Molecular Interactions and Properties of Biologically Active Compounds: Infrared and Raman Spectroscopic Studies

A. Bertoluzza*, C. Fagnano, G. Fini

Centro Studi Interfacoltà sulla Spettroscopia Raman, Sezione di Chimica e Propedeutica Biochimica, Dipartimento di Biochimica, Università di Bologna, Via Selmi 2, 40126 Bologna, Italy

and

M. A. Morelli

Dipartimento di Chimica »Ciamician«, Università di Bologna, Via Selmi 2, 40126 Bologna, Italy

The correlation between molecular interactions and properties of biomolecules is a pre-eminent problem in the biological field.

H-bond interactions in different classes of biomolecules, such as phospholipids, polypeptides, polynucleotides, polyamines and neurotransmitter-receptors, are here discussed by vibrational infrared and Raman spectroscopy.

Effects of the strength, non-stoichiometry, and of the water molecules on this type of bond have been considered and correlated with structural and biological modifications.

I. INTRODUCTION

Molecular interactions play a very important role in the biological field, since they determine specific biological properties and functions. The hypothesis regarding the existence of a close relation between the »specificity« of a molecular interaction and the »specificity« of biological properties is still to be established and is an important stage in the study of the correlation between structure and function of biologically active compounds.

For several years we have been carrying out Raman and infrared vibrational studies in this field.[1-11] The results of such studies are here compared and elaborated with new results to indicate correlations between molecular interactions and biological properties.

The hydrogen bond is the most »articulate« type of molecular interaction, even if dipole-dipole and van der Waals forces can contribute to the »specificity« of such an interaction.

The idea of a »static« hydrogen bond must be discarded and it is necessary to re-evaluate the possibility that such a bond »dynamically« organizes itself,

---

* Author for correspondence.

by modifying its energy (i. e. the common concept of »strength«), its polarizability, the protonic transfer, etc.

The properties of a hydrogen bond depend not only on the characteristics of the atoms which bind together via the hydrogen atom but also on the groups to which such atoms are directly bound. Furthermore, the non--stoichiometry of the interaction, as well as the presence of molecular species of the »habitat« (e. g. water) involved in the same interaction, can modify the properties of the hydrogen bond and, therefore, the biological properties determined by the bond itself.

Hence, the specificity of the hydrogen bond in the biological field can be modulated by several parameters, the importance of which will be discussed with the aid of some Raman and infrared results.

The commonest hydrogen bond in biological compounds is the OHO one. For such a bond, close correlations exist between $O \cdots O$ and $O—H$ distances, from which it is possible to distinguish the hydrogen bond in different types (Figure 1). »Normal or weak«, $O—H \cdots O$, when the $O \cdots O$ distance is more than 2.7 Å and the hydrogen atom is tightly bound to one oxygen atom; »symmetrically strong« $O—H—O$, when $O \cdots O$ distance is less than 2.5 Å and the hydrogen atom is located exactly equidistantly between the two oxygen atoms; »asymmetrically strong or intermediate« $O—H \cdots O$, when $O \cdots O$ distance is between 2.7 and 2.5 Å and the hydrogen atom, even if
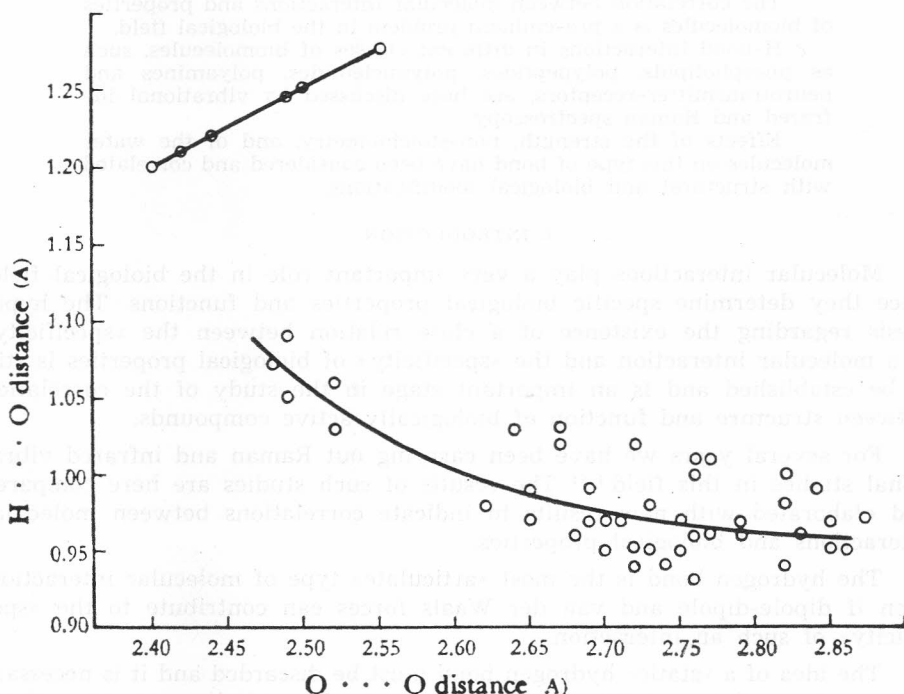

Figure 1. OH distance as a function of $O \cdots O$ distance as determined by neutron diffraction for a number of compounds containing OHO hydrogen bonds (from ref. 12).

shifted to the middle, is still more closely bound to one of the two oxygen atoms.

From the spectroscopic point of view, as the strength of the hydrogen bond increases, the ν_{OH} stretching mode gradually shifts to lower wavenumbers (Figure 2): at the same time, several Raman and infrared components (called A, B, C bands) appear, and their relation to proton vibration motions has been explained through deuteration studies.[13] For the strong symmetrical or almost symmetrical hydrogen bond, the infrared spectrum
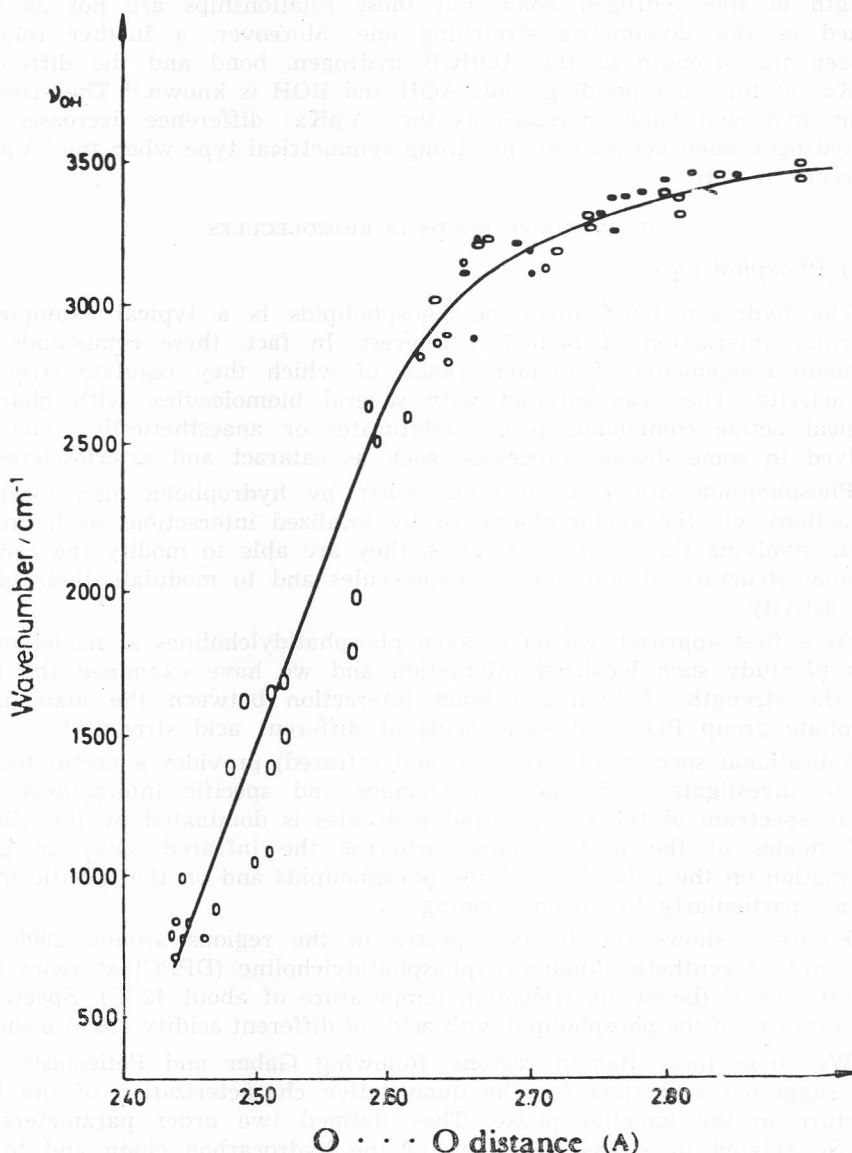

Figure 2. Frequency of ν_{OH} stretching as a function of O$\cdots$O distance (from ref. 13).

shows a single broad band (called D band) located between 1600 and 600 cm^{-1}, on which localized vibrations appear. »Negative bands« or »transmission windows« or »Evans holes« are generally superimposed on this broad band.

Most hydrogen bonds lie between these limiting cases and it is often possible to observe in the spectra the presence of both A, B, C and D components

The other vibrational modes of the hydrogen bond, that is the bending and the symmetric stretching ones, can also be used to determine the strength of the hydrogen bond, but these relationships are not as well defined as the asymmetric stretching one. Moreover, a further relation between the strength of the AOHOB hydrogen bond and the difference $|\Delta\,\mathrm{pKa}|$ of the corresponding acids AOH and BOH is known.[14] The strength of the hydrogen bond increases as the $|\Delta\,\mathrm{pKa}|$ difference decreases and the hydrogen bond becomes of the strong symmetrical type when the $|\Delta\,\mathrm{pKa}|$ difference is zero.

II. HYDROGEN BONDS IN BIOMOLECULES

2—1) Phospholipids

The hydrogen bond involving phospholipids is a typical example of molecular interaction of biological interest. In fact, these compounds are the main components of biomembranes, of which they regulate structure and activity. They can interact with several biomolecules, with pharmacological active compounds (e. g. barbiturates or anaesthetics)[15,16] and are involved in some disease processes, such as cataract and arteriosclerosis.[17]

Phospholipids are able to bind either by hydrophobic non localized interactions, via the apolar chains, or by localized interactions as hydrogen bonds, involving the polar head. Thus, they are able to modify the conformational structure of interacting biomolecules and to modulate their biological activity.

As a first approach we have taken phosphatidylcholines as model molecules to study such localized interaction and we have examined the type and the strength of hydrogen bond interaction between the main basic phosphate group PO_2^- and some acids of different acid strength.[7]

Vibrational spectroscopy (Raman and infrared) provides a useful technique to investigate conformational changes and specific interactions. The Raman spectrum of the phospholipid molecules is dominated by the vibrational modes of the acylic chains, whereas the infrared spectrum gives information on the polar head of the phospholipids and on the specific interactions, particularly hydrogen bonding.

Figure 3 shows the Raman spectra in the regions around 2900 and 1100 cm^{-1} of synthetic dipalmitoylphosphatidylcholine (DPPC) at room temperature, 20 °C (below its transition temperature of about 42 °C). Spectra of 1 : 1 mixtures of the phospholipid with acids of different acidity are also shown.

We chose these Raman regions, following Gaber and Peticolas[18], who first suggested a method for the quantitative characterization of the lipid structure in the lamellar phase. They defined two order parameters, S_{Γ} and S_{L}, related to »trans« structure of the hydrocarbon chain and to the lateral packing density of the hydrocarbon chains, respectively.
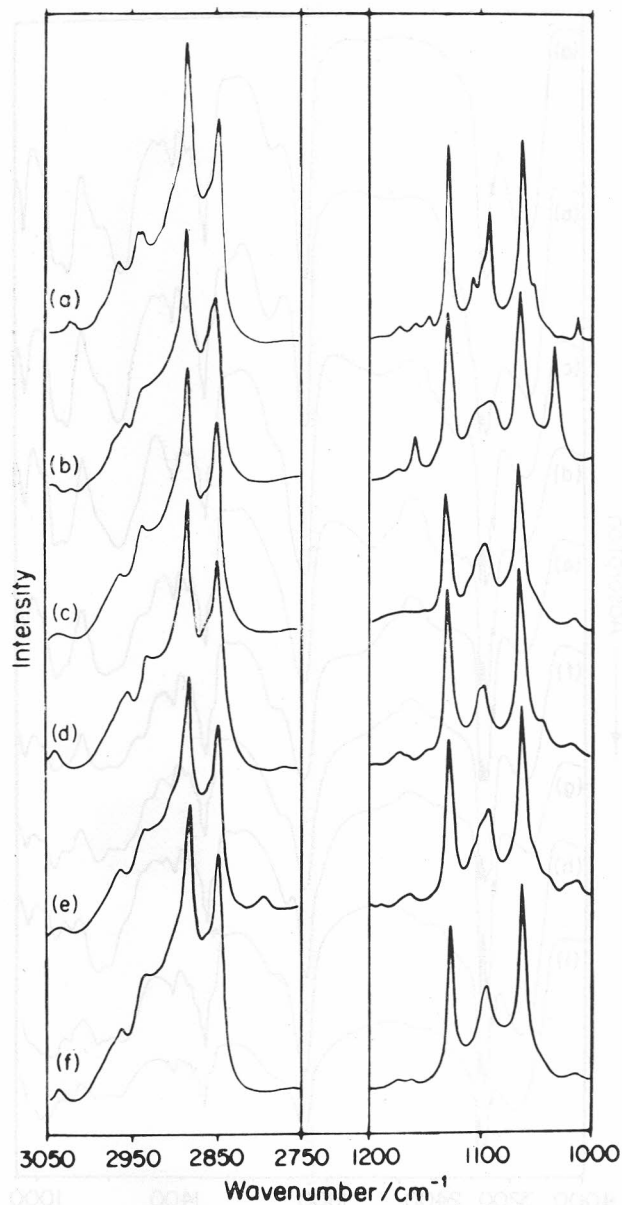
Figure 3. Raman spectra of DPPC and (1 : 1) mixtures of DPPC with some acids in the regions around 2900 cm^{-1} and 1100 cm^{-1}: (a) pure DPPC; (b) DPPC/o-chlorophenol; (c) DPPC/acetic acid; (d) DPPC/monochloroacetic acid; (e) DPPC/dichloroacetic acid; (f) DPPC/trichloroacetic acid. The values of S_T are: (a) = 1; (b) = 1.43; (c) =1.10; (d) = 1.46; (e) = 1.12; (f) = 1.05, and those of S_L: (a) = 0.43; (b) = 0.43; (c) = 0.37; (d) = 0.44; (e) = 0.40; (f) = 0.35.
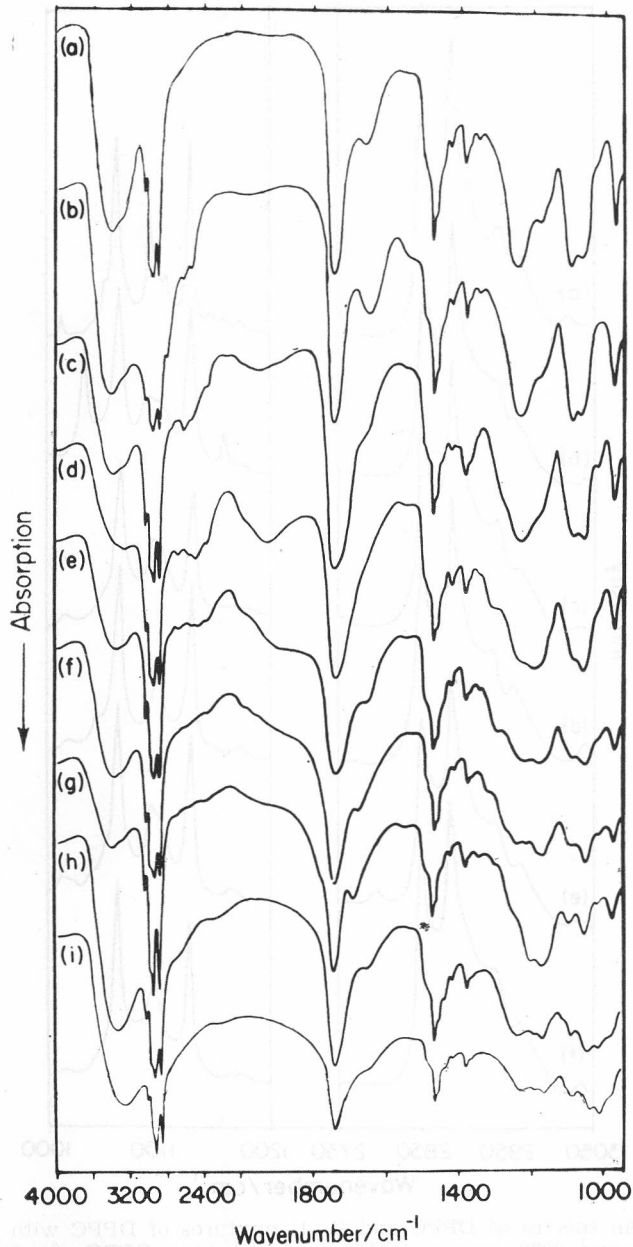
Figure 4. IR spectrum of: (a) pure egg yolk phosphatidylcholine (PC); (b) PC/methyl-amine hydrochloride (1 : 1); (c) PC/acetic acid (1 : 1); (d) PC/monochloroacetic acid (1 : 1); (e) PC/dichloroacetic acid (1 : 1), (f) PC/trichloroacetic acid (1 : 1); (g) PC/tri-fluoroacetic acid; (h) PC/hydrochloric acid (1 : 1); (i) PC in the presence of an excess of hydrochloric acid (approx 1 : 3).

These parameters in different modified forms are widely used in the analysis of membrane structure by means of Raman spectroscopy, although they are of value only in semi-quantitative analysis, as reported by us.[19,20]

By adding acids to phospholipid, the values of both order parameters change, indicating a modification of the crystalline structure to some more disordered phase. Indeed, the S_L parameter decreases on the addition of acids, indicating that the presence of acids varies the interchain interactions and slightly lowers the close packing of the chains.

We also found that the intensity of the 1130 cm^{-1} band decreases, indicating an increase of «gauche« conformations in the acyclic chains, if we take the CH$_2$ twisting mode at 1300 cm^{-1} as an intensity standard. The 1096 cm^{-1} band cannot be used as the intensity standard, since when acids were added, S_T, calculated according to Gaber and Peticolas, was larger than the value obtained for the pure phospholipid, suggesting the existence of a direct interaction between the PO$_2^-$ group of the polar head of phospholipid and acids, as it is better shown by the IR spectra.

The IR spectra between 4000 and 950 cm^{-1} of phosphatidylcholine and of 1 : 1 mixtures with acids of different acidity are shown in Figure 4. On addition of a very weak acid (methylamine hydrochloride, $pKa = 10.70$) to phosphatidylcholine, we did not observe any variation in the spectral features (Figure 4b).

When a stronger acid, such as acetic acid (Figure 4c; $pKa = 4.76$); monochloracetic acid (Figure 4d; $pKa = 2.85$) or dichloroacetic acid (Figure 4e; $pKa = 1.30$) is added, two bands appear at about 2600 and 1950 cm^{-1}, indicating the setting up of a strong asymmetrical or intermediate hydrogen bond (PO$_2$HO)$^-$ between the phosphate PO$_2^-$ headgroup of the phosphatidylcholine and the hydrogen atom of the carboxylic group. These two bands can be classified as B and C type bands. The A band cannot be observed in our spectra because it is partially overlapped by the strong ν_{C-H} modes of phospholipid.

With the addition of acid of increasing strength (that is, trichloroacetic acid, Figure 4f, $pKa = 0.20$; trifluoroacetic acid, Figure 4g, $pKa = -0.26$ and hydrochloric acid, Figure 4h, $pKa = -7.4$) the infrared spectra show a gradual decrease in the intensity of these new components at 2600 and 1950 cm^{-1}. They are no longer detectable in the spectrum when hydrochloric acid is added in excess (Figure 4i).

In addition, the intensity of a band at about 3250 cm^{-1}, which can be attributed partially to the asymmetric stretching mode of a weak hydrogen bond (PO$_2$—H$\cdots$A)$^-$ with the hydrogen localized on phosphate group, increases. This behaviour confirms a gradual proton transfer from the acid to the phosphate group as the strenght of the acid increases. Also the strong bands at 1240 and 1080 cm^{-1}, attributed to the asymmetric and symmetric PO$_2^-$ stretching modes are strongly affected by the addition of acids.

The frequency and the intensity of the 1240 cm^{-1} band decrease, the shift depending on the acid strength. By contrast, the intensity of the 1080 cm^{-1} component varies without any apparent relation to the acid strength.

In the presence of strong acids, a new component at about 1020 cm^{-1} appears in the spectra, whose intensity increases with an excess of acid,

attributable to the P—OH stretching vibration, arising from the transfer of the proton from the acid to the phosphate group. Consequently, the band at about 1250 cm^{-1} which appears in these spectra could be attributed to the uncoupled P=O stretching vibration.

The infrared spectra, after adsorption of acid vapour on a thin layer of phospholipid and subsequent gradual removal by pumping, were also recorded and are similar to those obtained with 1 : 1 molar ratio[7].

In particular, they show the presence of the two broad bands at about 2600 and 1950 cm^{-1} in the case of acetic acid ($pka = 4.76$) and the contemporary presence of a third band centred at about 1100 cm^{-1} in the case of dichloroacetic acid ($pKa = 1.30$). All these bands decrease in intensity as acidity increases, as in the case of trifluoroacetic acid ($pKa = —0.26$).

All this confirms our previous statement about changes in the hydrogen bond strength as a function of different pKa values. The hydrogen bond strength, in the case examined for phosphatidylcholines, attains a maximum for dichloroacetic acid and tends to decrease for weaker and stronger acids, suggesting the conclusion that asymmetrical, strong, polarizable hydrogen bonds are possible between the phosphate group of phosphatidylcholines and the OH group of carboxylic acids, whose pKa range from 2.85 to 0.20.

This situation can occur in many biological systems. The pKa of the carboxylic group in monoamino monocarboxylic aminoacids lies between 1.71 (cysteine) and 2.63 (threonine); indeed, they are comparable in strength to mono or dichloroacetic acid.

The γ-carboxylic group of aspartic acid and δ-carboxylic group of glutamic acid are considerably weaker ($pKa = 3.86$ and 4.25, respectively), but nevertheless stronger than acetic acid (pKa 4.7).

In polypeptides and proteins, the secondary group of acidic aminoacids has a mean value $pKa = 4.7$ and for terminal carboxylic groups the value is somewhat less. Therefore, in the primary interaction between phospholipids and biomolecules the existence of a strong, polarizable hydrogen bond can be expected, and the effect of these interactions could stabilize the structure of the biological system. Our Raman data show also the existence of conformational changes in the acylic chains of phospholipid molecules, and these effects can also help to explain the properties of phospholipids in biological systems.

2—2) Peptidic Group

Another typical example of hydrogen bond of variable strength is given by peptidic groups interacting with acid groups of different strength. In the simplest case of the amidic bond, we were able to observe the formation of a strong symmetrical O—H—O hydrogen bond when acetamide interacts with protons in the 2 : 1 molar ratio (acetamide-hemihydrochloride).[1] The infrared spectrum (Figure 5) shows a broad band with the maximum at about 850 cm^{-1}, Raman inactive (which does not appear in the acetamide and in the acetamide chloride spectra), due to the asymmetric stretching mode of a (O—H—O)$^+$ strong symmetrical hydrogen bond of the hemihydrochloride, according also to X-ray preliminary data.[21]

The assignment of the spectra is in accordance with a site symmetry Ci of the isolated hemiprotonated acetamide ion. The existence of strong,
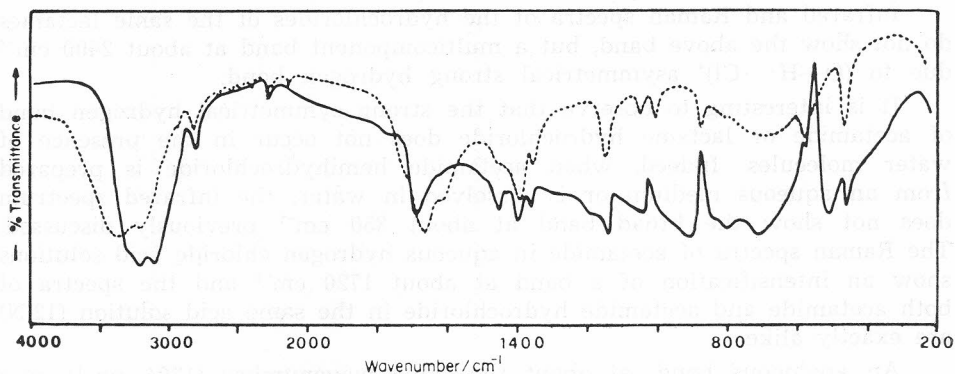
Figure 5. Infrared spectra of a) - - - - acetamide; b) ——— (acetamide)$_2$ HCl.

symmetrical, hydrogen bonds in the hemihydrochloride of polyamides, particularly in the δ-valerolactame and ε-caprolactame, was also observed.

For instance, the infrared spectrum of δ-valerolactame hemihydrochloride (Figure 6) shows a broad band at about 800 cm^{-1} with negative bands superimposed. Such a band is to be attributed to the asymmetric stretching mode of (O—H—O)$^+$ symmetrical (or nearly symmetrical) strong hydrogen bond.
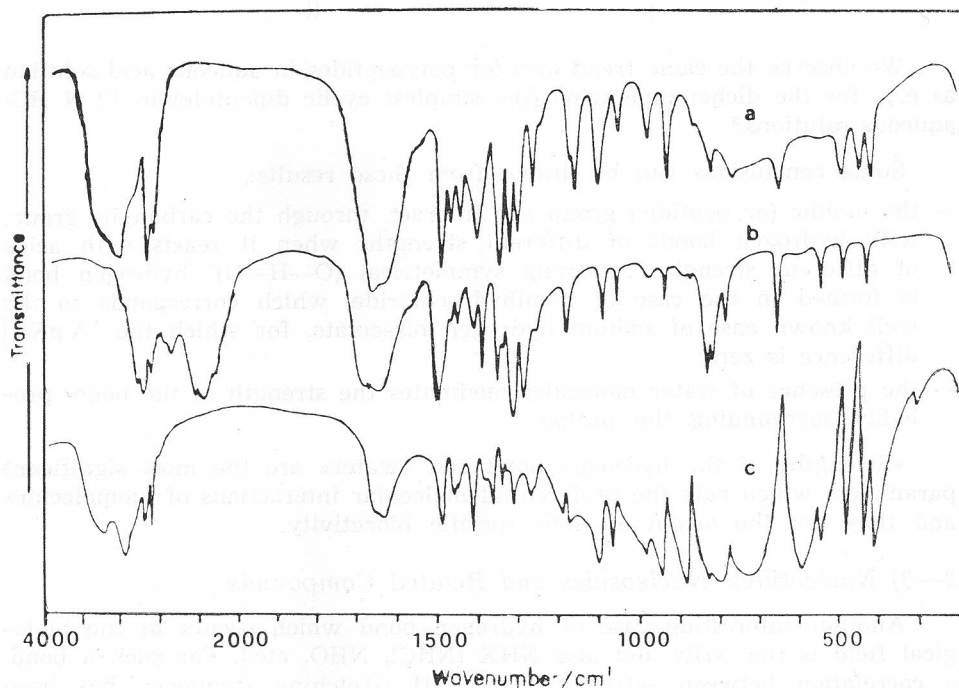
Figure 6. IR spectra of (a) δ-valerolactam (CH$_2$)$_4$CONH; (b) δ-valerolactam hydrochloride (CH$_2$)$_4$CONH·HCl.

Infrared and Raman spectra of the hydrochlorides of the same lactames do not show the above band, but a multicomponent band at about 2400 cm^{-1} due to $(O—H\cdots Cl)^+$ asymmetrical strong hydrogen bond.

It is interesting to observe that the strong symmetrical hydrogen bond of acetamide or lactame hydrochloride does not occur in the presence of water molecules. Indeed, when acetamide hemihydrochloride is prepared from an aqueous medium or is dissolved in water, the infrared spectrum does not show the broad band at about 850 cm^{-1} previously discussed. The Raman spectra of acetamide in aqueous hydrogen chloride acid solutions show an intensification of a band at about 1720 cm^{-1} and the spectra of both acetamide and acetamide hydrochloride in the same acid solution (12 N) are exactly alike.

An analogous band, at about the same wavenumber (1704 cm^{-1}), was also observed in the Raman spectrum of acetic acid in acid solution. Such a band can be attributed to the asymmetric carbonium-oxygen stretching mode of the ion (I) and, in the case of acetamide, to the asymmetric carbonium-oxygen and carbonium-nitrogen stretching mode of the oxonium ion (II), according to Meckle.[22]
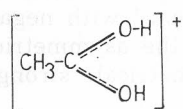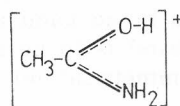
We observe the same trend also for polypeptides in aqueous acid solution as e. g. for the dichetopiperazine (the simplest cyclic dipeptide) in 12 N HCl aqueous solutions.[5]

Some conclusions can be drawn from these results:

— the amidic (or peptidic) group can interact, through the carbonylic group, with hydrogen bonds of different strengths when it reacts with acids of different strength. A strong symmetrical $(O—H—O)^+$ hydrogen bond is formed in the case of hemihydrochloride, which corresponds to the well known case of sodium hydrogen bisacetate, for which the $|\Delta pKa|$ difference is zero;

— the presence of water molecules modulates the strength of the bond, probably surrounding the proton.

»Strength« of the hydrogen bond and »water« are the most significant parameters which rule the preferential molecular interactions of biomolecules and they are the origin of their specific bioactivity.

2—3) Nucleotides. Nucleosides and Related Compounds

Another interesting case of hydrogen bond which occurs in the biological field is the NHN and also NHX (NHCl, NHO, etc.). For such a bond, a correlation between »strength« and NH stretching frequency has been found (Figure 7) (14). Also a correlation between strength and biological activity is to be expected.
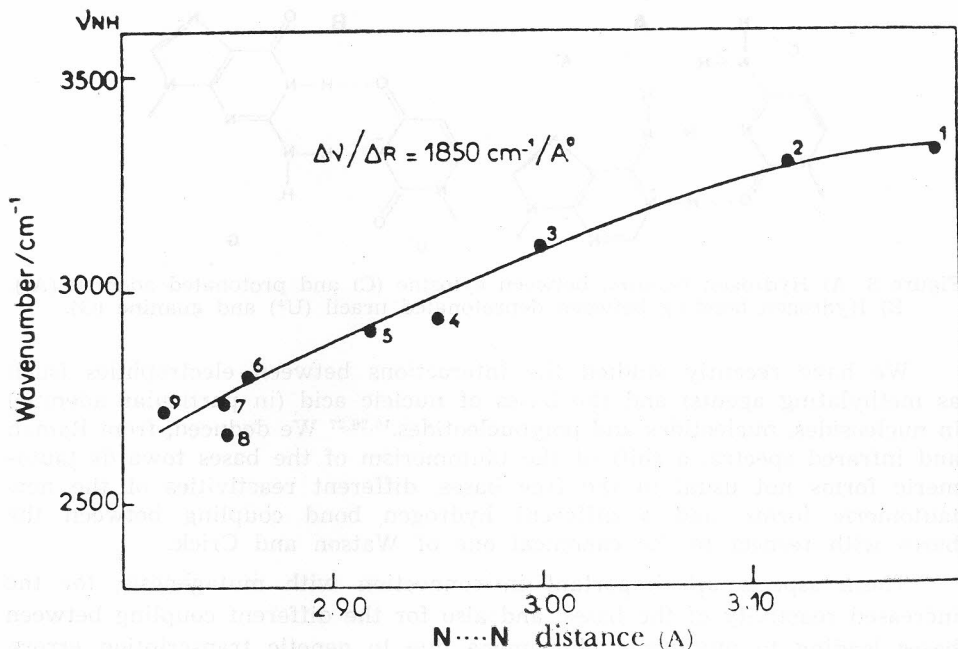
Figure 7. Relation between NH stretching frequency and N···N distance (from ref. 14).

Typical examples of such bonds are present in polynucleotides. The NHN (and also NHO) hydrogen bonds in polynucleotide pairs are of the asymmetrical strong or intermediate type[12] and contribute to the stabilization of the secondary structure of the polynucleotides. The formation of a strong or intermediate hydrogen bond in the base pairing of nucleic acid structure can be responsible for mutagenic phenomena, as suggested in the literature.[12,23,24]

Let us consider, for example, the adenine-uracil (or adenine thymine) pair, which is preferential in the Watson-Crick scheme.[25] The NHN hydrogen bond in poly U is characterized by an infrared NH stretching band at about 2750 cm^{-1}, typical of strong or intermediate hydrogen bond.

During the separation process involved in the replication, if the proton has been »cooperatively« exchanged between bases, from uracil to adenine, as a consequence of the proton tunnelling capability in a strong or intermediate hydrogen bond, a base pair which may be denoted as A*(adenine protonated) and U*(uracil deprotonated) is formed. If the strands now separate, and if the synthesis of a new strand is governed by the base pairing in a given strand, A* is no longer able to pair with U (uracyl) since its proton is not compatible. A hydrogen bonding will more likely occur with C (cytosine) (Figure 8A). Similarly, U* pairs more readily with G (gaunine) (Figure 8B). The newly replicated strand will thus contain an error.

The existence of a double-minimum potential function, typical of a strong or intermediate hydrogen bond and necessary for the exchange of the proton, may be then of great biological significance.
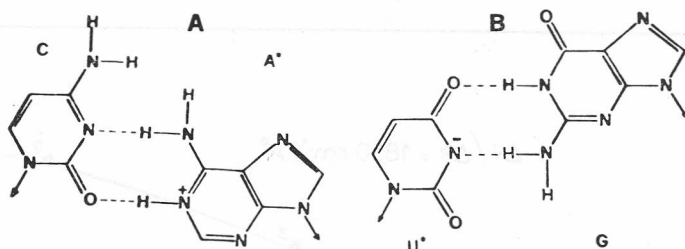
Figure 8. A) Hydrogen bonding between cytosine (C) and protonated adenine (A*).
B) Hydrogen bonding between deprotonated uracil (U*) and guanine (G).

We have recently studied the interactions between electrophiles (such as methylating agents) and the bases of nucleic acid (in particular adenine) in nucleosides, nucleotides and polynucleotides.[10,26,27] We deduced, from Raman and infrared spectra, a shift of the tautomerism of the bases towards tautomeric forms not usual in the free bases, different reactivities of the new tautomeric forms, and a different hydrogen bond coupling between the bases with respect to the canonical one of Watson and Crick.

These aspects are important in connection with mutagenesis, for the increased reactivity of the bases, and also for the different coupling between bases leading to mutagenic phenomena due to genetic transcription errors.

The methylating agent we used was dimethylsulphate. The main products of the interaction between dimethylsulphate and adenine were protonated adenine, 1-methyladenine and 1-methyladenine protonated, depending on the pH values.

Following the discussion on the C_6N stretching mode, the NH deformation modes and the bands of pyrimidine and imidazole rings, we can deduce[10]:
— an increased contribution of the imino protonated adenine structure to the resonance pattern of molecules of adenine hydrochloride:
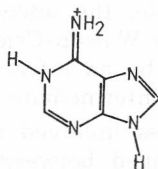
— an iminic resonating structure for 1-methyladenine, which derives from the iminic one through intermolecular hydrogen bond with hydrogen transfer from N_9 imidazole to N_6 iminic:
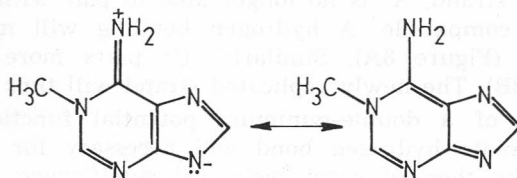
— an iminic protonated and resonating structure for 1-methyladenine hydro-chloride:
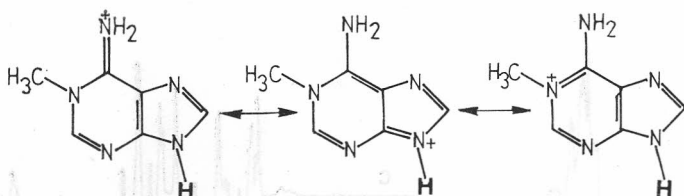
All these adenine derivatives shown a change of tautomerism toward the iminic form with double bond character between C_6 and N_6, which is less stable at room temperature for adenine. This change of tautomerism involves a different molecular reactivity due to the different distribution of the bonds in the molecular skeleton. But, what is most important with regard to the problem of mutagenesis and carcinogenesis, the products of protonation and methylation of the adenine are subject to interactions of hydrogen bonds between the bases in nucleic acids, different from the cano-nical interactions postulated by Watson and Crick.

For example, where adenine has a preferential Watson-Crick coupling with thymine, adenine hydrochloride shows a preferential pairing with cytosine. In the same way, the methylated and protonated-methylated deri-vatives of adenine can give rise to non canonical Watson-Crick type coup-lings which could be responsible for the origin of mutagenic and cancero-genic phenomena, as already pointed out.

2—4) Polyamines

Hydrogen bonds involving polyamines and those in the primary neuro transmitter-receptor interaction (see 2.5) are other examples of NHN or NHO hydrogen bonds of biological significance.

It is well known that polyamines (1,3 diaminopropane, putrescine, cada-verine, spermidine and spermine) have a physiological importance due to their close affinity with nucleic acids[28,29]. Because of their basic nature, poly-amines can interact with the phosphoric acid groups of nucleic acids. Alter-natively, they can interact in the protonated form with the phosphate group of nucleic acids. Among the polyamines, spermidine and spermine form the strongest bonds with nucleic acids and polynucleotides.

We carried out a vibrational spectroscopic study of polyamine-hydro-chloric acid, and polyamine-phosphoric acid interactions as a model for the polyamine-nucleic acid interactions[9].

In the case of the polyamine hydrochlorides we note the absence in both the Raman (Figure 9, 10) and infrared spectra (Figure 11, 12) of the group of bands around 3300 cm⁻¹ associated with asymmetric NH_2, and NH stretching modes. However, the componente below 3000 cm⁻¹ are more intense and there is a general broadening of the bands between 3000 and 2000 cm⁻¹, especially in the infrared spectra. This phenomenon is due to the protonation of the primary and secondary amine groups and the formation of (NHCl)⁻ medium-strong hydrogen bonds. In the case of polyamine phosphate hexa-hydrates, Raman and infrared spectra confirm the transfer of proton from phosphate to primary and secondary amine groups.
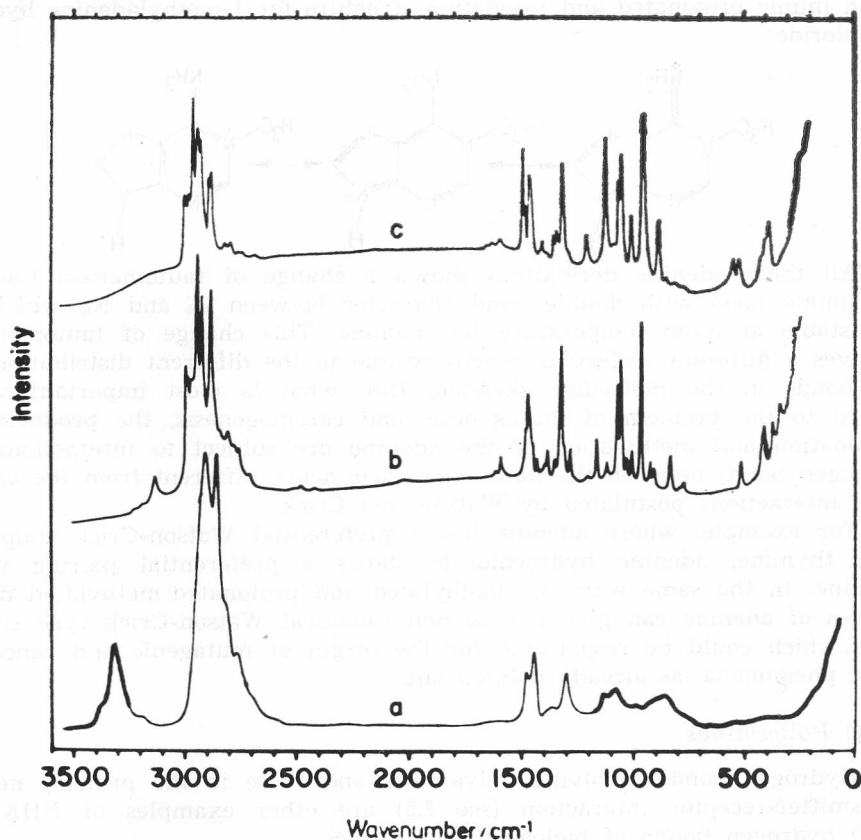
Figure 9. Raman spectra of: (a) spermidine; (b) spermidine trihydrochloride and (c) spermidine phosphate hexahydrate.

In this case, however, the Raman spectra of the compounds between 1300 and 700 cm^{-1} contain all the bands of the related hydrochlorides and in addition new bands at about 1180, 965 and 885 cm^{-1}, which are in good correlation with the Raman spectrum of crystalline bisodium acid phosphate (Na$_2$HPO$_4$). Consequently, the complex broadening (more marked and widespread in the infrared spectrum) between 3700 and 2000 cm^{-1} may be explained by the coexistence of a variety of different hydrogen bonds with increasing strength. That is, OHO weak hydrogen bonds between water molecules; NHO and OHO intermediate hydrogen bonds between the NH of protonated amine group and the oxygen of water, and the OH of water and the oxygen of phosphate group; and again OHO and NHO strong hydrogen bonds between the OH of acid and the oxygen of the phosphate group, and the NH of protonated amine group and the oxygen of the phosphate group, according also to an X-ray study of spermine phosphate hexahydrate (30).

We are now investigating the molecular interaction involving hydrogen bonds between polyamines and nucleic acids: the IR spectrum shows a large
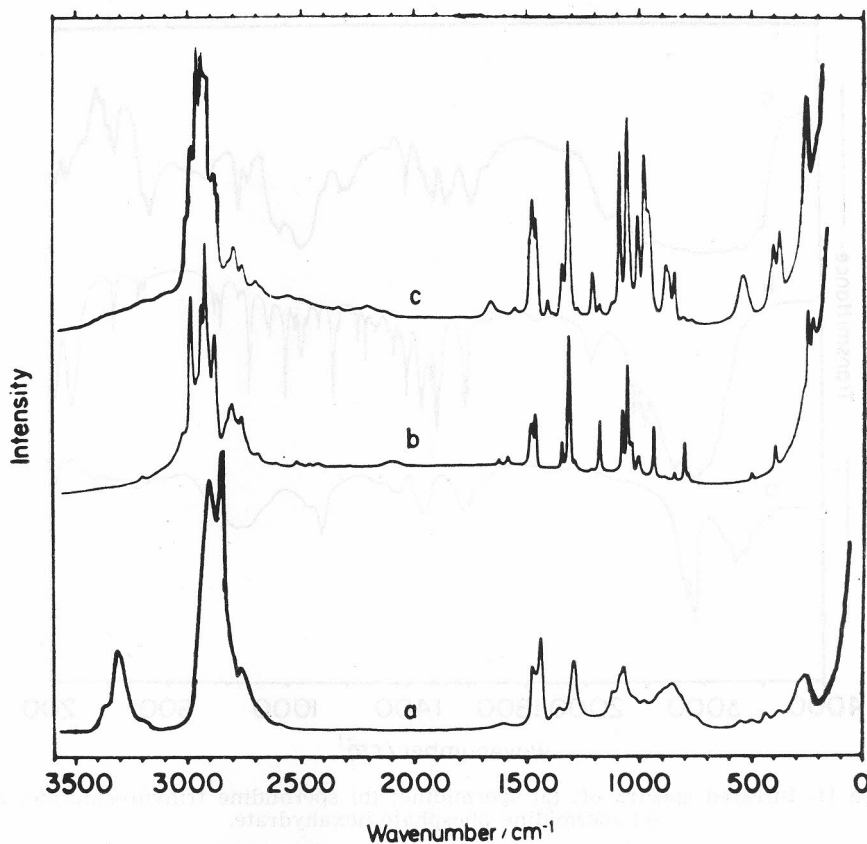
Figure 10: Raman spectra of: (a) spermine; (b) spermine tetrahydrochloride and (c) spermine phosphate hexahydrate.

and widespread infrared absorption in the stretching region for adduct DNA-spermine hydrochloride, offering thus a spectroscopic evidence of the presence of strong and medium strong hydrogen bonds.

2—5)(Neuro)Transmitter-Receptor Interactions

Hydrogen bonds are also involved in the primary transmitter-receptor interaction. A THR hydrogen bond interaction between a transmitter T and a receptor R has been postulated[31,32].

The amino NH_2 group, which is present in most of the neuro-transmitter molecules, is responsible for such an interaction, whereas the acidic group in the receptor molecule has not been well defined yet.

Furthermore, the transmitter-receptor interaction must be »flexible« and »reversible«, that is it must allow the transmission of an impulse through a perturbation which modifies the interaction strength, leaving the system in the initial conditions when the perturbation is removed. We think that this situation may be chemically mediated by a strong hydrogen bond.
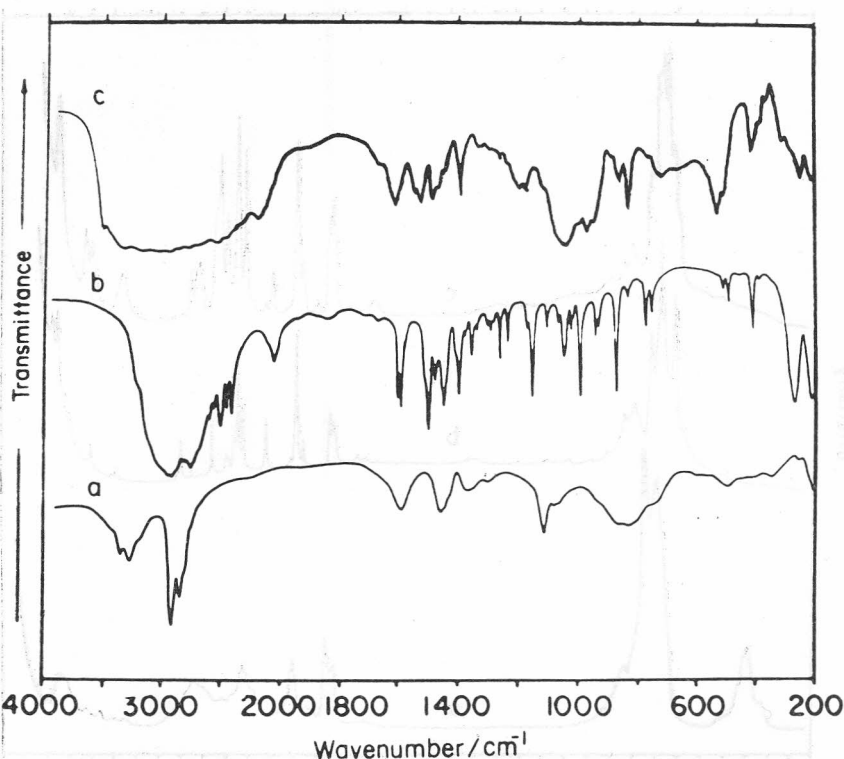
Figure 11. Infrared spectra of: (a) spermidine; (b) spermidine trihydrochloride; and (c) spermidine phosphate hexahydrate.

As a model for the serotonine-receptor system, we have studied the interactions between n-propilamine and acids examining the Raman and infrared spectra of stoichiometric complexes between propylamine ($pKa = 10.6$) and acids of increasing acidity, whose pKa varies from about zero to about the same value as protonated propylamine: these pKa values can well simulate acidic centres of biologically interesting molecules.

By studying the stretching modes of protonated and non-protonated basic and acid groups, as well as the behaviour of the asymmetric stretching vibration of the hydrogen bond, some results can be obtained.

— In the case of the interaction between propylamine and the strongest acid (trifluoroacetic acid), the proton completely transfers from the acid to the amino group and the resulting NHO hydrogen bonds is of the weak N—H$\cdots$A$^-$ type, as shown by the appearance in the spectrum of the adduct of a strong band at 1680 cm^{-1} at tributable to the ν_{as} CO$_2^-$ stretching vibration. A similar band appears in the Raman spectrum at 1475 cm^{-1}, arising from ν_s CO$_2^-$ vibration. This behaviour is in accordance with the difference $|\Delta pKa| = 10.87$.
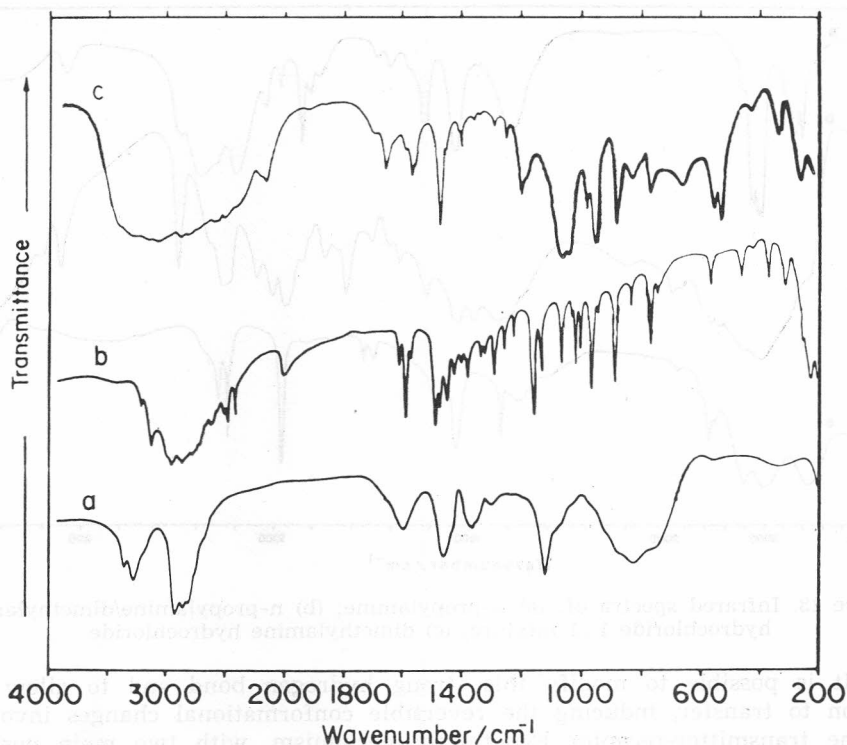
Figure 12. Infrared spectra of: (a) spermine; (b) spermine tetrahydrochloride and (c) spermine phosphate hexahydrate.

— As the strength of the acid decreases (dichloroacetic, acetic, ortho-chlorophenol), a less complete transfer of the proton from the acid to the amino group occurs and the spectrum shows the formation of a strong asymmetrical NHO hydrogen bond. The setting up of a hydrogen bond NHO of intermediate strength is observed in the spectrum of the complex by the broad IR absorption centered at about 2880 cm^{-1}, extending toward lower frequencies. The infrared spectrum shows also a band at 1655 cm^{-1} attributable to the bending mode δ NH$_3$ (the corresponding vibrational mode in the free aminic group was found at 1605 cm^{-1}) and a strong band at 1573 cm^{-1} with a shoulder at higher frequencies, typical of the o-chlorophenate ion. Raman spectrum shows a similar behaviour.

Only in the case of the weakest acid (dimethylamine hydrochloride, Figure 13) the analysis of the spectrum suggests the formation of a strong symmetrical, or nearly symmetrical, NHN hydrogen bond, according to the difference $|\Delta pKa|$, which is 0.1.

Indeed, in the infrared spectrum a large absorption appears, with a maximum at about 1500 cm^{-1}, in a close analogy to the D band of symmetrical, or nearly symmetrical, strong hydrogen bond. We observed a similar behaviour also for the 1 : 1 adducts between triptamine (and also for the serotonine) with the same acids used for the adducts with propylamine.
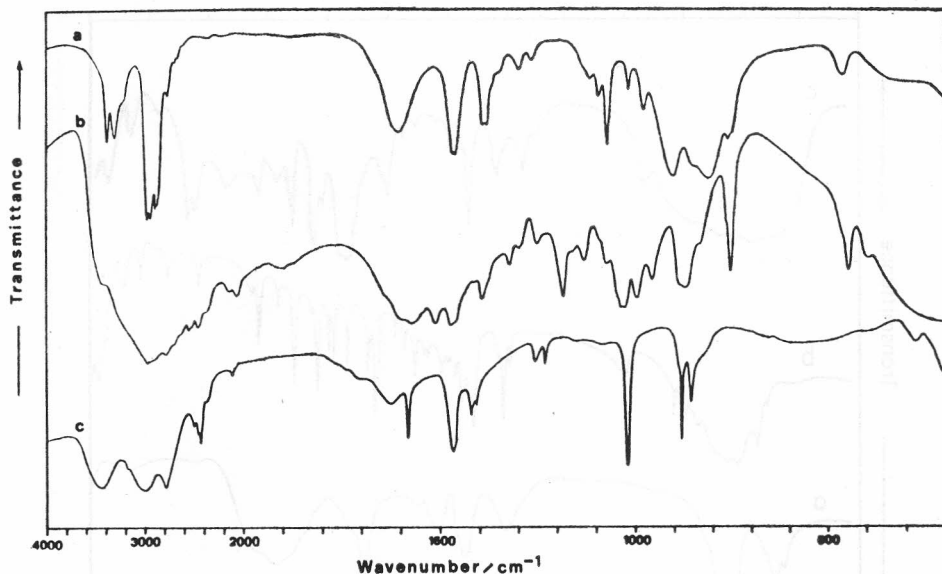
Figure 13. Infrared spectra of: (a) n-propylamine; (b) n-propylamine/dimethylamine
         hydrochloride 1 : 1 mixture; (c) dimethylamine hydrochloride.

It is possible to modify this strong hydrogen bond and to allow the
proton to transfer, inducing the reversible conformational changes involved
in the transmitter-receptor hypothized mechanism, with two main possibi-
lities. One, through the participation of water molecules in the hydrogen
bond interaction; the other, through a non-stoichiometry of the interaction.

The effect of water is shown in Figure 14. Spectrum 1 : 1 : 0 refers to
the stoichiometric 1 : 1 propylamine-propylamine hydrochloride adduct; it
is characterized by a large D band, centered at about 1500 cm⁻¹, typical of
a symmetrical, or nearly symmetrical, strong NHN hydrogen bond, the
$|\Delta pKa|$ being in this case exactly zero.

Upon adding water molecules to the system, the large infrared absorp-
tion decreases and the spectrum reverts to that of propylamine plus pro-
pylamine hydrochloride, showing a progressive weakening of the hydrogen
bond strength.

The progressive weakening of the hydrogen bond strength is also ref-
lected in the behaviour of the Raman bands arising from ν_{NH} (Table I) and
ν_{CN} stretching modes, bonds (Table II).

The effect of a non-stoichiometric interaction has been studied investi-
gating the non-stoichiometric addition compounds between propylamine and
propylamine hydrochloride with an excess of propylamine.

In this case too, the strength of the symmetrical or nearly symmetrical
strong NHN hydrogen bond of the 1 : 1 propylamine-propylamine hydrochlo-
ride adduct decreases as the polyamine content increases, and the spectra
tend to that of propylamine plus propylamine hydrochloride, that is with a
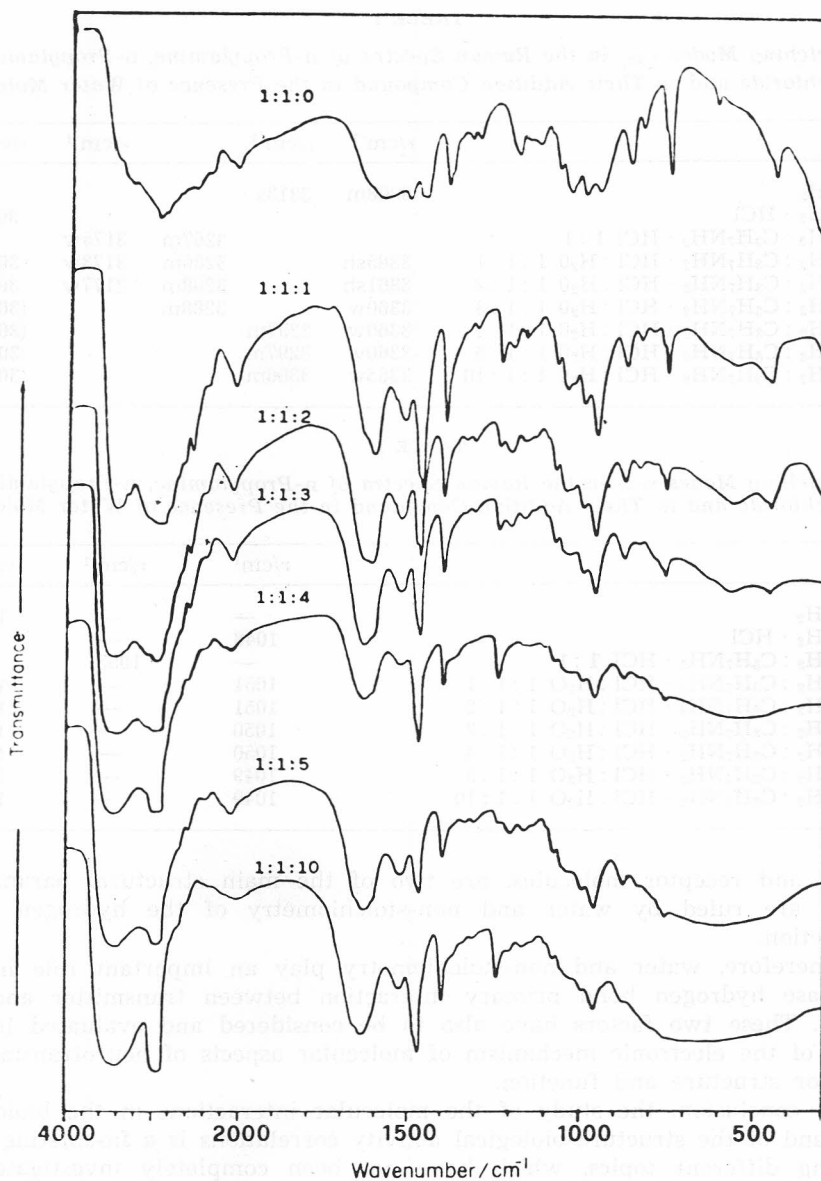transfer of the proton on one of the two nitrogen atoms.

Figure 14. Infrared spectra of n-propylamine/n-propylamine hydrochloride/water addition compounds in ratio: (a) 1 : 1 : 0; (b) 1 : 1 : 1; (c) 1 : 1 : 2; (d) 1 : 1 : 3; (e) 1 : 1 : 4; (f) 1 : 1 : 5; (g) 1 : 1 : 10.

The removal of the water, or of the non-stoichiometric propylamine in the 1 : 1 propylamine-propylamine hydrochloride complex, makes the hydrogen bond once again strong and reversible.

We believe that the reversible proton tunnelling, as well as the consequent reversible conformational changes which are involved on both trans-

TABLE I

Stretching Modes ν_{NH_2} in the Raman Spectra of n-Propylamine, n-Propylamine Hydrochloride and in Their Addition Compound in the Presence of Water Molecules

	ν/cm^{-1}	ν/cm^{-1}		ν/cm^{-1}	ν/cm^{-1}
$C_3H_7NH_2$	3368m	3313s			
$C_3H_7NH_2 \cdot HCl$					3050sh
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl$ 1:1			3267m	3175w	
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:1	3365sh		3280m	3173w	3055sh
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:2	3361sh		3288m	3177w	3060sh
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:3	3360w		3288m		(3060)
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:4	3360w	3297m			(3065)
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:5	3360w	3297m			(3065)
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:10	3365w	3306m			(3065)

TABLE II

Stretching Modes ν_{C-N} in the Raman Spectra of n-Propylamine, n-Propylamine Hydrochloride and in Their Addition Compound in the Presence of Water Molecules

	ν/cm^{-1}	ν/cm^{-1}	ν/cm^{-1}
$C_3H_7NH_2$	—	—	1071
$C_3H_7NH_2 \cdot HCl$	1048	—	—
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl$ 1:1	—	1055	—
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:1	1051	—	1070
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:2	1051	—	1070
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:3	1050	—	1067
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:4	1050	—	1067
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:5	1049	—	1067
$C_3H_7NH_2 : C_3H_7NH_2 \cdot HCl : H_2O$ 1:1:10	1049	—	1067

mitter and receptor molecules, are two of the main structural parameters which are ruled by water and non-stoichiometry of the hydrogen bond interaction.

Therefore, water and non-stoichiometry play an important role in the acid-base hydrogen bond primary interaction between transmitter and receptor. These two factors have also to be considered and evaluated in the study of the electronic mechanism of molecular aspects of neurotransmitter-receptor structure and function.

In conclusion, the study of the molecular interactions in the biological field and of the structure-biological activity correlations is a fascinating area offering different topics, which have not been completely investigated to date.

REFERENCES

1. A. Bertoluzza, C. Fagnano, M. A. Morelli, and R. Tosi, Rend. Accad. Naz. Lincei 58 (1975) 919.
2. A. Bertoluzza, C. Fagnano, M. A. Morelli, and R. Tosi, Rend. Accad. Naz. Lincei 60 (1976) 135.
3. A. Bertoluzza, C. Fagnano, M. A. Morelli, and R. Tosi, Rend. Accad. Naz. Lincei 60 (1976) 462.
4. G. B. Bonino and A. Bertoluzza, Rend. Accad. Naz. Lincei 61 (1976) 717.

5. A. Bertoluzza, C. Fagnano, M. A. Morelli, and R. Tosi, *Rend. Accad. Naz. Lincei* **62** (1977) 365.
6. A. Bertoluzza, M. A. Morelli, P. Finelli, and R. Tosi, *It. J. Biochem.* **27** (1978) 323.
7. A. Bertoluzza, S. Bonora, G. Fini, and M. A. Morelli, *J. Raman Spectrosc.* **11** (1981) 225.
8. A. Bertoluzza, S. Bonora, G. Fini, M. A. Battaglia, and P. Monti, *J. Raman Spectrosc.* **11** (1981) 430.
9. A. Bertoluzza, C. Fagnano, P. Finelli, M. A. Morelli, R. Simoni, and R. Tosi, *J. Raman Spectrosc.* **14** (1983) 386.
10. A. Bertoluzza, C. Fagnano, M. A. Morelli, R. Tosi, and D. A. Long, *J. Raman Spectrosc.* **18** (1983) 83.
11. A. Bartoluzza, S. Bonora, G. Fini, and M. A. Morelli, *Canad. J. Spectrosc.* **29** (1984) 93.
12. W. C. Hamilton and J. A. Ibers, *Hydrogen Bonding in Solids*, Benjamin, New York 1988.
13. D. Hadzi and S. Bratos, in *The Hydrogen Bond, ed. by* P. Shuster, G. Zundel and C. Sandorfy, vol. **II** p. 567, North Holland Publ. 1976.
14. A. Novak, *First European Crystallographic Meeting*, Bordeaux, 5—8th sept., 1973.
15. A. G. Lee, *Mol. Pharmacol.* **13** (1977) 474.
16. J. H. Chin and D. B. Goldstein, *Biomedicine* **28** (1978) 141.
17. E. Cotlier, Y. Obara, and B. Toffness, *Biochim. Biophys. Acta* **530** (1978) 267.
18. B. P. Gaber and W. L. Peticolas, *Biochim. Biophys. Acta* **465** (1977) 260.
19. A. Bertoluzza, S. Bonora, G. Fini, M. A. Morelli, and R. Simoni, *J. Raman Spectrosc.* **14** (1983) 395.
20. A. Bertoluzza, S. Bonora, G. Fini, and M. A. Morelli, *Canad. J. Chem.* **63** (1985) 1390.
21. N. Albert and R. M. Bagder, *J. Chem. Phys.* **29** (1958) 1193.
22. W. Kutzelnigg and R. Mecke, *Spectrochim. Acta* **18** (1962) 549.
23. M. D. Topal and J. R. Fresco, *Nature* **263** (1976) 285.
24. M. D. Topal and J. R. Fresco, *Nature* **263** (1976) 289.
25. J. D. Watson and F. H. C. Crick, *Nature* **171** (1953) 964.
26. A. Bertoluzza in *Spectroscopy of Biological Molecules*, ed. by C. Sandorfy and T. Theophanides, Riedel Publ. 1984, p. 91.
27. A. Bertoluzza, C. Fagnano, M. A. Morelli, and R. Tosi, *J. Raman Spectrosc.*, in press.
28. R. A. Campbell et al (Eds.), *Advances in Polymine Research, Vol.* **I.** Raven Press, New York (1978), C. M. Caldarera et al. (Eds.), *Advances in Polyamine Research*, Vol. **III.** Raven Press New York 1981.
29. D. R. Morris and J. J. Harada, *Polyamines Biomed. Res.* (1980).
30. Y. Itaka and Y. Husc, *Acta Cryst.* **18** (1965) 110.
31. D. J. Triggle in *Chemical Aspects of the Autonomic Nervous System*, Academic Press, London 1972.
32. A. Bertoluzza, A. M. Marinangeli, M. A. Morelli, L. Sperti, and A. Tinti, *Biophysical Aspects of Receptor Structure and Function*, Portorož, 22—26th Sept. 1982.

SAŽETAK

Molekulske interakcije i svojstva biološki aktivnih spojeva: studij infracrvenom i Ramanovom spektroskopijom

A. Bertoluzza, C. Fagnano, G. Fini i M. A. Morelli

Korelacija molekulskih interakcija i svojstava biomolekula istaknuti je problem u području biologije.

Interakcije preko vodikove veze u različitim razredima biomolekula, kao što su fosfolipidi, polipeptidi, polinukleotidi, poliamini i neurotransmiterski receptori, razmatraju se ovdje u svjetlu vibracijske infracrvene i Ramanove spektroskopije. Efekti jakosti, nestehiometričnosti i nazočnosti molekula vode na taj tip veze razmatra se i korelira sa strukturnim i biološkim modifikacijama.