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# Electrostatic Interaction Between Oligopeptides and Phosphate Residues by Determination of Absolute Raman Intensities

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The changed isotropic absolute Raman intensities of the phosphate residue in the complexes of positive charge oligopeptides, lys-lys, arg-arg, lys-aromat-lys, negative charge diethyl phosphoric acid (DEP) and polyriboadenylic acid{poly(rA)} were reported and discussed. Our measurements showed that the absolute intensities of phosphate stretch vibration in complexes were different according to the reaction partners. Due to the partial electrical charge and molecular structure of oligopeptides for the complex formation lysine can interact more strongly than arginine when the reaction partners have short chain and no steric hindrance. Owing to these reasons the intensity of phosphate stretching vibration is very sensitive according to the circumstance of reaction. From our results we could suggest that we can discriminate any one of the the lysine and arginine in the complicated biological molecule during interaction between nucleotides and proteins. The activity of reaction of two basical oligopeptides is not quite similar for complex formation in aqueous solution. The activity of dipeptides depends upon the structure of molecule and environment for complex formation. Aromatic ring contributes to electrostatic interaction in complexes. The amount of the absolute intensity for pure stacking interaction is smaller than electrostatic interaction in macromolecular complexes.

## Introduction

There is a vast interest in protein-nucleotide interaction because of the widespread importance of these interactions for, *e.g.*, gene expression, transcription, and other processes. On the molecular basis, the forces involved in the complexing process between protein and nucleic acid are commonly divided into

i) electrostatic interaction of positive charge on the protein by the negative charge of the nucleic acid back bone;

ii) dispersive forces originating from the interaction of aromatic side chains of the protein with the bases inside of the nucleic acid helix;

iii) formation of hydrogen bonds and other[1]. The knowledge of prefered mechanisms and sites of docking between proteins and DNA or RNA is one of the fundamental problems in molecular biology, therefore all kinds of physical probing have been used, such as; UV and ORD[2], NMR[3], X-ray analysis[4] and Raman spectroscopy[5]. An advantage of Raman spectroscopy might be, that molecular properties can be studied in aqueous solution and at conditions almost physiological. In macromolecules, these vibrations are usually highly localized on small numbers of atoms within specific groups such as the peptide bond, C-O stretches, C-H stretches or bends, C-S vibrations of cysteins and S-S vibration of cystines just to name some. A shift of the Raman band position, which corresponds to the frequency of the vibration, is a measure of the change of binding forces within the characteristic group of atoms involved in the observed vibration. It needs usually strong complexation or molecular rearrangement to express major shifts of frequencies. The intensity of a Raman band depends on the change of polarizability during the corresponding vibration. Intermolecular interactions, especially those of coulombic nature, cause changes of the polarity of the molecule and hence also effect the polarizability. This can be observed on intensity changes of the Raman bands, which are consequently a very sensitive probe of intermolecular interaction giving at the same time information about the site of the interaction because of the local character of the oscillators. In this paper, we want to concentrate only on electrostatic interaction and we used simple model systems for this purpose. We assembled the data of the intensities of symmetric phosphate stretch vibration in various complexes.

## Experimental

**Chemicals and Their Origine.** Diethylphosphoric acid (DEP)/Eastmann Kodak; the dipeptides, lys-lys. 2HCl 0.5 $H_2O$ and arg-arg. 3HAc/Serva; the potassium salt of polyriboadenylic acid (poly(rA)) (lyophil, "reinst", homopolymer) /Boehringer Mannheim FRG; lys-tyr-lys.2 formate, lys-phe-lys. acetate (research grad)/Serva; These chemicals were used without further purification.

**Sample Preparation.** Solutions were prepared with 0.05 mol/*l* NaCl in $CO_2$ free water. DEP solutions were kept at pH 11.5 by addition of NaOH in order to have DEP in the anionic form. Sample concentrations were adjusted to,

30 mmol/*l* before mixing. For Poly (rA), the concentration is referred to the repetition unit. Complexes were prepared by direct mixing of equal volumes *i.e.*, equimolar additions were made. For the mixing procedure we chose to add the peptide solution dropwise to the phosphate component, while this latter solution was strongly agitated. The final mixture was further agitated for some time and then left to settle for 15 min before it was taken to the spectroscopic measurements. The same procedure was used for preparation of oligopeptidepoly (rA) complexes (dipeptide, aromat ring containing tripeptide). All samples had a pH around 6.0-7.5. For the purpose of intensity calibration 10 mmol/*l* of potassium sulfate was added as an internal standard. In the present study we prefer internal standards over external standards, because we have to compare in our difference system the spectra of one component with the spectra of the complex solution, both of them standardized with the above mentioned internal standard.

**Spectra.** Raman spectra of the solutions were recorded using the spinning cell technique[6]. A Spex 1403 monochromator and Elscint single photon counting electronics worked under full computer control (IBM AT/02). For more details see Ref.[7]. The spectra were excited with the green line at 514.5 nm of an argon ion LASER, "coherent Labs model CR 6" and 400-1000 mW) at the sample. We used a standard slit width of 240 $\mu m$, 3 $cm^{-1}$ spectral resolution. Integrated intensities were scaled to the scattering activity of $\nu_1$ $SO_4^{2-}$ as an internal standard. The activity of the standard is taken as $32.8\times10^{-8}$ $cm^4$ $g^{-1}$ per molecule. All spectra were measured after most of the fluorescence "burnt out" whithin the spinning cell system by irradiation with LASER beam for a period of time (at least 1 hr).

**Nummerical Method.** The following paragraph is a very brief summary of the nummerical procedure used to convert experimental relative intensity reading into quantitative Raman scattering activities on a molecular level. The intensity readings are normalized to counts per second and divided by the instrument response function. Further particle density, temperature dependence of ground state population, and a Lorentz local field term are introduced in order to express the detected scattering power {DSP (ν)} as a function of the differential scattering activity {DSA (ν)}. The full elucidation and more details on the procedure are given in Ref.[7], and shall not be repeated here. The scattering activity $SA_j=g(45\ \bar{\alpha}_j')^2+7(\gamma_j')^2$ of the $j$-th normal mode can be evaluated by integration over the $j$-th band in the DSA ($d\nu$) spectra. These data are still not absolute because of unknown absolute incoming intensity $I_0$ and unknown absolute efficiency of the collection optics. The relative estimates of the scattering activity of the normal modes of the sample are converted into absolute values by calibration with the known scattering activity of a nearby band of a secondard. Using the difference method with the divided spining cell we were able to isolate intensities of vibrations in the following manner:

a) (complex solution spectra)-(solvent+peptide component)→phosphate component in complex
b) (phosphate component in complex)-(free phosphate) →Intensity and/or frequency changes of phosphate component

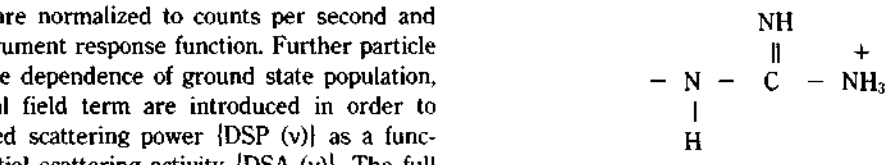
**Table 1.** The Isotropic Absolute Intensity of the Symmetric O-P-O Stretch Vibration in Complexes (in 0.05 mol/*l* NaCl solution)

| Substrate | Dipeptide | Raman shift ($cm^{-1}$) | Change of Raman scattering activity ($10^{-8}$ $cm^4$ $g^{-1}$) | pH |
|---|---|---|---|---|
| DEP | $(Lys)_2$ | 753 | −8.23 | 7.06 |
| DEP | $(Arg)_2$ | 753 | −6.68 | 7.09 |
| poly (rA) | $(Lys)_2$ | 816 | −2.94 | 6.04 |
| poly (rA) | $(Arg)_2$ | 816 | −8.96 | 6.01 |

In this scheme complex means equimolar mixtures of phosphate and peptide components in 0.05 mol/*l* NaCl solution; free phosphate is either diethylphosphate or polyriboadenylic acid in 0.05 mol/*l* NaCl. In order to reduce noise in the difference spectra the original spectra are treated with a "robust smoothing" routine[8] and with fourier transform methods. In the case of overlapping bands the profile was decomposed into a given number of Voigt profiles (Gauss-Lorentz product functions). The number of bands used in the fitting routine was restricted to the number of observed peaks and shoulders. The fitting routine was an interactive graphics procedure. All spectra were recorded in the wave number range from 500 to 1800 $cm^{-1}$.

## Results and Discussion

In the following discussion we concentrated only on the symmetric P-O stretch vibrations (O-P-O and $PO_2^-$) of complexes in our interest. For electrostatic interaction it is assumed that complex formation arises from reaction between the positive charge of the protonated base functions



, guanidinium ion,

or ($-\hat{N}H_3$), ammonium ion, on the oligopeptide and negative charge on the phosphate groups of the back bone in polynucleotide.

In order to understand the electrostatic interaction we experimented various reaction partners, for example, various phosphate molecules, various oligopeptides. We discussed only isotropic absolute intensity of symmetric phosphate residue in complexes. We known that the isotropic intensity relates to the number of the phosphate units[9]. Because of the physical background the isotropic intensity is a weak band and almost constant, while quadrapole intensity changes drastically.

**Effect of Dipeptide Complexes.** The activity of the reaction for the complex formation, as to be seen in Table 1 and 2, could be compared to absolute intensity between various complexes.

In Table 1, 2, the negative number in the Raman scattering activity implies that the intensity is decreased in the complex formation. Because the reference and free component inten-

**Table 2.** The Isotropic Absolute Intensity of the Symmetric $PO_2^-$ Stretch Vibration in Complexes (in 0.05 mol/l NaCl solution)

| Substrate | Dipeptide | Raman shift ($cm^{-1}$) | Change of Raman scattering activity ($10^{-8}$ $cm^4$ $g^{-1}$) | pH |
|---|---|---|---|---|
| DEP | $(Lys)_2$ | 1082 | −16.0 | 7.06 |
| DEP | $(Arg)_2$ | 1082 | −10.6 | 7.09 |
| poly (rA) | $(Lys)_2$ | 1094 | −1.29 | 6.04 |
| poly (rA) | $(Arg)_2$ | 1094 | −2.43 | 6.01 |

sity is subtracted from the complex intensity, zero means no intensity change and presumably no complexation. If the change of intensity increases into negative, the complex formation becomes stronger. We had described the details of calculations of the intensity in the numerical method paragraph. This is proportional to the degree of complex formation. We could assume that there is no steric hindrance in DEP complex, because the residue of DEP is a very small chain and hydrophobic alkylrest. In Table 2, the absolute intensity parallels coulomb's force for electrostatic interaction between two reaction partners. As to be seen in Table 2, the reaction of complex formation with lysine is stronger than with arginine.

On the basis of our spectroscopic observations, we can conclude, that the electrostatic interaction in the lysine complex differs from the arginine complex. We want to explain the reason in the following discussion of the poly (rA) complexes. We are interested in macro molecules, polyriboadenylic acid, which has sugar and base in the side chain of phosphate ester. Our question is, which phosphate residue is reacted strong in poly (rA) compared to DEP and how is the electrostatic interaction in macro molecules. We chose poly (rA), because it is the only polynucleotide of a well defined structure in aqueous solution at neutral pH. We measured the absolute intensity of the complex between oligopeptide and the back bone in polyriboadenylic acid. The results are shown in Table 1 and 2. Inspite of the same experimental conditions, it gave us a very weak absolute intensity, compared to the DEP complex.

How can we understand this phenomenon? This answer is clear to understand. The appearance of the decreased intensity of symmetric $PO_2^-$ stretch vibration in the poly (rA) complex is made reasonable with the following arguments: we could assume, that the reason comes from steric hindrance because of the bigger side chain (sugar/base) in phosphate ester. On account of big side chain of poly (rA), the distance between the two polar functional groups, P-O- and $N^+$-H, increase to such an extent, that the contact is not sufficient. We can propose several reasons, one of them is the electrostatic partial charge[10], which is the difference between O and N atom of function groups (guanidininium, ammonium ion and phosphate residue) in molecules. Another reason is the difference of the distance of binding between O atom on phosphate and N atom on oligopeptides. Arginine has a positive charge in there N atoms, which can be moved as meso form in function groups, while lysine has a positive charge in ammonium ion. Erfurth *et al.*[11] had postulated that oligopeptides (lysine, arginine) together with phosphate residue formed three hydrogen bonds in crystal respectively. Two N atoms of arginine from hydrogen bonds one with a $P\text{-}O^-$ group the other with an oxygen atom P-O ester linkage. The N atom of lysis side chain binds only with $P\text{-}O^-$ atom[11]. The length of hydrogen bond in lysine is about 2.8 Å for arginine 3.2 Å (X-ray structure analysis)[12].

Although the chemical nature of the studied dipeptides is very similar, *e.g.*, $pK_s$ and aliphat chain length, the charge density on N atoms is different. In all types of interaction the same intermolecular and interatomic forces are involved with different weights, depending upon the chemical nature: charge distribution and polarizabilities of the interacting species (coulombic interaction between charge and dipoles, polarization, dispersion and repulsive force *etc*). In addition to charge density on the oligopeptide side chains, the steric hindrance leads to different activity of oligopeptide complexes. Luis *et al.*,[13] observed no alternation of the intensity for symmetric $PO_2^-$ stretch vibration in poly (rA) complexes with polypeptides. They measured only crystalline samples contained in capillary tubes and therefore had no means of quantitative comparison. Due to a difference in the origine of DNA ionic strength and the ratio of peptide to DNA concentrations, it's very difficult to compare their results with this study. From our results the intensity of symmetric $PO_2^-$ stretch vibration changes clearly in aqueous solution. We could suggest, that lysine reacted strong than arginine with phosphate residue in DEP complexes. If there is no steric hindrance of the residue in ester of phosphates backbones, DEP is more favorable for complex formation with lysine than with arginine. The DEP complex reacts 1.5 fold stronger with lysine than with arginine. On the other hand the intensity of the symmetric $PO_2^-$ stretch vibration of poly (rA) complexes with arginine can react 1.9 times stronger than its with lysine. This result is inverted in the case of DEP complexes.

**Effect of Tripeptide Complexes.** From our results we are interested in another question. How is the effect of aromatic ring containing oligopeptide on electrostatic interaction? For this question we selected lysine tripeptide with containing aromat ring. The tripeptides (lys-aromat-lys) combines both the presence of aromatic residue capable stacking interaction and the presence of positive charges on $N^+H_3$ groups of the aliphatic residue with a very short peptide chain. We used aromatic ring, tyrosine and phenylalanine, because of their similar chemical structure. As we discussed, lysin has only one $N^+H_3$ on the terminal C atom of the side chain and reacts with $PO_2^-$ groups in the nucleotide unit. Using tripeptides containing an aromatic ring, the lys-aromat-lys, it would be of interest to study the effect of stacking interaction on electrostatic interaction.

In Table 3, 4 we can see the alteration of absolute intensity in oligopeptide complexes containing an aromatic ring. In order to compare intensities, we summarized various lysine oligopeptide complexes. All poly (rA) complexes with lysine containing an aromatic ring have a decreased intensity for symmetric $PO_2^-$ stretch vibration in Table 4.

It is known that the aromatic side chain reacts with bases in form of a sandwich stacking interaction. In tripeptide com-

**Table 3.** The Isotropic Absolute Intensity of the Symmetric OPO Stretch Vibration (in 0.05 mol/l NaCl solution)

| Substrate | Oligopeptide | Raman shift ($cm^{-1}$) | Change of Raman scattering activity ($10^{-8}$ $cm^4$ $g^{-1}$) | pH |
|---|---|---|---|---|
| poly (rA) | $(lys)_2$ | 816 | −2.94 | 6.04 |
| poly (rA) | (lys-tyr-lys) | 810 | −2.88 | 6.87 |
| poly (rA) | (lys-phe-lys) | 810 | −0.86 | 6.66 |

**Table 4.** The Isotropic Absolute Intensity of Symmetric $PO_2^-$ Stretch Vibration (in 0.05 mol/l NaCl solution)

| Substrate | Oligopeptide | Raman shift ($cm^{-1}$) | Change of Raman scattering activity ($10^{-8}$ $cm^4$ $g^{-1}$) | pH |
|---|---|---|---|---|
| poly (rA) | $(lys)_2$ | 1094 | −1.29 | 6.04 |
| poly (rA) | (lys-tyr-lys) | 1100 | −1.51 | 6.87 |
| poly (rA) | (lys-phe-lys) | 1100 | −1.71 | 6.66 |

plexes containing an aromatic ring the intensity decreases stronger than in dipeptide complexes without an aromatic ring. In Table 4, the intensity of pure electrostatic interaction amounts $1.29\times10^{-8}$ $cm^4$ $g^{-1}$. The effect of pure stacking interaction on the electrostatic interaction is $0.22\times10^{-8}$ $cm^4$ $g^{-1}$ in tyrosine tripeptide complex and $0.42\times10^{-8}$ $cm^4$ $g^{-1}$ in phenylalanine tripeptide complex. Because the stacking interaction combined with the electrostatic interaction the reaction is stronger than electrostatic interaction by itself. The stacking interaction between bases and the aromatic ring stabilizes the structure and at the same time two polar function groups are brought near to the electrostatic interaction. The effect is not similar between the electrostatic- and stacking-interaction when we compare the intensity amount of aromatic ring and that of lysine per unit. We observed, that electrostatic interaction is stronger than stacking interaction. The intensity of symmetric OPO stretch vibration in complexes related to the degree of comformational change of P-O ester in phosphate residue. In DEP complexes with lysine the decreased intensity of the symmetric OPO stretch vibration is greater than in complexes with arginine but in poly (rA) complexes the effect is inverted. In tripeptide complexes containing an aromat ring the decreased intensity of the symmetric OPO stretch vibration is less than in the dipeptide complexes without containing an aromat ring. This means that the conformation of phosphate residue in the complexes containing an aromat ring is stabilized by stacking interaction. Comparing tyrosine- and phenylalanine-complexes, phenylalanine reacted more favorable to the sandwich binding and therefore the conformation of the symmetric OPO in phosphate residue changed less.

**Change in Structure of Phosphate Residue.** The change of structure of phosphate residue in complexes will be very interesting to observe in aqueous solution. In general the molecular structure of complex can be distinguished from one another by the frequency of Raman scattering near 800 $cm^{-1}$, largely due to the symmetric OPO stretch vibration of the phosphatediester. In structure A, a strong sharp line occurs at 807 $cm^{-1}$ and in structure B there is a weak broad line at $830\pm5$ $cm^{-1}$, in structure C 790-850 $cm^{-1}$ and in structure Z $745\pm3$ $cm^{-1}$ [14,15]. From DEP complex with dipeptide we can assume, that it is near the *Z* form. The Raman shift of symmetric OPO stretch vibration in the DEP complex is 753 $cm^{-1}$. The structure of DEP complexes is irregular in the aqueous solution. This is clear because the DEP me complex is unstable and has a zig zag form, while the poly (rA) complex with dipeptide lies between the A and B form, symmetric OPO stretch vibration at 816 $cm^{-1}$. The structure of the tripeptide complex containing aromatic ring lies near the A form, symmetric OPO stretch vibration at 810 $cm^{-1}$.

In our experiment we have proved that in aqueous solution, th structure of the macromolecular complex{poly (rA) complex with tripeptide} is clearly stabilized and closer to the A form than the structure of the small molecular complexa{DEP complex with dipeptide}.

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