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ASPECTS OF LOW FREQUENCY VIBRATIONS (20-350 cm^{-1}) FROM WATSON-CRICK BASE PAIRING IN AN AQUEOUS SOLUTION OF tRNA.

O. Faurskov Nielsen. P.-A. Lund[a], Lone S. Nielsen[b] and E. Praestgaard[c]

University of Copenhagen, Chemical Laboratory V, The H. C. Ørsted Institute, 5, Universitetsparken, DK-2100 Copenhagen Ø, Denmark.

[a]Danish National Institute of Occupational Health, Baunegårdsvej 73, DK-2900 Hellerup. [b]Copenhagen University Hospital, DK-2600 Glostrup.

[c]Roskilde University, Postbox 260, DK-4000 Roskilde, Denmark

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Summary: The low frequency Raman spectrum from 20 cm^{-1} to 350 cm^{-1} of tRNA from Escherichia coli in an aqueous solution is studied. A band at ca. 115 cm^{-1} is assigned to displacements of atoms in hydrogen bonds between Watson-Crick base pairs. Temperature studies between room temperature and ca. 90°C are performed showing near reversibility of the base pairing upon temperature. The couplings between low frequency vibrations of tRNA and formamide, N-methylformamide and N,N-dimethylformamide are discussed in terms of physiological effects.

The concept of low frequency vibrations and coupling between these have been thought to be of importance for structural changes and reactions of biological systems (1-10). Investigations have been performed to determine experimentally vibrational frequencies for biologically active molecules (11-13). Most of the interesting in vivo reactions are performed in aqueous solutions or gel states, which unfortunately are very unsuited for investigations by ordinary IR and Raman methods at frequencies below 200-300 cm^{-1}. In order to solve this problem we used an $R(\bar{\nu})$ representation of the low frequency Raman spectrum in studying water (14) and aqueous solutions of γ-aminobutyric acid (15),nucleosides (16), nucleotides (17-19) and formamides (20,21). The $R(\bar{\nu})$ representation could be used

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even to obtain information about the low frequency vibrational spectrum of water in aqueous gels of agarose and κ-carrageenan (22) and cellulose (23). We proposed the importance for in vivo systems of intermolecular coupling for some of the experimentally observed frequencies (15,18). Most of the low frequency bands observed were broad, but of particular interest was the assignment of a rather sharp band at ca. 115 cm^{-1} to a selfassociate of guanosine-5'-monophosphate (18,19). A more precise description of these modes is desirable and studies of some model compounds including isotopically substituted acetic acid (24), formamide and other simpler amides have been undertaken (20,21,25). Between 100 cm^{-1} and 200 cm^{-1} was observed a mode, which could be assigned to an "out of plane" motion of atoms involved in hydrogen bonding between selfassociated molecules.

The aim of the present paper is to show that the $R(\bar{\nu})$ representation permits investigation of low frequency vibrational modes of aqueous solutions of tRNA from Escherichia coli. Representations similar to the $R(\bar{\nu})$ representation are generally useful in studies of low frequency vibrations as discussed in refs. 24,26,27, and 28 and references cited therein.

METHOD

A sample of tRNA from Escherichia coli was obtained from Boehringer Mannheim (no. 15518 in the 78/79 catalog). An aqueous solution was obtained by dissolving 80 mg of tRNA in 800 µl redistilled water (pH ∿ 7). This solution was cleaned for particles by ultracentrifugation at 85.000 rpm for ½ hrs. using a Beckman Airfuge ultracentrifuge. Ca. 400 µl of the centrifuged solution was transferred to a 10 mm (o.d.) NMR tube, which was used as cell. Raman spectra from 20 to 350 cm^{-1} were recorded on a Coderg PHl spectrometer using perpendicular illumination, cooled PM tube (EMI 9558) and a Coderg CPH 100 photon counting system. The vertically polarized 514.5 nm line of a Spectra Physics model 165 Ar^{+}-laser (400 mW) was used as exciting source in a horizontal scattering plane (I_{VV} configuration). A microprocessor sampled the number of counts per step in frequency (8.4 step per cm^{-1}) with a 24 bits dynamical range. Calculations were performed on either an RC 4000 or an RC 8000 computer. The spectral slit width was 4 cm^{-1} and the registration speed 10 cm^{-1} per minute. Temperature experiments were performed by thermostatting the cell with water and the temperature should be accurate to ± 1°C. $R(\bar{\nu})$ curves were calculated from the intensity in the Stokes side of the Raman spectrum, $I(\bar{\nu})$:

$$R(\bar{\nu}) \propto \bar{\nu}[1 - \exp(-h\bar{\nu}c/kT)]I(\bar{\nu})$$

where the symbols have their usual meaning (14-26). All spectra showed some fluorescence and a background correction was performed as described previously (14-22,23,26) by subtracting a mean intensity value found in a 10 cm^{-1} interval around the lowest intensity from all intensities in the $I(\bar{\nu})$ curve prior to calculation of $R(\bar{\nu})$. The $R(\bar{\nu})$ curves were smoothed by a running mean smoothing procedure (29) and in order to improve the signal to noise ratio two $R(\bar{\nu})$ curves from separate runs were finally added for each temperature. The results are shown in Fig. 1 where the highest intensity in an $R(\bar{\nu})$ curve for each temperature was normalized to the same height and curves at different temperatures (A-C) were then placed above each ether.

RESULTS AND DISCUSSION

Fig. 1A (26°C) and Fig. 1B (50°C) show a rather sharp band with a maximum at 115 cm^{-1} and a weaker and broader shoulder at 70-80 cm^{-1}. The band at 115 cm^{-1} has disappeared completely at 92°C (Fig. 1C) at which temperature the $R(\bar{\nu})$ curve is dominated by the broad band with maximum at ca. 70 cm^{-1}. Recently (17-19), we assigned broad bands with maxima between 65 and 100 cm^{-1} to modes involving displacements of water as well as displacements of atoms in the nitrogen containing bases of the nucleic acids. By hydrogen bonding between 5'-GMP molecules (self-association) a rather sharp band appeared at ca. 115 cm^{-1} and this band was preliminary assigned to a mode involving atoms in the hydrogen bond. Our recent investigations of model compounds (20, 21,24,25) confirm this assignment. It is thus straightforward to assign the band at 115 cm^{-1} in Fig. 1A and Fig. 1B to a mode involving atoms in the Watson-Crick hydrogen bonds between bases within tRNA, and most probably an "out-of-plane" motion as shown in Fig. 2, in analogy with results from the model compounds (20,21,24,25). The hydrogen bonds are know to break at temperatures between 40°C and 60°C (11,30) and the disappearance of the 115 cm^{-1} band in the spectrum at ca. 90°C thus strongly corroborates the assignment of this band to a mode involving hydrogen bonding between the bases. The broad band at ca. 70 cm^{-1} is assigned to stacks of bases non hydrogen bonded to each other in complete agreement with our previous observations on mononucleotides (17-19).

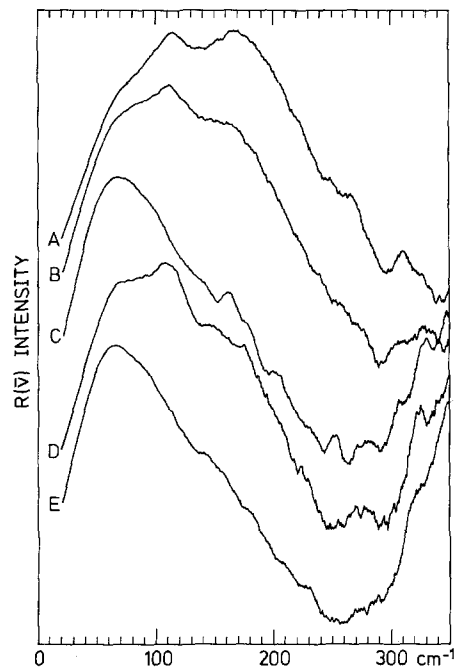

Figure 1 Smoothed $R(\bar{\nu})$ curves of a ca. 10% aqueous solution of tRNA from Escherichia coli. All spectra were obtained in I_{VV}-configuration. The maximum intensity in a given curve was normalized to the same height for all curves and the curves were placed above each other. A: 26°C, B: 50°C, C: 92°C, D: 26°C, and E: 90°C, see text.

This band at a reduced intensity is also present at 26°C and 50°C indicating the presence of some bases non hydrogen bonded to each other.

The band at ca. 170 cm^{-1} in Fig. 1A-C is in accordance with previous results (14,28) assigned to water. The intensity was reported to decrease drastically with increasing temperature (27,31,32), whereas the frequency was lowered only 10 cm^{-1} from room temperature to 80°C (28). Accordingly, the intensity of the 170 cm^{-1} band decreases from Fig. 1A to Fig. 1C.

The band with a maximum at 115 cm^{-1} assigned to the Watson-Crick base pairing appears again in Fig. 1D, which shows the $R(\bar{\nu})$ curve after cooling down from 92°C to 26°C within a period of ca. 12 hrs. and the previously reported (11,30) reversibility upon temperature seems supported. However, the band at 115 cm^{-1} in Fig. 1D is somewhat

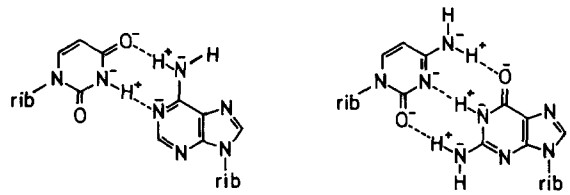

Figure 2 An approximate description of an out of plane motion involving atoms in the hydrogen bond. The plus and minus signs mean in and out of the plane, respectively.

broader than the corresponding band before heating (Fig. 1A), which might indicate that although hydrogen bonds again are formed between bases, some minor structural differences may exist between heated and non heated tRNA. The sample was again heated to 90°C and as shown in Fig. 1E the 115 cm^{-1} band disappeared and only the band at 70-80 cm^{-1} due to stacking is observed.

The high frequency part of the individual $R(\bar{\nu})$ curves differ somewhat. This is due to a small change in fluorescence upon heating, changing the background correction a little. The intensity of the water band at ca. 170 cm^{-1} may also depend somewhat upon this background correction whereas the influence upon bands at lower frequencies is insignificant (26).

The method described in this communication allows a direct experimental observation of a vibration of atoms directly involved in the Watson-Cric base pairing of tRNA from Escherichia coli and the structural changes could directly be followed. Similar modes (Fig. 2) are expected for other RNA molecules with base pairing. Although the importance of vibrational coupling between low frequency modes, even at frequencies lower than those investigated in this work has been emphasized from theoretical viewpoints (1-10) we believe that the experimental determinations of low frequency modes by $R(\bar{\nu})$ technique may be helpful in clarifying these aspects. A specific example shall be given. In order to achieve a maximal dynamical coupling between different molecules, these should show nearly coinciding frequencies. Neat liquid formamide

(20,21) exhibits two bands of nearly equal intensity at 192 cm^{-1} and 110 cm^{-1}. N-methylformamide (20,25) shows below 200 cm^{-1} a strong band at 112 cm^{-1} and N,N-dimethylformamide (20,25) a strong band at 65 cm^{-1}. The 110 cm^{-1} band for formamide and the 112 cm^{-1} band for N-methylformamide are both very close to the vibrational frequency at 115 cm^{-1} for the mode shown in Fig. 2. Formamide and especially N-methylformamide are known to show teratogenic effects (33-35) and a plausible mechanism for the physiological effect of these molecules is a vibrational coupling through the 110-115 cm^{-1} bands, which would perturb the hydrogen bonding between the bases in nucleic acids. This hypothesis is supported by the fact that N,N-dimethylformamide (20) and tRNA show no resonance frequencies in this frequency region and accordingly N,N-dimethylformamide is reported to show no teratogenic effects. Furthermore, many chemicals and derivatives of these are known to show mutagenic effects for which the detailed mechanism is unknown, but vibrational coupling might explain some of these effects, and an experimental determination of the low frequency vibrations is thus important. Formamide and N-methylformamide are known to show an anticancer effect (36-45), which is easily understandable from the coupling mechanism described above. We hope that further aspects of a detailed knowledge of the low frequency vibrations due to hydrogen bonding might give a better selection of drugs used in therapy of some cancer forms.

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