported here [35].

3.2. Phosphate stretching vibrations

In Figs. 1–3, the IR bands at approximately 1090 and 1230 cm^{-1} arise from the symmetric and antisymmetric phosphate stretching vibrations, respectively. These bands have been used as markers for DNA backbone conformations. The antisymmetric phosphate stretching mode is generally the more sensitive to backbone conformational change [26,36–39]. This band occurs near 1226 cm^{-1} for B-family helices and at 1241 cm^{-1} for helices in the A conformation. We observed

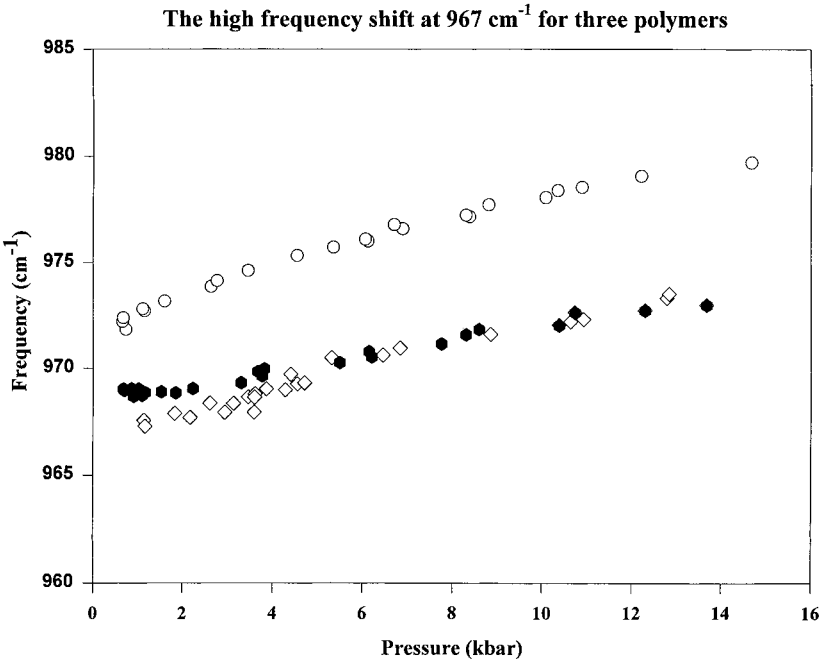

Fig. 4. The pressure-induced frequency shift of the hydration-sensitive deoxyibose band: (o), poly(dT); (◇), poly(dA); (●), poly(dA)poly(dT).

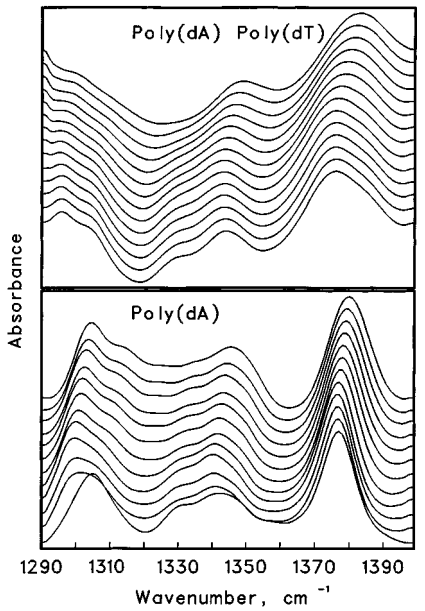

Fig. 5. The spectral region of poly(dA)poly(dT) (for the individual spectra, the pressures are the same as those in Fig. 3) and poly(dA) (for the individual spectra, the pressures are the same as those in Fig. 1) for in-plane adenine ring vibrations. By analogy with the behaviour of the Raman band at this frequency, the peak at 1301 cm^{-1} is expected to become sharper and less intense with increased base stacking.

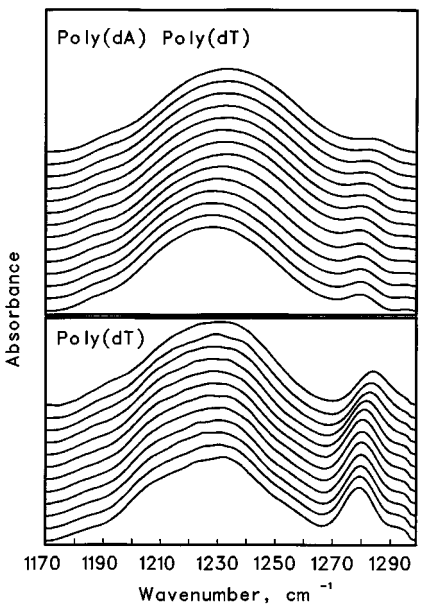

Fig. 6. The spectral region of poly(dA)poly(dT) (for the individual spectra, the pressures are the same as those in Fig. 3) and poly(dT) (for the individual spectra, the pressures are the same as those in Fig. 2) sensitive to deformation of thymine. The band at 1281 cm^{-1} is consistent with the B conformation; this band appears at 1273 cm^{-1} in the A conformation.

absorption at or near 1226 cm^{-1} in Figs. 1–3 in all spectra further confirming that the B conformation is conserved at pressures up to 12 kbar.

3.3. Adenine

The intensity of the absorption bands of nucleic acids often decrease as a consequence of a structural transition from a random coil to an ordered helical form. This is known as hypochromicity and it arises from a mutual cancellation of transition moments of bases in the ordered structure. A hypochromic effect in the Raman bands of adenine in-plane ring vibrations at 730 cm^{-1} and 1301 cm^{-1} upon helix formation has been reported [15–17]. These two bands are also characteristic of adenine in IR spectra of DNA [46]. In our analysis, we have made the assumption that they will also display stacking-dependent hypochromicity and that they are useful indicators of adenine stacking in polynucleotides. No pressure-induced hypochromicity of the band at $\sim$730 cm^{-1} for poly(dA) or poly(dA)poly(dT) in Figs. 1 and 3 is evident. However, the 1301-cm^{-1} band exhibits a qualitative reduction of the intensity and a change in the peak shape (Fig. 5). We attribute this observation to a pressure-induced increase in base stacking.

3.4. Thymine

The conformation-sensitive mode involving the N1–C6–H and C5–C6–H deformations of thymine appear at 1281 cm^{-1} for poly(dT) and poly(dA)poly(dT) (Fig. 6). This is consistent with a B conformation as this band appears at 1273 cm^{-1} in the A conformation [28]. The relative intensity of the bands also decreases with increasing pressure.

4. Discussion

The frequencies, bandwidths, intensities and shapes of vibrational bands reflect the structural

and dynamic properties of molecules. Infrared spectroscopy provides a useful method to investigate molecular structure and dynamics under a wide range of experimental conditions. One of the most successful exploitations of this technique is in the study of the effect of hydrostatic pressure on the conformational stability of proteins and lipid membranes (for a general review, the reader is referred to the chapters in Wong [18]). The data presented here are the first measurements of the effect of high pressure on nucleic acids using IR spectroscopy.

Our previous studies on the influence of high pressure on the thermodynamics and kinetics of nucleic acids at high pressures have revealed that duplex and triplex structures are stabilized by pressure [11–14]. We have also shown that, with increasing pressure, the rate of helix formation increases and the rate of helix denaturation decreases [1,2]. The thermodynamic and kinetics results were obtained by measuring changes in the UV absorption resulting from the helix–coil transition. We found no evidence of large-scale structural changes in the duplex form caused by pressure; however, in this spectral region, it is not possible to address the possibility of less dramatic conformational changes, such as a transition between the B and A forms.

Analysis of spectral parameters at elevated pressure is complicated due to experimental effects. In the diamond anvil cell, there is often a general intensity decrease with increasing pressure due to the reduction of the sample thickness. A small shift to higher frequencies (≤ 0.3 cm^{-1}/kbar) because of the decrease in the atomic interaction distances is also common. However, with these caveats, the data presented here yield several interesting results.

We have acquired FT-IR spectra for poly(dA)poly(dT) and its component single strands, poly(dA) and poly(dT) at pressures up to 12 kbar (water freezes at higher pressures). The positions of the bands arising from the phosphate and deoxyribose vibrations are consistent with the molecules remaining in the B form under these conditions. The resistance of these polymers toward pressure-induced conformational changes may arise due to a combination of structural stability and hydration effects. The duplex polymer, poly(dA)poly(dT) exhibits many unusual physical properties. For example, it is structurally rigid because of the system of bifurcated hydrogen bonds between adjacent base pairs. The base pairs also exhibit an unusually high degree of propeller twist [23]. The extent to which the data we report here can be extrapolated to DNA polymers with other sequences is a matter of conjecture; however, the data are consistent with our previous studies on the influence of hydrostatic pressure on the stability and kinetics of DNA helices.

Although the single stranded polymers, poly(dA) and poly(dT), are less conformationally constrained than the duplex, their sugar conformations in solution maintain the conformation observed in the duplex right-handed B conformation [40,41]. The sugar conformation of these molecules is unaltered at high pressure despite their greater flexibility.

One of our motivations for carrying out this study was to assist in the interpretation of our data on the influence of hydrostatic pressure on the stability and kinetics of DNA helices. We have shown previously that the activation volume of the DNA helix formation is negative, the molar volume of the transition state is smaller than that of the two interacting single strands [1,2]. Experimentally, the negative activation volume is manifested by an acceleration of the rate of helix formation at high pressure. The smaller volume of the transition state may be a result of hydration effects, electrostriction effects or conformational changes. Estimations of the role of hydration in the stabilization of DNA have shown that hydration decreases the electrostatic repulsion between adjacent, negatively charged phosphates, increases the attraction between base and phosphate residues and has a marked influence on the stacking of neighbouring bases [42]. These changes are consistent with the results of spectroscopic and kinetic studies carried out at high pressure.

The absence of significant changes in the frequencies of the bands arising from backbone vibrations implies that, to a first approximation, the backbone conformations of the three DNA polymers do not change with increasing pressure. At

high pressure the polymers will adopt the conformation with the greatest extent of hydration. Semenov et al. [34] have shown that shifts in the band at 967 cm^{-1} to higher frequencies correlates with increasing DNA hydration [34]. Pressure causes this band to shift to higher frequencies with the single stranded polymers exhibiting a larger shift than the duplex polymer. This observation together with the absence of any large pressure-induced changes in the backbone conformation underscore the importance of hydration in the structure of right-handed B helices,

The stabilization of ordered structures by high pressure is also reflected in the adenine and thymine vibrational bands. Hypochromicity and frequency shifts in the in-plane base ring modes are useful for investigating base stacking and helix formation in nucleic acids [16,34]. In spectra of the single and double stranded polymers we have shown that the base stacking markers for adenine (1301 cm^{-1}) and thymine (1281 cm^{-1}) become sharper and less intense with increasing pressure. Both of these changes are indicative of increased base stacking. Studies with model compounds in aqueous solutions have shown that stacking of aromatic compounds proceeds with a negative volume change, and thus is favoured by elevated pressure [43]. We propose that the negative activation volume of helix formation observed in previous studies may be related to this effect: high pressure decreases the extent of random coil in the single stranded forms shifting the coil $\rightleftarrows$ helix equilibrium of the individual single strands toward the helix state. This accelerates the nucleation and helix propagation step of the reaction leading to double stranded helix formation by forcing the single stranded sections of the nascent helix to adopt a conformation resembling a double stranded helix. This idea appears to be consistent with the results of Vesnaver and Breslauer [44] from their investigation of the contribution of single stranded structure to the energetics of duplex formation at atmospheric pressure. They demonstrated that single strands could exhibit intramolecular interactions that enthalpically poise them for duplex formation. Prior to association at 25°C, the two complementary single strands already possess more than 40% of the total enthalpy that stabilizes the final duplex state. Kumar [45] has put forward a proposal that relates the stability of polymeric nucleic acid duplexes to the internal pressure of solutions, the lack of significant structural changes at elevated hydrostatic pressures appears to be consistent with this hypothesis.

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References

[1] M.-C. Lin, R.B. Macgregor, Jr., Biochemistry 35 (1996) 11846–11851.
[2] M.-C. Lin, R.B. Macgregor, Jr., Biochemistry 36 (1997) 6539–6544.
[3] C.-G. Heden, T. Lindahl, I. Toplin, Acta Chem. Scand. 18 (1964) 1150–1156.
[4] F. Hughes, R.F. Steiner, Biopolymers 4 (1966) 1081–1090.
[5] B. Weida, S.J. Gill, Biochim. Biophys. Acta 112 (1966) 179–181.
[6] T.E. Gunter, K.K. Gunter, Biopolymers 11 (1972) 667–678.
[7] S.A. Hawley, R.M. MacLeod, Biopolymers 13 (1974) 1417–1426.
[8] S.A. Hawley, R.M. MacLeod, Biopolymers 16 (1977) 1833–1835.
[9] H. Høiland, A. Skauge, I. Stokkeland, J. Phys. Chem. 88 (1984) 6350–6353.
[10] E. Nordmeier, J. Phys. Chem. 96 (1992) 1494–1501.
[11] J.Q. Wu, R.B. Macgregor, Jr., Biochemistry 32 (1993) 12531–12537.
[12] J.Q. Wu, R.B. Macgregor, Jr., Biopolymers 35 (1995) 369–376.
[13] R. Najaf-Zadeh, J.Q. Wu, R.B. Macgregor, Jr., Biochim. Biophys. Acta 1262 (1995) 52–58.
[14] R.B. Macgregor, Jr., Biopolymers 38 (1996) 321–327.
[15] E.W. Small, W.L. Peticolas, Biopolymers 10 (1971) 1377–1416.
[16] S.C. Erfurth, W.L. Peticolas, Biopolymers 14 (1975) 247–264.
[17] F.S. Parker, Applications of Infrared, Raman, and Resonance Raman Spectroscopy in Biochemistry, Plenum Press, New York, 1983, pp. 349–359.
[18] P.T.T. Wong, in: R.E. Margulis, A.M. Zimmerman (Eds.), Current Perspectives in High Pressure Biology, Academic Press, London, 1987, pp. 287–314.
[19] L.J. Peck, J.C. Wang, Nature 292 (1981) 375–378.

[20] D. Rhodes, A. Klug, Nature 292 (1981) 378–380.
[21] F. Strauss, C. Gaillard, A. Prunell, Eur. J. Biochem. 118 (1981) 215–222.
[22] W. Saenger, Principles of Nucleic Acid Structure, Springer-Verlag, New York, 1984, pp. 271–312.
[23] H.C. Nelson, J.T. Finch, B.F. Luisi, A. Klug, Nature 330 (1987) 221–226.
[24] V.A. Buckin, B.I. Kankiya, N.V. Bulichov, et al., Nature 340 (1989) 321–322.
[25] T.V. Chalikian, A.P. Sarvazyan, G.E. Plum, K.J. Breslauer, Biochemistry 33 (1994) 2394–2401.
[26] H.K. Mao, P.M. Bell, Science 191 (1976) 851–853.
[27] P.T.T. Wong, D.J. Moffatt, F.L. Baudais, Appl. Spectrosc. 39 (1985) 733–735.
[28] E. Taillandier, J.-P. Ridoux, J. Liquier, et al., Biochemistry 26 (1987) 3361–3368.
[29] G.A. Thomas, A.H.-J. Wang, in: F. Eckstein, D.M.J. Lilley (Eds.), Nucleic Acids and Molecular Biology, vol. 2, Springer-Verlag, Berlin, 1988, pp. 1–30.
[30] E. Taillandier, in: R.H. Sarma, M.H. Sarma (Eds.), Structure and Methods, vol. 3, Adenine Press, New York, 1990, pp. 73–78.
[31] J. Liquier, E. Taillandier, R. Klinck, E. Guittet, C. Gouyette, T. Huynh-Dinh, Nucleic Acid Res. 23 (1995) 1722–1728.
[32] C. Dagneaux, J. Liquier, E. Taillandier, Biochemistry 34 (1995) 16618–16623.
[33] E. Taillandier , J. Liquier, Method Enzymol. 211 (1992) 307–335.
[34] M.A. Semenov, D.A. Matveyev, T.V. Bol′bukh, V.Y. Maleyev, Biophysics 39 (1994) 631–640.
[35] W. Pohle, M. Fritzsche, Nucleic Acids Res. 8 (1980) 2527–2535.
[36] D.M. Loprete, K.A. Hartman, Biopolymers 30 (1990) 753–761.
[37] D.M. Loprete, K.A. Hartman, Biochemistry 32 (1993) 4077–4082.
[38] P. Garirga, D. Garcia-Quintana, J. Manyosa, FEBS Letters 358 (1995) 27–30.
[39] A.P. White, J.W. Powell, Biochemistry 34 (1995) 1137–1142.
[40] D.M. Cheng, M.M. Dhingra, R.H. Sarma, Nucleic Acid Res. 5 (1978) 4399–4416.
[41] Z.E. Kahana, B.F. Erlanger, Biochemistry 19 (1980) 320–324.
[42] D. Perahia, M.S. Jhon, B. Pullman, Biochim. Biophys. Acta 474 (1977) 349–362.
[43] A.J.W.G. Visser, T.M. Li, H.G. Drickamer, G. Weber, Biochemistry 16 (1977) 4883–4886.
[44] G. Vesnaver, K.J. Breslauer, Proc. Natl. Acad. Sci. USA 80 (1991) 3569–3573.
[45] A. Kumar, Biochemistry 34 (1995) 12921–12925.
[46] E. Taillandier, J. Liquier, J.A. Taboury, in: R.J.H. Clark, R.E. Hester (Eds.), Advances in Infrared and Raman Spectroscopy, vol. 12, Wiley Heyden, New York, 1985, pp. 65–114.