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Effect of the electronic coupling in dinucleotides and oligonucleotides of adenine and thymine from synchrotron radiation absorption and circular dichroism

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Abstract

Absorption and circular dichroism measurements were performed on specifically chosen DNA mononucleotides, dinucleotides and oligonucleotides with combinations of adenine (A) and thymine (T), namely 2′-deoxyadenosine 5′-monophosphate (dAMP), thymidine 5′-monophosphate (TMP), dApdA, dTpdT, dApdT, dTpdA and dAp(dTp)$_n$dA ($n = 1$, 2 or 3) to study the importance of electronic coupling between bases for various bands. The coupling is stronger in the VUV region than in the UV region. In the VUV, it is possible to distinguish between dApdT and dTpdA sequences whereas in the UV the spectra are almost identical. A simple sequence dependent nearest neighbour model is used to explain the observations from both types of spectroscopy, underlining the importance of not only the nature of nearest neighbour nucleobases but the sequence as well.

Keywords: Synchrotron radiation; Circular dichroism; Absorption spectroscopy; Vacuum ultraviolet; Exciton coupling

1. Introduction

The nature of excited states of DNA bases governs the photostability of DNA with respect to UV damage. One protection mechanism is fast nonradiative relaxation from excited states to the electronic ground state [1] and another is delocalisation of the excitation energy over several nucleobases [2,3] to prevent subsequent photochemical reactions. Therefore the electronic coupling between nucleobases in DNA is essential for nonradiative deexcitation of electronically excited states [2,3]. The formation of excimers in which the excitation energy is shared between two stacked bases limits the excitation energy to one strand at a time leaving the other strand undamaged.

Numerous studies, including a series of theoretical works [4–10], have been performed to look for the signature of electronic coupling between bases in various cases of polynucleotide single and double strands [4–16]. Most of the studies have focused in the UV region of absorption [13–16], particularly in and around the 260-nm region. The CD spectroscopic measurements show clear sign of exciton coupling in this region [15,16]. There have been attempts to look at the effect of exciton coupling in the absorption [16], but due to the weakness of the coupling and the broad width of the band, such effects are difficult to see at room temperature. Also as pointed out by our recent study, the coupling is state dependent and very strong in states accessed by VUV radiation [17]. In the present paper we examine the influence of coupling on the nucleobases as a function of the nearest neighbour. Another highlight of the work presented here is the sequence dependence of absorption and CD. Again, in this case, the measurements in the UV region show very small differences, whereas the signal in the VUV region is very sensitive to the sequence. The sequence dependence of the CD signal of nucleotide dimers has already been studied in much detail in the UV region

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[18]. This work extends it to the VUV range and with good statistics due to high intensity of radiation from synchrotron radiation sources compared to conventional CD spectrometers [19–21].

2. Experimental

Absorption and circular dichroism spectroscopy of oligonucleotides in aqueous solution at neutral pH was carried out. The samples used were mono- and dinucleotides of adenine and thymine (dAMP, TMP, dApdA and dTpdT), mixed dinucleotides (dApdT and dTpdA) and mixed oligonucleotides ($dAp(dTp)_n dA$ with $n = 1, 2$ or 3). All the oligonucleotides were purchased from DNA technology, Aarhus, and the mononucleotides from Sigma-Aldrich. The oligonucleotide samples contain no salt according to the supplier and the largest "impurity" is uncoupled monomers. Concentrations were determined from the measured absorbance at 260 nm and calculated extinction coefficients at 260 nm by the nearest neighbour method [18,22]. The extinction coefficients were calculated at other wavelengths by Beer-Lambert law. Nucleotides were dissolved in 10 mM phosphate buffer (NaP_i) at pH 7.4 and 100 mM NaF. The estimated concentrations of various oligomers are given in the figure captions. A quartz cell type QS124 with a path length of 0.1 mm (Hellma GmbH, Germany) was used for the measurements. The experiments were performed at the UV1 beam line at the ASTRID synchrotron radiation source at the University of Aarhus, Aarhus, Denmark. The same setup was used for measuring the absorption and CD spectra. The range of wavelengths used was between 170 and 330 nm. Several scans were acquired and averaged. A typical CD run lasted for about 35–40 min. Care was taken to check the consistency between first and last scan in order to check for possible evaporation of the solvent or photodegradation. Spectra of buffer and NaF (reference solution) were subtracted from the spectra of the nucleotides for both absorption and CD measurements.

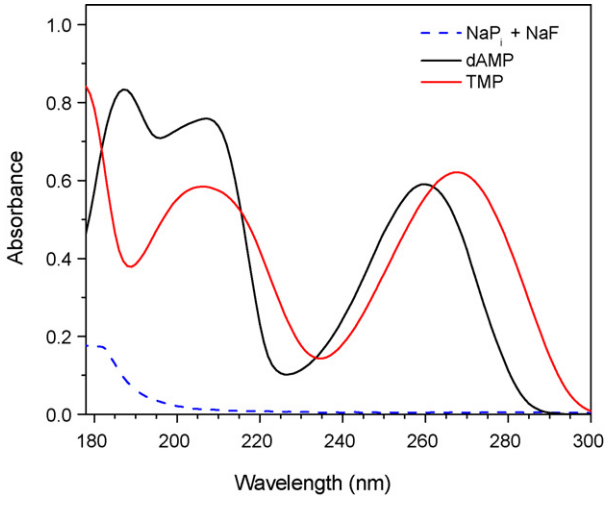

Fig. 1. Absorbance of buffer and salt solution (10 mM NaP_i + 100 mM NaF), dAMP (4.02 mM) and TMP (6.46 mM).

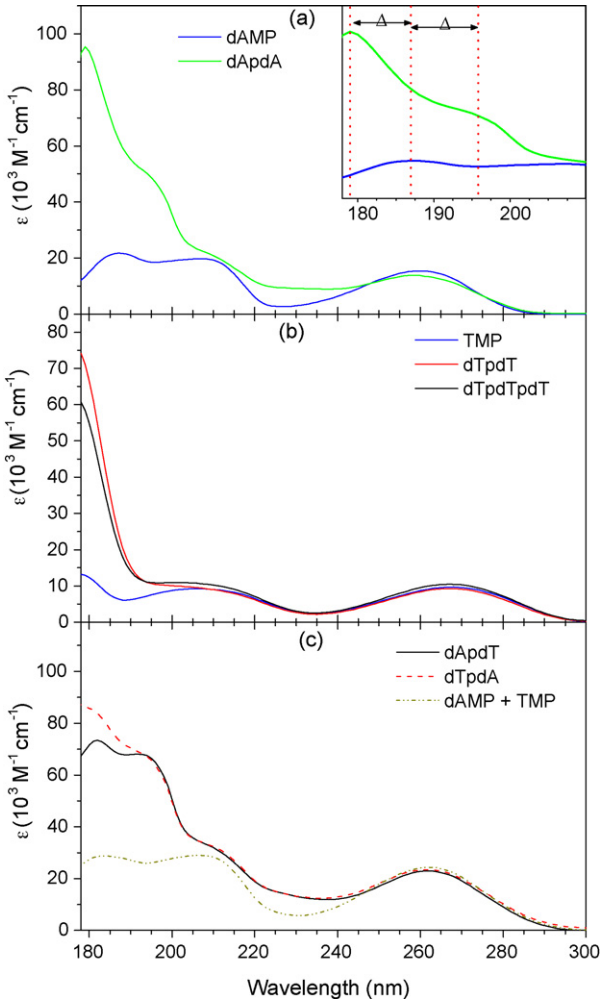

Fig. 2. Absorption (a) for dAMP/2 (4.02 mM) and dApdA/2 (0.88 mM) (b) for TMP (6.46 mM), dTpdT/2 (0.69 mM) and dTpdTpdT/3 (0.70 mM) (c) for dApdT (0.71 mM) and dTpdA (0.73 mM) along with the sum of monomer spectra for comparison. The inset shows the approximate peak positions and exciton splitting.

3. Absorption spectroscopy

3.1. Monomers and dimers

Absorption spectra of dAMP (2′-deoxyadenosine 5′-monophosphate) and TMP (thymidine 5′-monophosphate) after subtracting the reference solution spectrum are shown in Fig. 1 along with the reference solution spectrum for comparison. The data below 178 nm are considered unreliable due to substantial absorption by water. The absorption by the nucleotides is at least 2.5 times larger than that of the reference solution. The absorption spectra of dAMP and TMP are in excellent agreement with those previously reported [23]. The detailed assignments of various bands have been discussed in detail elsewhere [23]. The main features clearly observable in the dAMP spectrum are the bands at 187, 208 and 260 nm and valleys at 195 and 228 nm. For the TMP spectrum, there are bands at 178, 205 and 268 nm and valleys at 188 and 235 nm. We will consider how these features evolve

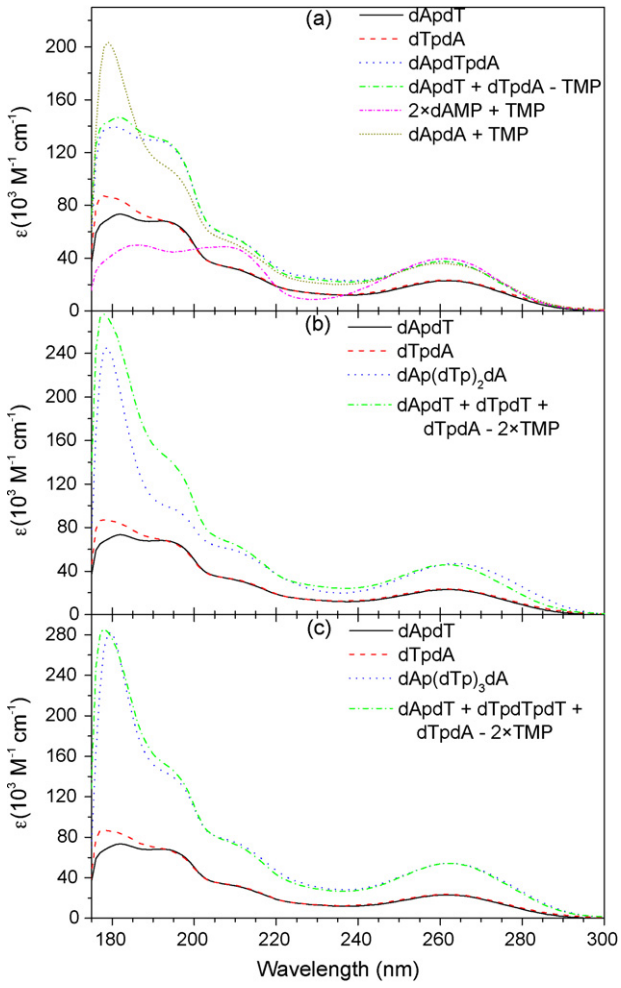

Fig. 3. (a) Absorption spectra for dApdTpdA (0.71 mM) along with the dApdT, dTpdA and various different ways in which the dApdTpdA spectrum can be generated for generic spectra. (b) and (c) Spectra for dApdTpdTpdA (0.65 mM) and dApdTpdTpdTpdA (0.68 mM), respectively.

as we extend the nucleotide with one more nucleoside in the chain.

Extinction coefficients per nucleobase for dAMP and dApdA as a function of wavelength are plotted in Fig. 2a. The peak at 260 nm and the shoulder at 208 nm are very similar in both cases. At 260 nm a small amount of broadening is seen in the dApdA spectrum which is ascribed to exciton coupling [16]. The dimer absorbs to some extent at 228 nm in contrast to the monomer. This could be due to possible increase in the oscillator strength of the 230-nm nπ* transition [14,23]. In the VUV region, on the other hand, the changes are more drastic. As can be seen from the inset, apart from the nearly 4.5-fold increase in the absorption per nucleotide in dApdA, the 187-nm band appears to have split into two equally spaced bands, each shifted by 8 nm (0.3 eV) from the monomer value of 187 nm (6.6 eV), with the higher energy transition with highest oscillator strength. It is well known from CD measurements that exciton coupling occurs at 187 nm [17]. Interestingly, the signal in the VUV of dApdA is significantly larger than that previously measured of dried films of dApdA [24,25]. The difference cannot be ascribed to absorption by the buffer solution or NaF (see Fig. 1).

TMP, on the other hand, displays very little, if any, difference in the UV region from dTpdT (Fig. 2b). In the VUV region the 178-nm band increases nearly five times but shows no sign of splitting or significant broadening. The CD spectroscopy does, however, indicate exciton coupling in this range (*vide infra*).

The mixed type dimers dApdT and dTpdA, Fig. 2c, have identical spectra in the UV range but differ from each other in the range from 178 to 200 nm. Surprisingly the exciton splitting observed in dApdA appears to be preserved in both heterodimers. Hence we conclude, first of all, that in the VUV region we can distinguish between dApdT and dTpdA, and, secondly, that the state corresponding to the 187 nm band in adenine undergoes exciton splitting by the presence of either A or T in any position. Note that a simple addition of dAMP and TMP spectra reproduces signal at the 260-nm band but fails to match the rest of the spectra in the lower wavelength region.

In the following we will use the spectra of dAMP, TMP, dTpdA and dApdT as basis spectra, *S*, for the empirical calculation of spectra of oligonucleotides and compare with experiment.

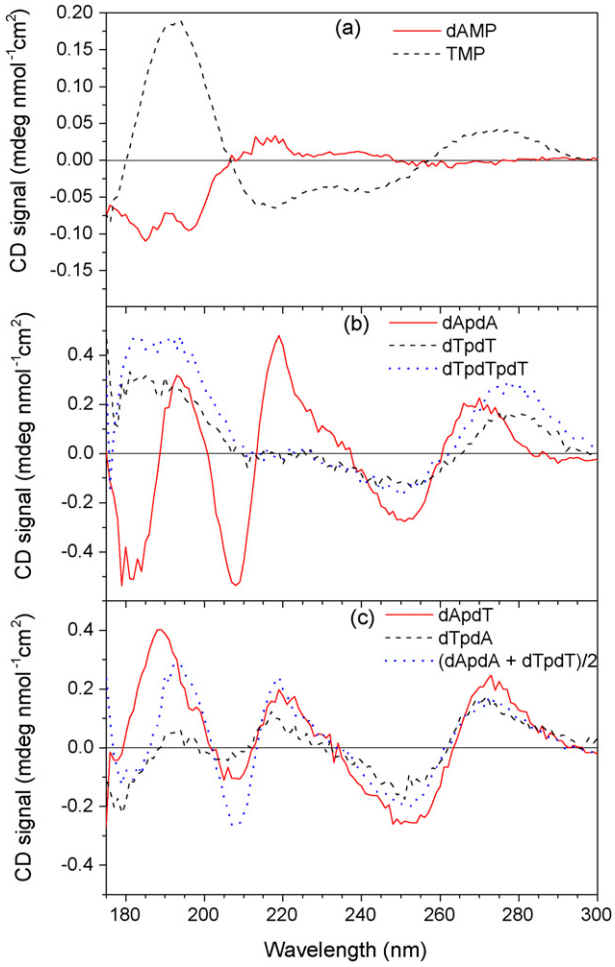

Fig. 4. VUV and UV CD spectra of dAMP and TMP (a), dApdA, dTpdT and dTpdTpdT (b) and the mixed dimers dApdT and dTpdA (c).

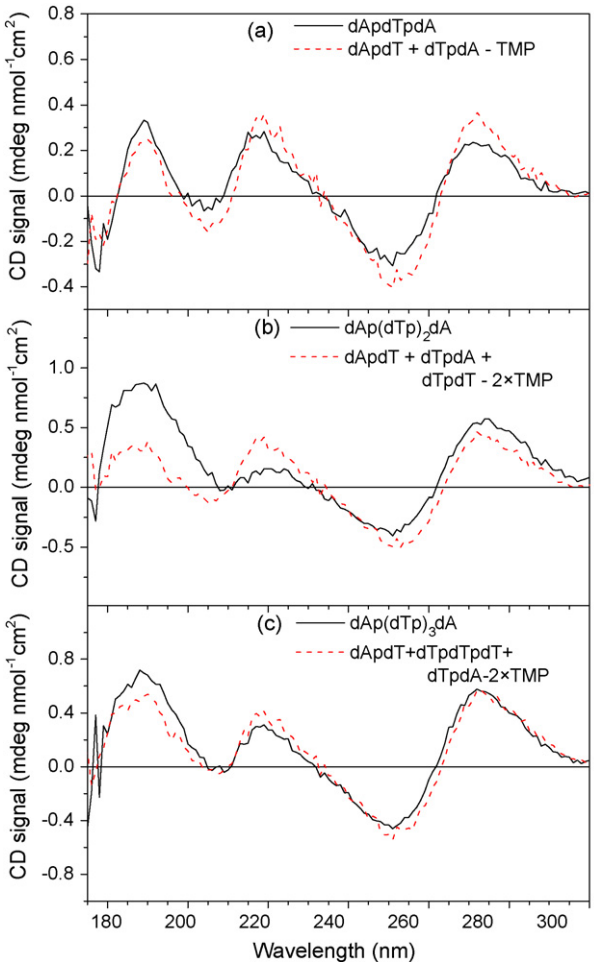

Fig. 5. CD spectra for the oligonucleotides dApdTpdA (a), dApdTpdTpdA (b) and dApdTpdTpdTpdA (c).

3.2. Oligonucleotides

We have looked at oligonucleotides with two adenines that are separated by one or more thymine. The absorption spectrum of dApdTpdA is plotted in Fig. 3a along with those of dApdT and dTpdA for comparison. It is evident that the exciton coupling at 187 nm still exists for dApdTpdA. We have calculated the dApdTpdA spectrum, S(dApdTpdA) by three different methods as illustrated in Fig. 3a. The sum of $2 \times S$(dAMP) and S(TMP) or the sum of S(dApdA) and S(TMP) does not match the dApdTpdA spectrum. Instead S(dApdT) + S(dTpdA) − S(TMP) successfully generates the observed dApdTpdA spectrum. The combinations $2 \times S$(dApdT) − S(TMP) and $2 \times S$(dTpdA) − S(TMP) were also tried and were in reasonably good agreement but not as good as the other. It should be mentioned that all the calculated spectra properly reproduce the 260-nm band.

Spectra of dAp(dTp)$_2$dA and dAp(dTp)$_3$dA are shown in Fig. 3b and c, respectively. Again spectra can be reproduced from sum spectra, S(dApdT) + S(dTpdA) + S(dTpdT) − $2 \times S$(TMP) and S(dApdT) + S(dTpdA) + S(dTpdTpdT) − $2 \times S$(TMP), respectively.

4. Circular dichroism measurements

4.1. Monomers and dimers

CD in the VUV and UV range for dAMP, TMP, dApdA and dTpdT have been extensively studied in the past [10,15]. CD spectra of monomer S(dAMP and TMP), dimer S(dApdA and dTpdT) and mixed dimer S(dTpdA and dApdT) are plotted in Fig. 4(a)–(c). Both dApdA and dTpdT spectra display the signatures of exciton coupling (Fig. 4b). The coupling is seen to be much stronger in the case of adenine than that of thymine. In the UV region the two spectra of dApdT and dTpdA show qualitatively the same features but in the VUV there is a clear difference between the two, which again, indicates that the position of nearest neighbour and not just the type, determines the CD signal in the VUV region. An attempt to generate dApdT and dTpdA spectra using the dApdA and dTpdT spectra is successful only above 216 nm.

4.2. Oligonucleotides

The spectra for dApdTpdA, dApdTpdTpdA and dAp(dTp)$_3$dA are plotted in Fig. 5(a)–(c), respectively. The nearest neighbour method using both dApdT and dTpdA interaction reproduces the observed CD spectra for dAp(dTp)$_n$dA ($n = 1, 2, 3$) (Fig. 5). Thus, the spectrum of dAp(dTp)$_n$dA can be reproduced from the sum of spectra,

$$S(\mathrm{dAp(dTp)}_n\mathrm{dA}) = S(\mathrm{dApdT}) + S(\mathrm{dTpdA}) + S(\mathrm{dT}_n) - 2 \times S(\mathrm{TMP}).$$

Other sum spectra with either dApdT or dTpdA alone, fail to match the data, highlighting the sequence dependent interaction of two different bases.

5. Conclusion

Exciton coupling between nucleobases can be observed more clearly in the VUV region than in the UV region both from absorption and CD spectroscopy. The dApdT spectra are different from those of dTpdA, most prominently in the VUV region, which indicates that the sequence matters. Thus, spectra of oligonucleotides S(dAp(dTp)$_n$dA) could be calculated satisfactorily only by using both dApdT and dTpdA basis spectra. At the same time, the presence of the thymine base between two adenine bases considerably reduces the coupling between the two adenines but does not completely switch it off. Coupling in the VUV was also found to occur between A and T neighbours.

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References

[1] C.E. Crespo-Hernández, B. Cohen, B. Kohler, Nature 436 (2005) 1141–1144.
[2] D. Markovitsi, F. Talbot, T. Gustavsson, D. Onidas, E. Lazzarotto, S. Marguet, Nature 441 (2006) E7.
[3] C.E. Crespo-Hernández, B. Cohen, B. Kohler, Nature 441 (2006) E8.
[4] B. Bouvier, T. Gustavsson, D. Markovitsi, P. Millié, Chem. Phys. 275 (2002) 75–92.
[5] E. Emanuele, D. Markovitsi, P. Millié, K. Zakrzewska, Chem. Phys. Chem. 6 (2005) 1387–1392.
[6] B. Bouvier, J.P. Dognon, R. Lavery, D. Markovitsi, P. Millié, D. Onidas, K. Zakrzewska, J. Phys. Chem. B 107 (2003) 13512–13522.
[7] H.-H. Ritze, P. Hobza, D. Nachtigalova, Phys. Chem. Chem. Phys. 9 (2007) 1672–1675.
[8] E.B. Starikov, Modern Phys. Lett. B 18 (2004) 825–831.
[9] F. Santoro, V. Barone, R. Improta, Proc. Natl. Acad. Sci. 104 (2007) 9931–9936.
[10] D.M. Markovitsi, T. Gustavsson, F. Talbot, Photochem. Photobiol. Sci. (2007) 717–724.
[11] J. Eisinger, R.G. Shulman, Science 161 (1968) 1311–1319.
[12] A. Steinschneider, B. Leshem, J. Mol. Biol. 67 (1972) 333–337.
[13] D.E. Joyce, T. Kurucsev, Biophys. Chem. 2 (1974) 273–277.
[14] Ch. Zimmer, E. Birch-Hirschfeld, R. Weiss, Nucleic Acid Res. 1 (1974) 1017–1030.
[15] R.W. Wilsont, P.R. Callis, J. Phys. Chem. 80 (1976) 2280–2288.
[16] A.I. Kononov, V.M. Bakulev, V.L. Rapoport, J. Photochem, Photobiol. B: Biol. 19 (1993) 139–144.
[17] U. Kadhane, A.I.S. Holm, S.V. Hoffmann, S. Brøndsted Nielsen, Phys. Rev. E submitted for publication.
[18] C.R. Cantor, M.M. Warshaw, H. Shapiro, Biopolymers 9 (1970) 1059–1077.
[19] (a) B.A. Wallace, Nat. Struct. Biol. 7 (2000) 708–709;
(b) B.A. Wallace, J. Synchrotron Radiat. 7 (2000) 289–295;
(c) A.J. Miles, B.A. Wallace, Chem. Soc. Rev. 35 (2006) 39–51;
(d) B.A. Wallace, F. Wien, A.J. Miles, J.G. Lees, S.V. Hoffmann, P. Evans, G.J. Wistow, C. Slingsby, Faraday Discuss. 126 (2003) 237–243;
(e) N. Ojima, K. Sakai, T. Fukazawa, K. Gekko, Chem. Lett. (2000) 832–833;
(f) N. Ojima, K. Sakai, K. Matsuo, T. Matsui, T. Fukazawa, H. Namatame, M. Taniguchi, K. Gekko, Chem. Lett. (2001) 522–523.
[20] A.I.S. Holm, E.S. Worm, T. Chakraborty, B.R. Babu, J. Wengel, S.V. Hoffmann, S. Brøndsted Nielsen, J. Photochem. Photobiol. A: Chem. 187 (2007) 293–298.
[21] S. Brøndsted Nielsen, T. Chakraborty, S.V. Hoffmann, Chem. Phys. Chem. 6 (2005) 2619–2624.
[22] M.J. Cavaluzzi, P.N. Borer, Nucleic Acids Res. 32 (2004) E13.
[23] C.A. Sprecher Jr., W.C. Johnson, Biopolymers 16 (1977) 2243–2264.
[24] S. Onari, J. Phys. Soc. Jpn. 26 (1969) 214.
[25] T. Ito, M. Saito, Radiat. Phys. Chem. 37 (1991) 681–690.