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A library of IR bands of nucleic acids in solution

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Abstract

This review presents a compilation and discussion of infrared (IR) bands characteristic of nucleic acids in various conformations. The entire spectral range 1800–800 cm^{-1} relevant for DNA/RNA in aqueous solution has been subdivided into four sections. Each section contains descriptions of bands appearing from group specific parts of nucleic acid structure, such as nucleobase, base–sugar, sugar–phosphate and sugar moiety. The approach allows comparisons of information obtained from one spectral region with another. The IR band library should facilitate detailed and unambiguous assignment of structural changes, ligand binding, etc. in nucleic acids from IR spectra. Section 2 is aimed at highlighting specific features that are useful for following major changes in nucleic acid structures. Section 2 also concerns some recent results, where IR spectroscopy has been used to obtain semi-quantitative information on coexisting modes of sugar pucker in oligonucleotides.

Keywords: FTIR; Nucleic acids; Spectral library; Sugar pucker; Band assignment

1. Introduction

This review presents a library and a discussion of IR bands observed in Fourier transform infrared (FTIR) studies of nucleic acids in aqueous solution. The collection of assigned IR bands should be helpful in the interpretation of IR spectra obtained in the study of structure and interaction in nucleic acids. Marker bands for different conformational families (*B*, *A* and *Z* backbone conformers, *N*- and *S*-type of sugars) have been coordinated with marker bands for base-specific interactions, (e.g. base pairing) and conformations (anti/syn). Several useful IR reviews for nucleic acids have been published in the past [1–5]. However, these earlier reviews focus on specific issues of base composition, base pairing and backbone conformation, and accordingly the information content in each collection is restricted to IR bands relevant to the specific issue. The present library combines the information given in these earlier collections [1–5], but also expands the outline by adding data obtained from additional studies.

IR spectroscopy has been the method of choice for studying various issues concerning structure and interaction in nucleic acids. IR studies have been performed on single-stranded [6], double-stranded [7–11] and triple-stranded [12–18]

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Table 1A
The wavenumbers and spectral assignments of selected IR bands in nucleic acids. The comment column gives the basis for the assignment. Typical relative intensities are given in parentheses for certain bands[a]

Wavenumber (cm^{-1})	Assignment	The 1800–1500 cm^{-1} region in D_2O In-plane base vibrations Sensitive to effects of base pairing and base stacking Comment
1715	G*G–C/ts	C6=O6 str of G involved in Hoogsteen third strand binding [5]
1712	T*A–T/ts	C2=O2 str of T involved in reverse Hoogsteen third strand binding [5]
1710	C^+*G–C/ts	C2=O2 str of protonated C involved in Hoogsteen third strand binding [5]
1698–1691	U, T/ss	C2=O2 str of U/ss or ds (medium) [1,2]
	U, T/ds	C2=O2 str of T/ss or ds (medium) [5]
		Calc: C2=O2 str of U [3]
1689–1678	G/ds	C6=O6 str of G/ds (medium) [2,5]
1677–1672	U/ds	C4=O4 str U/ds (medium) [2]
1673–1660	G/ss	C6=O6 str of G/ss (strong) [2,5]
		Shifts to 1689–1684 upon duplexation [2,5]
		Calc: C6=O6, C4=C5 in-phase str coupled with C5–C6 out-of phase str of G [3]
1671–1655	T/ss	C4=O4 str of T/ss (strong) or T/ds (medium) [5,15]
	T/ds	Decrease in intensity upon duplexation [5]
1657–1653	U/ss	C4=O4 str of U/ss (strong) [1,2]
		Shifts to 1672 and decrease in intensity upon duplexation [2]
		Calc: C4=O4 str of U, coupled with C4–C5 str [3]
1655–1647	C/ss	C2=O2 str of C/ss (strong) or ds (medium) [1,2,5]
	C/ds	Small downshift (1652 to 1649) and decrease in intensity upon duplexation [2,5]
		Calc: Almost pure C2=O2 str of C [3]
1645–1641	T/ds	In-plane ring vib of T/ds [5,15]
1632	T/ss	In-plane ring vib of T/ss [5,15]
		Shifts to 1644–1641 upon duplexation [5,15]
1632–1622	A/ss	C=N; C=C ring vib of A/ss (strong) and ds (medium) [1,2]
	A/ds	Decrease in intensity upon duplexation [2,5]
		Shifts 1626 to 1622 upon duplexation in DNA [5] and 1628 to 1631 upon duplexation in RNA [2]
		Calc: C4=C5, C5–C6 out-of-phase ring vib of A [3]
1624–1616	C/ss	In-plane ring vib of C/ss (medium) or ds (weak) [1,2]
	C/ds	Calc: C4–C5, C5=C6 out-of-phase ring vib of C [3]
1618–1615	U/ss	In-plane ring C=C str of U/ss (weak) [1,2]
		Calc: suspected combination band (1258+364/352) of U [3]
1590–1575	G/ss	C=N ring vib of G/ss (strong) and ds (weak) [2]
	G/ds	Decrease in intensity upon duplexation [2,5]
		Calc: C4=C5, C5–C6 in-phase ring vib of G [3]
1585–1582	C	In-plane ring vib of C (weak) [2]
1579–1576	A	In-plane ring vib of A (weak) [2]
		Calc: C4=C5, C5–C6 in-phase ring vib of A [3]
1568–1564	G/ss	C=N ring vib of G/ss (medium) and ds (weak) [2]
	G/ds	Decrease in intensity upon duplexation [2,5]
		Calc: C6=O6, C5–C6 in-phase str coupled with C4=C5 out-of phase str of G [3]
1527–1520	C/ss	In-plane vib of C/ss (medium) and ds (weak) [2]
	C/ds	Drastic decrease in intensity upon duplexation [5]
		Calc: skeletal out-of-phase vib of C [3]

[a] *Abbreviations:* G: guanine; A: adenine; C: cytosine; U: uracil; ts: triple-stranded; ds: double-stranded; ss: single-stranded; calc: calculation; str: stretch; vib: vibration; def: deformation; symm: symmetric; asymm: asymmetric; d: deoxyribose; r: ribose.

Table 1B

Wavenumber (cm^{-1})	Assignment	The 1500–1250 cm^{-1} region in H_2O Base–sugar vibrations Sensitive to glycosidic bond rotation, backbone conformation and sugar pucker Comment
1506–1498	C	In-plane vib of C (medium) [2] Calc: skeletal out-of-phase vib of C [3] Calc: N1C6H, N1C1′H, C4N4, C2N3, C1′N1 vib of dC [51]
1495-1476	A, G	Ring vib of A (medium) and G (weak) [3] N7C8H bend of A/G [12,16] Calc: NC8H, N7=C8, C2=N3 vib of dA [52] Calc: N7=C8, C8N9, C4=C5, C4C9, NC8H vib of dG [51]
1485–1477	T	Calc: C4C5, N1C2, N3C4 vib of dT (weak) [52]
1474	U	U (medium) [3] Shifts to 1467 in D_2O Calc: alternate bond str of U [3]
1457–1453	A /*B*-form /*A*-form	A [3] Calc: N1=C6, C6N6 vib of dA in *B*/*A*-form [52]
1438–1434	A /*Z*-form	A bases in *Z*-form helices [5] Calc: N1=C6, C6N6, C5C6N vib of dA in *Z*-form [52]
1425–1420	*S*-type /*B*-form	C2′-endo deoxyribose in *B*-form helices [4,5,35] Calc: C8N9, C1′N9, N3C4N9 vib of dA in *B*-form [52] Calc: C8N9, C5N7, N3C4 vib of dG in *B*-form [51]
1418–1408	*N*-type /*A*-form	C3′-endo deoxyribose in *A*-form helices [4,5,35] Calc: C8N9, C1′N9, N3C4N9 vib of dA in *A*-form [52]
1413–1408	*N*-type /Z-form	C3′-endo deoxyribose in *Z*-form helices [4,5,35] Calc: NC8H, C8N9, N1=C6, C1′N9 vib of dA in *Z*-form [52] Calc: HC2′H, N9C8, C5N7 vib of dG in *Z*-form [51]
1400	RNA	In-plane C2′OH in RNA [2]
1399–1390	U	U [27] Calc: C4C5H, C6C5H bend of rU [53]
1389–1374	T	Calc: CH_3 symm def of dT [52]
1381–1369	Purine /anti	Purine in anti conformation (sugars: C2′/C3′-endo) [4,5,35] Shifts to 1355 for purine in syn conformation Calc: C1′N9, C6N6, N9C1′H vib of dA in *B*/*A*-form [52] Calc: C1′N9, N9C1′H, N7=C8 vib of dG in *B*/*A*-form [51]
1365–1360	dC /anti	Cytidine in anti conformation (sugars: C2′/C3′-endo) [35] Calc: HC3′O3′, C4N4, N1C1′H vib of dC [51]
1357–1352	Purine /syn	Purine in syn conformation (sugars: C3′-endo) [4,5,35] Compare 1320 Calc: C1′N9, C6N6, N9C1′H vib of dA in *Z*-form [52] Calc: C1′N9, C5C6, N9C1′H, C5N7, C2′C1′H vib of dG in *Z*-form [51]
1344	dA /*S*-type	N7C8H vib of dA in C2′-endo/anti [27] Calc: NC8H, C2=N3, NC2H, C8N9 vib of dA in C2′-endo/anti [52]
1335	dA, rA, dT, rU /*N*-type	dA, rA, dT, rU in C3′-endo/anti [27] dT: compare 1275 Calc: NC8H, C2=N3, NC2H, C8N9 vib of dA in C3′-endo/anti [52] Calc: C5′O5′, C4′C5′ vib of dT in C3′-endo/anti [52]
1328	dT /*S*-type	dT in C2′-endo/anti [27] Compare 1281 Calc: C4′C5′, C4′O1′ vib of dT in C2′-endo/anti [52]

Table 1B *(Continued)*

Wavenumber (cm^{-1})	Assignment	The 1500–1250 cm^{-1} region in H_2O Base–sugar vibrations Sensitive to glycosidic bond rotation, backbone conformation and sugar pucker Comment
1320	dG /syn	G in syn conformation (sugars: C3′-endo) [5,24,35] Compare 1355 Calc: C4′O1′, C4′C5′, C1′O1′, C5C6 vib of dG in *Z*-form [51]
1306–1300	A	A [3] Calc: HC2′H, C4′C5′ vib of dA [52]
1297–1285	C	$C4NH_2$ str (strong) of C [1,54] Calc: N3=C4, C4C5, N1C2, C2N3, N1C6, C5=C6 vib of dC [51]
1281	dT, dU /*S*-type	CN3H bend of dT, dU in C2′-endo/anti [27] Compare 1328 Disappears in D_2O due to exchange of the labile H of CN3H Calc: CN3H, $C5CH_3$, N1C6H vib of dT in C2′-endo/anti [52]
1275	dT, rU /*N*-type	CN3H bend of dT, rU in C3′-endo/anti [27] Compare 1335 Calc: CN3H, $C5CH_3$, N1C6H vib of dT in C3′-endo/anti [52]
1265–1264	GC /*Z*-form	GC helices in *Z*-form [5,24]

Table 1C

Wavenumber (cm^{-1})	Assignment	The 1250–1000 cm^{-1} region in H_2O/D_2O Sugar–phosphate vibrations Sensitive to backbone conformation Comment
1245–1235	*A*-form	Main *A*-form marker Antisymmetric PO_2^- str [1,2,4,5]
1225-1220	*B*-form	Main *B*-form marker Antisymmetric PO_2^- str [1,2,4,5]
1221	Ribose	Ribose vib [27] Difficult to separate from 1225 *B*-form marker
1216-1213	*Z*-form	Main *Z*-form marker Antisymmetric PO_2^- str [4,5,31,35]
1188-1175	*A*-form	*A*-form marker [4,5,28] Sugar-phosphate backbone vib with a fairly high contribution from the sugar moiety in C3′-endo/anti type of puckering [28]
1135	Ribose	Ribose C1′C2′OC3′ str (strong) [2]
1123	*Z*-form	*Z*-form [5]
1119–1116	Ribose	Ribose C1′C2′OC3′ str (strong) [2,27]
1090–1085	Backbone	Symmetric PO_2^- str [1,2] Insensitive of *B*-to-*A* transition [27]
1069–1044	Furanose	CO str of backbone [2] Strongly enhanced in *Z*-form DNA [5,35] Calc: (1069) symmetric CO str of the backbone [49,55,56] Calc: (1049) antisymmetric CO str of the backbone [49,55,56]
1020–1010	Furanose	Furanose vib Strongly enhanced in *Z*-form DNA [5,35]

Table 1D

Wavenumber (cm^{-1})	Assignment	The 1000–800 cm^{-1} region in H_2O/D_2O Sugar vibrations Sensitive to sugar conformation Comment
995	Ribose	Ribose-phosphate main chain vib [2] Assigned to a vib involving the 2′-OH group [27] Disappears in D_2O
970	RNA backbone	Ribose-phosphate main chain vib [2] Disappears in D_2O
970–950	DNA backbone	CC str of the backbone [2,49,55] *B*-form: singlet at 970 *A*-form: triplet at 977, 968 and 952 *Z*-form: triplet at 970, 951 and 925 [35] Calc: vib mainly involving C4′C5′H, O5′C5′H [50,57]
938	*B*-form	AT base pairs in *B*-form helices [58]
930–924	*Z*-form	Z-form [5] Calc: same vib as 865–860 [57]
917–916	Ribose	Ribose ring vib [27] For DNA/RNA hybrids both 917 and 899 bands are present
899–890	Deoxyribose	Deoxyribose ring vib [27] Decrease in intensity in *Z*-form DNA [24] Calc: vib mainly involving C4′C3′H, C2′C3′H [50,57]
882–877	*N*-type	Main *N*-type (C3′-endo/anti) sugar marker [4,5] Calc: C4′C5′H, O5′C5′H, C4′O1′, C5′O5′ [50]
865–860	*N*-type	Main *N*-type (C3′-endo/anti) sugar marker [4,5] Coupled furanose-phosphodiester chain vib Calc: C4′C5′H, O5′C5′H, C5′O5′ [57] Calc: PO5′, C2′C3′H, C4′C3′H, C3′C4′, C5′O5′, C2′C3′ [50]
842–820	*S*-type	Main S-type sugar marker [4,5] Coupled furanose-phosphodiester chain vib Calc: C4′C5′H, O5′C5′H, C5′O5′, C3′C4′ [57] Calc: PO5′, C4′C5′H, C3′C4′, O5′C5′H [50] Calc: suggested coupling to antisymmetric OPO str [1,49]
815–802	*N*-type	Main *N*-type (C3′-endo/anti) sugar marker [4,5] Coupled furanose-phosphodiester chain vib Calc: C3′C4′, C5′O5′, C4′C5′H [57] Calc: C3′C4′, PO5′, C4′C5′H, O5′C5′H [50] Calc: suggested coupling to symmetric OPO str [48,49]
800–750		Out-of-plane base vib (weak in H_2O, strong in D_2O) [2,59,60]

nucleic acid structures, various RNA structural elements [19,20], backbone conformational substates (*BI*/*BII*) [21,22] and transitions (*B* to *A*, *B* to *Z*) [23–36], in the study of nucleic acid hydration [37–39] and interactions between nucleic acids and metal ions [40–42] or drugs [43–45]. The studies range from monomeric to macromolecular forms of nucleic acids in the state of crystals, powders, fibers and solution. This great range of variation is possible since IR spectroscopy is not limited by either the existing state (gaseous, liquid or solid) or size (diatomic or macromolecule) of a given molecule. The FTIR technique is ideal for systematic studies of nucleic acids, (e.g. sequence variations, covalent modifications) since it is fast, non-destructive and only requires small amounts of sample [7,22].

IR band assignments of complex nucleic acids have been obtained experimentally by spectral comparisons with simple model molecules, where

assignments have been firmly established. The assignments have been extended by variations of nucleic acid base composition, investigations of the effects of deuteration, changes in pH and isotopic labeling (^{15}N and ^{18}O), as well as from investigations of polarization effects in oriented films [1–3]. In addition, calculations have been performed to resolve the extensive coupling between the different vibrational modes that are expected in a complicated macromolecule like the nucleic acid. In the present library we have indicated where assignments have been obtained by experiments and by calculations, respectively.

Our library covers the mid IR spectral range 1800–800 cm^{-1}, which is the region of interest in IR studies of nucleic acids in aqueous solution. Due to interfering vibrations of H_2O at 1645 cm^{-1} and D_2O at 1450 cm^{-1} and 1210 cm^{-1}, spectra are generally recorded in both H_2O and D_2O solutions. The results are subsequently combined to gain insight into the full 1800–800 cm^{-1} region.

The compilation, presented in Tables 1A, B, C and D, has been divided into four spectral regions, each containing marker bands reflecting either nucleic acid interactions and/or conformation. In the 1800–1500 cm^{-1} region, bands originating from the base vibrations of the nucleic acid appear, acting as extremely sensitive markers for base pairing and base stacking effects. In the 1500–1250 cm^{-1} region, vibrational coupling between the base–sugar entities give rise to nucleoside-specific information, reflecting glycosidic bond rotation, backbone conformation and sugar pucker. In the 1250–1000 cm^{-1} region, vibrations along the sugar–phosphate chain give rise to strong markers of backbone conformation. In the 1000–800 cm^{-1} region, sugar/sugar–phosphate vibrations result in reliable markers for the various sugar puckering modes.

The IR marker bands occurring at different frequency regions provide an instant snapshot of various portions of nucleic acid structure, giving information on base pairing/base stacking, nature of helix, sugar pucker etc. These marker bands respond to changes in nucleic acid state by changes in position and/or intensity and/or polarization. As an IR spectrum contains a high number of overlapping bands, a critical evaluation of the entire 1800–800 cm^{-1} spectral range is necessary in order to accomplish a firm interpretation of bands. In addition, not all marker bands are equally sensitive (see below) and, therefore, minor differences in structure and/or interaction pattern can be revealed only in the fine analysis of the spectrum [7,22].

The comments in Tables 1A, 1B, 1C and 1D are self-explanatory. The span in wavenumber given for each specific band is approximate, and has been obtained both from the references given in Tables 1A, 1B, 1C and 1D, but also from the references given above as examples of FTIR spectroscopy applied in the study of nucleic acids [6–45].

2. Comments and discussion

The following section is aimed at highlighting only certain marker bands that are of key importance in the study of major features/changes in nucleic acid structure. The compiled IR bands are summarized in Tables 1A, 1B, 1C and 1D.

2.1. The 1800–1500 cm^{-1} region — base vibrations, recorded in D_2O

In the 1800–1500 cm^{-1} region, IR bands originating from nucleobase vibrations appear which are extremely sensitive to base stacking and base pairing interactions. The bands mainly originate from in-plane double bond base vibrations, which include C=C, C=N and C=O stretch. The hydrogen to deuterium exchange of the labile NH_2 group in D_2O solution causes a shift in the amino group vibrations otherwise seen in H_2O out of this wavenumber region. Bands appearing in this region can be effectively used to monitor DNA/RNA structural changes that involve alteration of base stacking and base pairing. Effects of ligand binding to specific bases can also be followed by changes of marker bands in this region.

2.1.1. Double-helical structures

The presence of base paired structures can be inferred by inspection of peak-positions and band ratios in this region. Base pairing of guanine is

reflected by a shift of the guanine C6=O6 vibration from 1673–1660 cm^{-1} to 1689–1678 cm^{-1} and a simultaneous decrease in intensity of the guanine ring vibration doublet at approximately 1590–1564 cm^{-1} [2,5]. Base pairing of thymine is reflected by a decrease in intensity of the thymine 1671–1655 cm^{-1} C4=O4 vibration, and a simultaneous shift of the thymine ring vibration at 1632 cm^{-1} to 1645–1641 cm^{-1} [5]. Base pairing of uracil is reflected by a shift of the uracil C4=O4 vibration from 1657–1653 cm^{-1} to 1677–1672 cm^{-1}, accompanied by a decrease in intensity [2].

2.1.2. Thermal denaturation

Thermal denaturation of DNA/RNA can be followed by intensities of the IR bands as a function of temperature. A double strand to single strand transition results in a decrease in the intensity of the band at approximately 1696–1684 cm^{-1} with a concomitant increase of the band at approximately 1677–1653 cm^{-1}. The band at approximately 1696–1684 cm^{-1} arises due to the C6=O6 stretch of base paired guanine plus C2=O2 stretch of uracil and thymine. The band at 1677–1653 cm^{-1} arises mainly from the stretching vibrations of C6=O6 of free, (i.e. non-base paired) guanine, C2=O2 of free cytosine and C4=O4 of free uracil or thymine. The intensity ratio of these two bands is used to follow the thermal denaturation profile of DNA/RNA [2,20]. The band at 1632–1622 cm^{-1}, arising from C=N, C=C adenine ring vibrations, has been used to monitor the thermal denaturation of adenine-rich regions in DNA/RNA structures. The doublet in the 1590–1564 cm^{-1} region, due to ring vibrations of guanine, has been used to follow the changes in guanine-rich regions. These two bands can, therefore, resolve any difference in the denaturation behavior of adenine-rich and guanine-rich regions independent of each other [20], which gives FTIR an advantage over UV absorption studies, since the latter cannot resolve any difference in the melting behavior of adenines and guanines. The changes in the guanine doublet in the 1590–1564 cm^{-1} region can also give information on the changes in environment of guanines in a particular nucleic acid structure [2,20].

2.1.3. Triple-helical structures

DNA/RNA triple helices are of particular interest because of their potential biological applications. Two types of triple helices can be formed, pyrimidine*purine–pyrimidine and purine*purine–pyrimidine. The asterisk denotes the third (Hoogsteen or reverse Hoogsteen) strand. In the first class, we have T*A–T (or U*A–U for RNA triple helix) and C^+*G–C base triples, whereas base triples like G*G–C and A*A–T belong to the second class. IR spectroscopy has been extensively used to determine the formation of triple helices and to characterize the binding scheme of the third strand (Hoogsteen or reverse Hoogsteen), along with the nature of sugar conformation prevailing in the different strands. We will only highlight a few IR characteristics of DNA/RNA triple helices, as the details of triple helix characterization by IR have been the subject of a separate review [5]. Only bands in the 1800–1500 cm^{-1} will be discussed, since derivation of the sugar conformation in individual strands requires discussion of specific triple helices, which is beyond the scope of this review.

For a triple-helical structure formed by T*A–T base triplets with an antiparallel orientation of the third strand, a new band is detected approximately 1712 cm^{-1} which is absent in the A–T duplex spectrum. This is accompanied by a decrease in the adenine band at 1632–1622 cm^{-1}. It was already known for both a Watson–Crick base paired A–T duplex and for a Hoogsteen type of base pairing scheme of T*A–T, where the C2=O2 thymine carbonyl groups are free, that the band of the C2=O2 stretching vibration appears at 1698–1691 cm^{-1}. The 1712 cm^{-1} band has been attributed to H-bonded C2=O2 stretching vibration of thymines of the third strand in T*A–T triplex, where the third strand is involved in a reverse Hoogsteen type base pairing scheme [5].

For C^+*G–C base triplets, the formation of a triple helix results in the appearance of a new band at approximately 1710 cm^{-1} with a concomitant decrease in the cytosine band located at 1527–1520 cm^{-1}. The 1710 cm^{-1} in this case has been assigned to the C2=O2 stretching vibration of protonated cytosines belonging to the third strand. For G*G–C triplets a new band appears at 1715

cm^{-1}, assigned to the free C6=O6 stretching vibration of third strand guanines involved in a Hoogsteen base pairing scheme with parallel orientation. However, for purine*purine–pyrimidine triple helices with a mixed A and G sequence in the third strand, a band is observed at 1687 cm^{-1} [5] characteristic of the reverse Hoogsteen type G*G–C and A*A–T triplets.

2.2. The 1500–1250 cm^{-1} region — base–sugar vibrations, recorded in H_2O

In the 1500–1250 cm^{-1} region, vibrations localized to the base and base–sugar entities give rise to marker bands sensitive to glycosidic bond rotation, backbone conformation and sugar puckering modes. This region can be used to obtain information about nucleoside-specific interaction and conformation.

2.2.1. Interaction involving the N7 sites of purines

The band at 1495–1476 cm^{-1} is assigned to purine imidazolic ring vibrations, and depends strongly on the bending of N7C8H. Any changes in the position and/or intensity of this band reflect interactions on N7 sites, as for example in the case of triple helix formation [16] or in the case of changes in hydration in the major groove of nucleic acid structures [6,12].

2.2.2. Anti/syn conformation

The traditional marker band reflecting the orientation of the glycosidic bond in the nucleoside, appears at approximately 1381–1369 cm^{-1} in the case of purines in anti conformation and shifts to 1357–1352 cm^{-1} in the case of purines present in the syn conformation [4,5,35]. The corresponding marker band for cytidine in anti conformation appears at approximately 1365–1360 cm^{-1} [35].

2.2.3. Sugar conformation

The bands at 1344 and 1328 cm^{-1} characterize adenosine and thymidine with *S*-type or C2′-endo sugar conformation, respectively. The 1281 cm^{-1} band is also due to thymidine (or uridine) with *S*-type sugars. Thus, the observation of both the bands at 1328 cm^{-1} and 1281 cm^{-1} allows an unambiguous assignment of thymidine in *S*-type sugar conformation [5,27]. Similarly, the observation of two bands at 1335 and 1275 cm^{-1} help in assigning thymidine with *N*-type sugars. The band at 1335 cm^{-1} arises due to vibrations of adenosine, thymidine and uridine with *N*-type or C3′-endo sugar conformation, while the band at 1275 cm^{-1} is due to thymidine (or uridine) with *N*-type sugars [5,27].

The values of the wavenumbers for the different sugar conformations in this region have been obtained mostly from experiments with simple sequences. As the position of these bands may be very sensitive to sequence context, conclusions with respect to the sugar conformation may be doubtful for arbitrary sequences. This may also be the case for some other infrared bands in the 1500–1250 cm^{-1} region.

2.3. The 1250–1000 cm^{-1} region — sugar–phosphate vibrations, recorded in H_2O/D_2O

In the 1250–1000 cm^{-1} region, vibrations along the sugar–phosphate chain give rise to marker bands sensitive to nucleic acid backbone conformation (*A*-, *B*- or *Z*-form). Part of this region (1150–1000 cm^{-1}) and the next full region (1000–800 cm^{-1}) can be recorded in both H_2O and D_2O, and thus, allows for comparison of bands observed in D_2O with those observed in H_2O. The spectra recorded in D_2O solution include the effects of the H to D exchange of the labile H of NH, NH_2 and OH groups in nucleic acids. In the 1150–1000 cm^{-1} region, such a comparison becomes particularly important for assigning vibrations arising from riboses, as the labile H of 2′-OH is capable of exchange in D_2O.

2.3.1. Backbone conformation

The antisymmetric PO_2^- stretching band is a characteristic marker for nucleic acid backbone conformation, independent of nucleobase vibrations and sugar pucker. In the *B*-form double helix, it appears at approximately 1225 cm^{-1}, in the *A*-form at approximately 1240 cm^{-1} and in the *Z*-form at approximately 1215 cm^{-1} [1,2,4,5]. This band is routinely used to follow the structural transitions between the *A*-, *B*- and *Z*-helical forms of DNA. In the case of RNA, a contribution from

a ribose vibration appearing at approximately 1221 cm^{-1} [27] overlaps the *B*-form marker (1225–1220 cm^{-1}), and thus makes it difficult to estimate any share of *B*-helical conformation in ribonucleotides. The 1188–1175 cm^{-1} band, arising from sugar–phosphate backbone vibrations with a contribution from the sugar moiety in C3′-endo/anti conformation [28], acts as a marker for *A*-form helices, and can be used in conjunction with the 1245–1235 cm^{-1} band to identify *A*-form geometry in nucleic acids. The symmetric PO_2^- stretching mode, appearing at 1090–1085 cm^{-1}, is rather insensitive to the *B*-to-*A*-helical transition, and is often used as an internal standard for spectral normalization [2,27]. Backbone vibrations with strong contributions from a C–O stretch appearing at 1069–1044 cm^{-1}, are also less sensitive to conformation, even though the intensity of the band is increased in the case of *Z*-form helices [5,35].

2.4. The 1000–800 cm^{-1} region—sugar vibrations, recorded in H_2O/D_2O

In the 1000–800 cm^{-1} region, vibrations along the sugar–phosphate backbone result in bands particularly sensitive to the various nucleic acid sugar puckering modes (*N*- and *S*-type). For *N*-type of sugars, three marker bands appear at 882–877, 865–860 and 815–802 cm^{-1}, while for *S*-type of sugars there is one broad band appearing at 842–820 cm^{-1} [4,5,46]. Studies of the relative intensities of the sugar conformational marker bands at approximately 865–860 cm^{-1} and 842–820 cm^{-1} have been used to evaluate relative amounts of *N*- and *S*-type sugars in oligonucleotides of varying base sequence [7,26].

2.4.1. Particular sensitivity to sugar conformation

The transition of a poly(rA)poly(dT) film from a heterogeneous conformation [poly(rA) in *A*-form, poly(dT) in *B*-form] to an *A*-family type of geometry, as induced by a lowering of the relative humidity (RH), is reflected by a non-simultaneous disappearance of two different and commonly used *S*-marker bands. The 841 cm^{-1} band (characteristic of *S*-type of sugars) disappears at 81% RH, while the 1281 cm^{-1} band (characteristic of thymidine with *S*-type of sugars) disappears at 32% RH [27,47]. These non-simultaneous transitions emphasize the need for careful inspection of the entire IR spectrum in the interpretation of IR data. The observations also suggest a greater sensitivity to sugar conformation for the vibrations occurring in the 900–800 cm^{-1} sugar region as compared to the 1500–1250 cm^{-1} base–sugar region. Studies have shown it possible, by careful inspection of the 900–800 cm^{-1} region, to reveal minor but significant changes in DNA sugar pucker as a consequence of biologically relevant modifications in base sequence or functional groups of DNA oligonucleotides. A varying tendency for *A*-form features as a result of variations in base sequence of *B*-DNA duplexes has been revealed by estimates of the relative ratios of the *N*- and *S*-markers appearing in this region [7]. In addition, it was recently found that cytosine methylation in GC-rich deoxyribonucleotide duplexes gave rise to a subtle splitting of the *S*-type 842–820cm^{-1} marker band, suggesting the coexistence of two different major sugar puckers within the *S*-family in the methylated DNA sequences. The results were interpreted in terms of localized transitions between the *BI* and *BII* sub-conformational states of the *B*-DNA backbone as a consequence of methylation [22].

2.4.2. Contributions from OPO vibrations

Bands occurring in the 840–800 cm^{-1} region have been suggested to show a fairly high contribution from stretching motions of the OPO group. Combined experimental and calculated results of dialkylphosphate anions as simple models of the nucleic acid backbone have shown that antisymmetric and symmetric stretching vibrations of the bridging phosphodiester group occur in this region [1,48,49]. Such a contribution could be important in the study of for example backbone conformational substates, differing in the value of the dihedral angles ε and ζ [22]. However, calculations of a backbone model that includes the sugar moiety have assigned the 842–820 cm^{-1} *S*-marker band and the 815–802 cm^{-1} *N*-marker band to vibrations along the P-O5′-C5′-C4′-C3′ part of the sugar–phosphate backbone, thus excluding the O3′-P of O3′-P-O5′ from these vibrations. The

authors argued that the complete absence of P-O3′ group in this vibration could be due to consequences of lowered symmetry of the OPO group when included in the nucleic acid phosphodiester chain [50].

3. Concluding remarks

IR spectroscopy applied to biological macromolecules, like nucleic acids, gives group-specific structural information, rather than structure at atomic resolution like X-ray crystallography or high resolution NMR. However, the information content of an IR spectrum is high. The spectra are easy to obtain and require relatively small amounts of samples. IR spectroscopy can be applied regardless of state of the sample or molecular size. An added advantage is that sample heterogeneity or dynamic equilibria between structural states show up as in a snapshot which may be assessed and possibly interpreted. The present library of IR band positions and assignments should hopefully facilitate the interpretation of IR spectra in structural studies of nucleic acids.

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