IR study of base stacking interactions

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ABSTRACT

For D₂O solutions of 1,3-dimethyluracil, cytidine, caffeine, inosine and 2'-deoxyadenosine the concentration dependence of IR spectra (1800-1400 cm⁻¹) have been found which reflects stacking association of these compounds. A method is proposed to use this data to obtain thermodynamic parameters of association and the molecular spectra in the monomer and associated forms. The homoassociation constant for 1,3-dimethyluracil was estimated as $k=0.65$. Stacking is shown to change radically the spectra, inducing a high-frequency shift of carbonyl vibrations and a decrease in the intensity of skeletal stretching vibration bands. This allows one to distinguish between the stacking interactions and hydrogen bonding. A conclusion is made about the considerable contribution of stacking interactions into the change of IR spectra of DNA and other polynucleotides following conformational transitions.

INTRODUCTION

In aqueous solutions the nucleic bases and their derivatives associate forming the interplanar contacts. The so-called stacking interaction takes place /1/. This type of interaction is of great interest for physico-chemistry of nucleic acids, since the stacking makes a substantial contribution into the stability of the secondary structure of polynucleotides /2-5/. A study of this phenomenon is also necessary to understand the chemical reactivity of the plane molecules and to solve a number of problems of prebiological evolution. Since the IR spectroscopy is one of the most informative methods for studying the structure of compounds and intermolecular interactions, the question arises as to the presence and peculiarities in the behaviour of interplanar interactions in IR spectra as well as the possibilities of applying this

method for stacking studies. On the other hand, the above mentioned is necessary for gaining detailed information about the structure of polynucleotides from their IR spectra. The effect of various intra and intermolecular interactions on the IR spectra of polynucleotides was discussed in a number of works /6-17/. In some papers the analysis of polynucleotide IR spectra was performed considering only base-pairing /17-22/ and a number of important for molecular biology conclusions have been made. The problem of interpretation of polynucleotide spectra can not be solved without regard to the effect of stacking interactions. Eyster and Prohovsky calculated normal vibrations of poly(A).poly(U) double helical complex but failed to obtain the effect of stacking on the spectrum in the range from 1800 to 1400 cm^{-1} /9/. Tsuboi et al. /7,8/ made an attempt to reveal experimentally the stacking effect, comparing IR spectra of dinucleosidephosphates and corresponding monophosphates. They concluded that the stacking of bases does not induce drastic changes of the IR spectra as compared to the changes of polynucleotide IR spectra following conformational transitions. This conclusion does not seem convincing to us, since the selfassociation of nucleotides which is substantial for such systems /23/ was not taken into consideration and on the other hand, it seems that measurements of IR spectra were not taken sufficiently correctly (see Methods). In the present work to elucidate the IR spectroscopic appearance of stacking interactions we investigated the selfassociation of nucleic bases and nucleosides in aqueous solutions in which no H-bonding of bases is found /24/ and, as opposed to nucleotides the effect of phosphate groups is ruled out. With this purpose we made concentration-dependent measurements of IR spectra for the purine derivatives - caffeine, inosine, 2-deoxyadenosine and pyrimidine ones-1,3-dimethyluracil and cytidine.

MATERIALS AND METHODS

1,3-dimethyluracil was synthesized as described elsewhere /25/. Caffeine (pharmaceutical preparation, USSR) was twice recrystallized from water, crystallized water was re-

moved by sublimation at 100°C. Inosine, cytidine ("Reanal", Hungary) and 2'-deoxyadenosine ("Fluck" Co. Switzerland) have been used without additional purification. Heavy water (D_2O) (produced by the "Isotope" plant, USSR) contained 99.8% of the basic substance. To substitute the labile protons by deuterium cytidine, inosine and 2'-deoxyadenosine were lyophilized from D_2O solutions.

The solutions were prepared by diluting the stock solutions by means of weighing, the error being less than 0.1%. The concentration of the stock solution was determined with spectrophotometer VSU-2P (GDR) using the molar extinction coefficients given in literature: for 1,3-dimethyluracil /26/, cytidine /27/, caffeine /28/, inosine /29/, 2'-deoxyadenosine /27/.

The IR spectra were measured on IR Spectrophotometer Perkin Elmer M-180 in CaF_2 cells with the optical path length from 9.9 to 100 μm . The thickness of the cells was estimated by the interference method with the error less than 1%. The cells were thermostated at $25 \pm 0.1^\circ C$.

In the studied range from 1800 to 1400 cm^{-1} D_2O has rather high absorption even when measurements are taken in thin cells /6/, therefore for quantitative measurements the solvent absorption must be carefully compensated by means of the reference cell. For the accuracy of spectral measurements it is essential that PD, the ionic force in the sample and reference cells and especially the effective thickness of the layer and isotope content of D_2O are equal. To reach the equality of the layer thickness we used the reference cells with the optical path length 0.1-1% less than for the sample cell, since the dissolved substance decreases the concentration of the solvent. Compensation of D_2O absorption in the range near 1550 cm^{-1} was controlled from the D_2O band at 3845 cm^{-1} . The control of the compensation of the absorption of light water admixture in the range of 1460 cm^{-1} was controlled from the vibration band of HOD molecules at 3420 cm^{-1} . Non-compliance with these methodic requirements distorts the IR spectra which, for example in studying the 5'AMP + 5'UMP solutions /7/ appears as the crossing of the solution spectra

with the line of zero absorption. Unfortunately the details of the compensation are not reported in the above paper.

Theory and Data Processing for Determination of Constants and Spectroscopic Appearance of Stacking. In the case of nonassociating substance in the solution, for the molar extinction coefficient (ϵ) the Lambert-Beer's law is valid:

$$\epsilon(A) = \text{const}$$

where A is the concentration of the substance.

In the case of association it is expected that

$$\epsilon(A) = \sum_{n=1}^{\infty} \epsilon_n A_n / A$$

where ϵ_n is the extinction coefficient of associate containing n molecules, A_n is the concentration of this aggregate.

A_n values are determined by the thermodynamics of association process

$$A_1 + A_n \xrightleftharpoons{K_n} A_{n+1}$$

and the values of ϵ_n characterize spectroscopic effect of association.

To describe the experimental dependence $\epsilon(A)$ the following model has been used. Isodesmic process of stacking association is assumed. In this case the constants of association are equal at each stage $K_1 = K_2 = K_n = K$. This assumption is most commonly used to describe the stacking /31-33/. Further, we assume that the molecular spectrum is affected only by the nearest neighbours. Then with the formation of an aggregate the value of ϵ will change by an amount $\Delta\epsilon$ for each face of the monomer involved in a stack.

A similar model has been used in /32-35/ where the stacking was investigated by NMR, UV spectroscopic and ultrasonic methods. From this model we have:

$$A_1 = [2K + 1/A - 1/A(1 + 4KA)^{1/2}] / 2K^2 \quad (1)$$

$$\epsilon = \epsilon_m + 2\Delta\epsilon KA_1 \quad (2)$$

$$[(\epsilon - \epsilon_m)/A]^{1/2} = (K/2\Delta\epsilon)^{1/2} [2\Delta\epsilon - (\epsilon - \epsilon_m)] \quad (3)$$

where A_1 is the concentration of monomers, ϵ_m is the molar extinction coefficient of monomer.

Equation (3) is the linear function between the experimental values $[(\epsilon - \epsilon_m)/A]$ and $\epsilon - \epsilon_m$, where ϵ_m is found from the experimental dependence $\epsilon(A)$ by extrapolation to the infinite dilution. The value of the stacking constant is determined from the slope and intersection points of this line. Concurrently with value K from equation (3) we obtain the value of spectral parameter $\Delta\epsilon$ and can calculate the extinction coefficient of the molecule in stacking associate by

$$\epsilon_s = \epsilon_m + 2K\Delta\epsilon \quad (4)$$

If value K is known there is no need to process the data from Eq.(3) for all frequencies (ν) in order to calculate the spectrum $\epsilon_s(\nu)$. In this case values $\epsilon_s(\nu)$ and $\epsilon_m(\nu)$ may be readily calculated from the two experimental spectra $\epsilon'(\nu)$ and $\epsilon''(\nu)$ at two concentrations A' and A'' . From equations (2,4) we have:

$$\epsilon_m = \frac{\epsilon' A'' - \epsilon'' A'}{A'' - A'} \quad (5)$$

$$\epsilon_s = \frac{\epsilon'' - \epsilon' + K(\epsilon' A'' - \epsilon'' A')}{A'' - A'} \quad (6)$$

where A_1 values are found from Eq. (1).

RESULTS

For all the investigated compounds we found the concentration dependence of IR spectra. Fig.1 illustrates this effect for 1,3-dimethyluracil and caffeine. Increasing the concentration causes substantial changes in the spectra within the whole range from 1800 to 1500 cm^{-1} . Fig.2 shows the concentration dependence of the molar extinction coefficient $\epsilon_{1700 \text{ cm}^{-1}}$ of 1,3-dimethyluracil and the treatment of these data by Eq. (3). It is seen from the figure that the experimental data are well linearized in the coordinates $x = \epsilon - \epsilon_m$ $y = [(\epsilon - \epsilon_m)/A]^{1/2}$, which supports our model. The obtained value of stacking constant $K = 0.65 \pm 0.13$ is in a good agreement with

Fig.1. Effect of concentration on IR spectra of the nucleic bases in neutral D_2O solution. A, 1,3 dimethyluracil at the concentration of 0.04 M (—), 1 M (····). B, caffeine at the concentrations of 0.013 M (—), 0.078 M (····).

the value $K=0.56$ obtained by osmometry technique /36/.

Experimentally obtained concentration dependences of IR spectra reflect the differences in the spectra caused by small changes in the degree of association. For example, the spectra of caffeine (Fig.1) measured in the range of 0.01-

Fig.2. Concentration dependence of $\epsilon_{1700\text{cm}^{-1}}$ of the absorption band for C(2)=O vibration of 1,3-dimethyluracil in D₂O solution. B, the same data plotted in coordinates $x = \epsilon - \epsilon_m$ $y = [(\epsilon - \epsilon_m)/A]^{1/2}$ according to eq.3.

0.08 M limited by the sensitivity of the method and the solubility, correspond to the change of monomers content from 85% to 40%. To determine a complete spectroscopic effect of association it is necessary to carry out an extrapolation of the measurements within some limited concentration range for the case of zero concentration when the molecules are in monomer state and infinite concentration, when all the molecules are associated. This procedure was carried out by (5) and (6) with a step of 1 cm^{-1} . Below we present the results of calculations of monomer and stack spectra.

1,3-dimethyluracil. In the range from $1800\text{--}1500\text{ cm}^{-1}$ of the spectrum three bands at 1615 , 1653 and 1691 cm^{-1} are observed which arise from the vibrations of C=O groups and the base ring [7]. The spectra of the monomer and stacking associate (Fig.3) were calculated from the spectra of the

Fig.3. IR spectra of molecules in a monomer and in stacking associate. — monomer, stack. Calculations of the spectra from (5)(6). See details in the text.

0.04 M and 1 M solutions and $K=0.65$. As seen from Fig.3A when the molecule transits from a free state to a stack there occur marked changes in the IR spectrum of 1,3-dimethyluracil, characterized by the band shift of carbonyl vibrations towards high frequency region. When the bands at 1614, 1652,

1692 cm^{-1} disappear, the new bands at 1623, 1658 and 1701 cm^{-1} become evident.

Cytidine. In the cytidine spectrum the bands of 1652, 1617, 1524 and 1505 cm^{-1} are observed. According Tsuboi et al. /7/ these bands are assigned, respectively, to the valent vibration of C(2)=O group, skeletal vibration of C=C bonds and the skeletal vibrations of CN bonds. To calculate the monomer and associate spectra we took $k=0.95$ /36/ and the spectra of 0.699 M and 0.0658 M solutions. As seen from Fig.3D, the change of the IR spectra of cytidine during stacking association is characterized by a decrease in the intensity of skeletal vibration bands, the decrease in the $\epsilon_{1505}/\epsilon_{1524}$ ratio, a slight low frequency shift of CN vibrations and the absence of the shift for the C=O vibration band.

Caffeine. The spectra of the monomer and stack obtained from the spectra of caffeine at the concentration of 0.08 and 0.013 M are presented in Fig.3B. For calculations the stacking constant $k=9.4$ was taken /37/. The caffeine stacking causes alteration of intensities and a typical shift of the maxima of carbonyl towards the high-frequency region:
1641 $\rightarrow$ 1657 cm^{-1} , 1691 $\rightarrow$ 1702 cm^{-1} .

Inosine. The inosine spectrum reveals a strong band near 1673 cm^{-1} assigned to C(6)=O valent vibration /38/. The spectra of inosine molecules in a free state and in associate (Fig. 3E have been obtained at $k=1.8$ /39/, and from the experimental spectra for the 0.12-0.012 M solutions. As seen from Fig.3, the stacking leads to a high-frequency band shift of inosine C=O vibration by 14.5 cm^{-1} .

2-deoxyadenosine. The spectra of the monomer and stack were calculated from the experimental spectra at the concentration of 0.059 and 0.015 M. The stacking constant was taken to be 10. This value is an average of the results of different authors /33,35,39,40/. As seen from Fig.3 a change of IR spectra of deoxyadenosine following stacking is characterized by a drastic decrease in the absorption band of skeletal vibration of the adenine residue near 1628 cm^{-1} /7/ and a small high-frequency shift of this band.

DISCUSSION

Our results indicate convincingly that stacking interaction markedly changes the IR spectra of valent carbonyl vibrations and skeletal vibrations of CC and CN bonds of the base ring. There occurs not only the change of intensities but the shift of bands especially for C=O vibrations not observed earlier by Tsuboi et al. /7,8/. It was shown for cytidine, 1.3-dimethyluracil, caffeine and inosine that this shift of the valent vibrations of C=O group occurs only towards high-frequency region, in contrast to the hydrogen bonding characterized by the known low-frequency band shift of C=O group. A similar situation is observed for the NMR method /1/, where chemical shifts of protons occur in different directions. This allowed extensive applications of NMR method for distinguishing two different types of association of plane heterocycles due to the formation of hydrogen bonds or interplanar contacts. Since the application of NMR method is limited to compounds with weak ring current such as pyrimidines, we believe that the method of IR spectroscopy could find applications for a solution such problems in studying purines, pyrimidines and other heterocyclic compounds. We hope also that our results would stimulate theoretical calculations of IR spectroscopic effect of stacking. The IR spectrum contains extensive information about the structure of a molecule, and therefore such calculations would make IR spectroscopy a convenient tool for determination of the space arrangement of molecules in the stacking associate. The data we obtained about spectroscopic appearance of the stacking on a simple model of association of free bases and nucleosides in the aqueous solution allow one to approach the analysis of IR spectroscopic effect of conformational transitions of polynucleotides. The change in the IR spectra of polynucleotides during the transition from the non-ordered into highly-ordered structure is characterized by the high frequency shift of the carbonyl band and a decrease in the band intensity of CC and CN vibrations. This has been observed for DNA and RNA /18/, poly U /41/, poly(A)·poly(U) /6,19,42), poly(I)·poly(C),

2 poly(I)·poly(A) /43-46/, poly(G)·poly(C) /6,19,47/ and other complexes of polynucleotides. Comparing these data with our results shows that many typical features of spectroscopic effects observed when the polynucleotides form an ordered structure are also manifested due to selforganization of free bases in the aqueous solution. Similar changes are observed in the value as well. These results indicate the necessity of considering the spectroscopic effect of stacking when discussing the results of works /17-22/, where the spectra of DNA, RNA and other polynucleotides were analyzed with the account of bases pairing only.

From the literature data one can see that hydrogen bonds also contribute to the IR spectra of polynucleotides. This is evidenced by the absence of a high-frequency shift of 1692cm^{-1} band for poly U in the complex with poly A /6,19/, in contrast to selfspiralization of poly U /41/, as well as by some spectral differences for double- and triple-helical complexes of polynucleotides /42/. The contribution of the hydrogen bonding in poly G·poly C spectrum has been proved in /15/. Our experimental data make a basis for a detailed analysis of structure of DNA and other polynucleotides on the basis of a separation of contributions of stacking and hydrogen bonds of different bases and atomic groups in the spectrum by means of a quantitative analysis of band intensity dependences.

The preliminary results of this work were reported in /48/.

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