

Clusters of a protected amino acid with pyrazole derivatives: β -sheet model systems in the gas phase

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Abstract. In this paper we report on the structure of isolated clusters formed by the protected amino acid Ac-Phe-OMe and pyrazole or 5-methyl-3-aminopyrazole (MAP). Ac-Phe-OMe contains phenylalanine (Phe) which is protected at the terminal positions by introducing an acetyl and a methyl group. From our previous gas phase investigations it is known that the conformation of Ac-Phe-OMe is similar to the one of an amino acid in a β -sheet structure. Experimental studies in the liquid phase show that pyrazole derivatives are suitable agents to form very stable aggregates with β -sheet model peptides in order to prevent an aggregation with other peptide molecules. The structures of the Ac-Phe-OMe/(pyrazole, MAP) clusters are investigated by applying Resonant 2-Photon Ionization (R2PI) and infrared/R2PI spectroscopy as well as Hartree-Fock and DFT calculations. Different structural arrangements of the clusters are discussed. In the case of MAP at least two different isomers are observed experimentally.

PACS. 42.62.Fi Laser spectroscopy – 87.15.By Structure and bonding – 33.20.Ea Infrared spectra

1 Introduction

To get a description of the conformational landscape and the intermolecular binding of protein building blocks, isolated amino acids and peptides are investigated in the gas phase by applying mass, isomer, and state selective spectroscopical methods. One of three common amino acids containing an aromatic chromophore is phenylalanine (Phe). The first molecular beam experiments on the free amino acid Phe have been performed by Levy and coworkers [1] using laser induced fluorescence (LIF) spectroscopy. According to these experiments five different isomers of Phe have been predicted. By applying UV/UV hole-burning [2] and IR/R2PI [3–7] spectroscopy in combination with *ab initio* calculations, Simons and coworkers [8] have been able to obtain six different isomers of Phe which show prominent peaks in the R2PI [8] or LIF [1] spectrum.

In contrast to free amino acids protected amino acids contain no free acid (COOH) and basic (NH₂) groups. Thus intramolecular hydrogen bonds are avoided and the molecules can be good candidates for β -sheet model systems. In a recent publication [9] we investigated the structure of the protected amino acid Ac-Phe-OMe (see Fig. 1a) by applying R2PI and IR/R2PI spectroscopy as well as HF (Hartree-Fock) and DFT (Density Functional Theory) calculations. Ac-Phe-OMe is obtained from phenylalanine by

introducing an acetyl (Ac) group to protect the base NH₂ and by using a methyl group to protect the COOH group. Ac-Phe-OMe is a simple model system which contains a –CO–NH–CHR–CO– moiety, the key structural element of peptides. It is shown in reference [10] that Ramachandran plots [11] used for the description of peptide back-bone structures can also be applied to characterize protected amino acids like Ac-Phe-OMe, *i.e.* different back-bone conformations of peptides lead to characteristic values of the angles $\phi = \langle(C', C^\alpha, N, C_3)$ and $\psi = \langle(O_2, C', C^\alpha, N)$, *cf.* Figure 1a. The R2PI spectrum of Ac-Phe-OMe is dominated by only one isomer [9]. From the IR/R2PI spectrum and from DFT calculations it could be concluded that the most stable conformer of Ac-Phe-OMe has a conformation as the same amino acid in a β -sheet structure. In our report on the dimer (Ac-Phe-OMe)₂ (see Fig. 1b) we introduced the first example of a hydrogen bonded cluster of a (protected) amino acid and the first β -sheet model system in the gas phase [9].

Clusters of small protected peptides with pyrazole derivatives dissolved in chloroform have been investigated [12,13] by using ¹H-NMR spectroscopy. From this work it can be derived that the peptides within the peptide/(amino)pyrazole clusters have a β -sheet conformation. One aim of this work was to find appropriate pyrazole derivatives (called template molecules) to inhibit the growth of a pathogenic β -sheet. The development to larger β -sheet aggregates causes BSE/Creutzfeldt-Jakob

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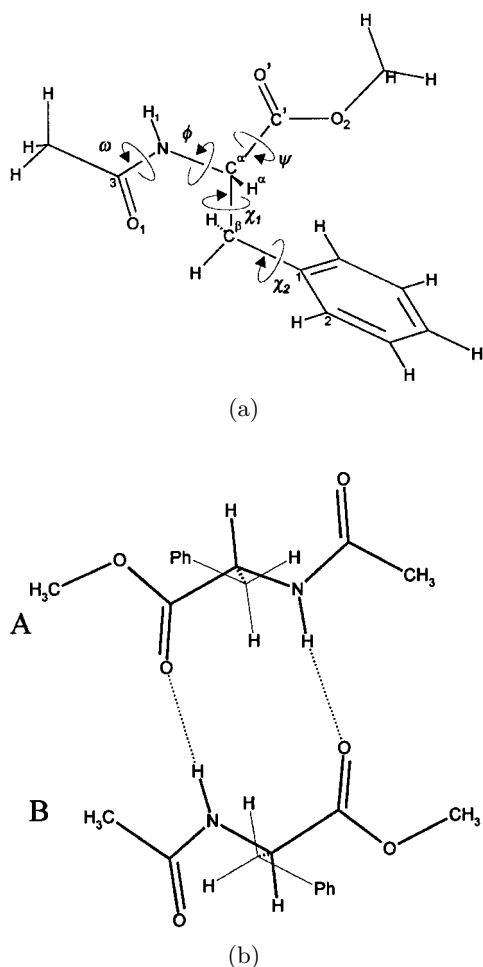


Fig. 1. Structures of (a) Ac-Phe-OMe and (b) the (Ac-Phe-OMe)₂ dimer.

and other prion diseases [14]. In a recent publication clusters of ferrocenoyl-dipeptides with 3-aminopyrazole dissolved in chloroform have been investigated by using IR spectroscopy [15]. The spectroscopical results also indicate the formation of a β -sheet arrangement.

In contrast to investigations in solution the examination of isolated clusters containing peptides and pyrazole derivatives in the gas phase offers the possibility to investigate the relative strength of different hydrogen bonds of size and isomer selected species. Furthermore the clusters of different tautomers can be analyzed by using IR/UV double resonance techniques. The different tautomers cannot be distinguished by NMR or pure IR spectroscopy of dissolved clusters, since the process of tautomerism is too fast on the time scale of the NMR spectroscopy and the different tautomers (as well as the existence of more than one isomer) cannot be distinguished without using a double resonance technique.

In this paper we report on the structure of clusters between the protected amino acid Ac-Phe-OMe and pyrazole as well as 5-methyl-3-aminopyrazole (see Fig. 2). These clusters are simple model systems to describe the inter-

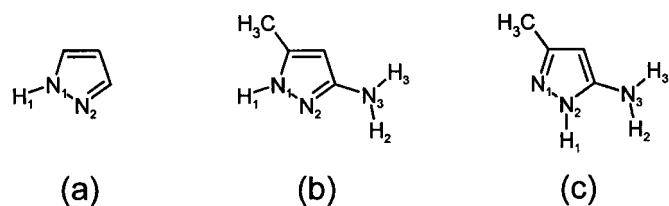


Fig. 2. Structures of (a) pyrazole and the two tautomers of 5-methyl-3-aminopyrazole (MAP): (b) MAP(1) and (c) MAP(2). The values calculated for some relevant geometrical parameters are given in Table 1.

action between a pyrazole derivative and a molecule with a β -sheet related conformation. To get a better comparison with larger systems we start our investigations with the cluster of Ac-Phe-OMe with pyrazole. In the case of 5-methyl-3-aminopyrazole (MAP) two different tautomers have to be taken into account (*cf.* Fig. 2) which can both form clusters with Ac-Phe-OMe.

2 Experimental set up

The experimental set up has been described in detail in reference [16]. Thus only a short description is given: the R2PI and IR/R2PI spectra were measured in a vacuum system consisting of a differentially pumped linear time-of-flight mass spectrometer and a pulsed valve (General Valve Iota One, 500 μm orifice) for skimmed jet expansion ($X/D = 130$). A frequency-doubled dye laser (Lumonics HD 300), pumped by a Nd-YAG laser (Lumonics HY 750), was used for the excitation to the S_1 state and for ionization. The IR light (2.8–3.2 μm) was generated with a LiNbO₃ crystal by difference frequency mixing of the fundamental (1064 nm) of a seeded Nd-YAG laser (Spectra-Physics PRO-230) and the output of a dye laser (Sirah, Precision Scan) pumped by the second harmonic (532 nm) of the same Nd-YAG laser. The IR output is amplified by an optical parametric amplification of the output of the IR-laser (2.8–3.2 μm) and the fundamental of the Nd-YAG laser [16]. Since the time delay chosen for the two lasers is not longer than 100 ns, all lasers have been spatially overlapped. In order to obtain IR/R2PI spectra the IR laser is fired 100 ns prior to the UV lasers.

Ac-(L)Phe-OMe (Bachem, purity >99%) was used without further purification. Pyrazole and 5-methyl-3-aminopyrazole (Aldrich) were purified by sublimation and distillation, respectively. Substance and valve are heated to 120 °C. Helium was used as carrier gas (2000 mbar).

3 Results and discussion

3.1 Ac-Phe-OMe/pyrazole cluster

The R2PI spectrum of the Ac-Phe-OMe/pyrazole cluster in the range from 0 to 700 cm^{-1} relative to the electronic origin at 37536 cm^{-1} is shown in Figure 3a. The corresponding IR/R2PI spectrum obtained *via* the electronic

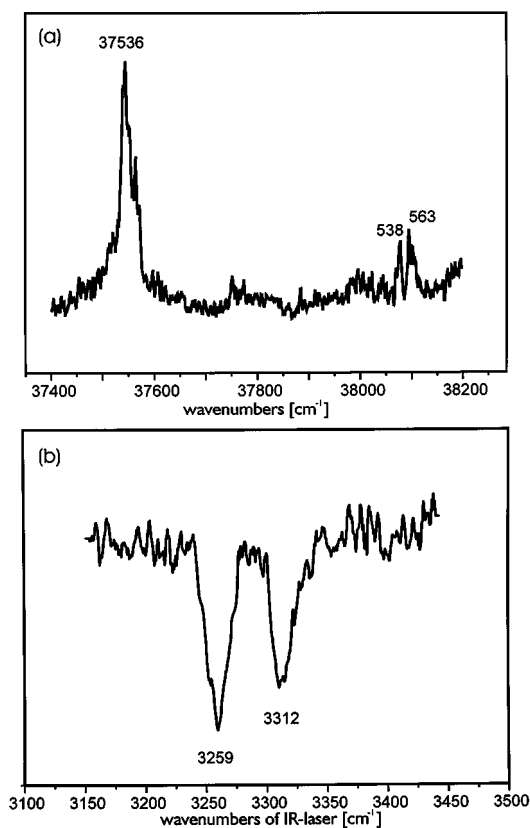


Fig. 3. (a) R2PI spectrum of the Ac-Phe-OMe/pyrazole cluster in the range from 0 to 700 cm^{-1} relative to electronic origin at 37536 cm^{-1} . (b) IR/R2PI spectrum of Ac-Phe-OMe/pyrazole in the range from 3150 to 3460 cm^{-1} obtained *via* the electronic $S_1 \leftarrow S_0$ transition at 37536 cm^{-1} .

origin is given in Figure 3b. Two strong transitions at 3259 and 3312 cm^{-1} are observed. The frequencies of the NH stretching modes of isolated Ac-Phe-OMe and pyrazole are 3458 [9] and 3508 cm^{-1} [17], respectively. Due to $\text{C}=\text{O} \cdots \text{H}-\text{N}$ or $\text{N} \cdots \text{H}-\text{N}$ hydrogen bonds between pyrazole and Ac-Phe-OMe strong red-shifts of the NH stretching frequencies are expected. The two transitions observed in the IR/R2PI spectrum are strongly red-shifted with respect to the free NH stretching frequencies at 3458 and 3508 cm^{-1} , respectively. If both transitions in the IR/R2PI spectrum can be correlated with NH stretching modes a doubly hydrogen bonded structure (see Fig. 4a) should be formed.

Our investigations on the Ac-Phe-OMe monomer [9] have shown that this species has a beta-sheet related structure (β_L) with a relative position of the phenyl ring in an anti or gauche (+) position leading to a $\beta_L(a)$ or a $\beta_L(g^+)$ arrangement. Starting from one of these beta-sheet related structures, the corresponding clusters with pyrazole have been calculated at the DFT level using the B3LYP functional and the 6-31G(d) basis set. In order to find the minimum energy structures full optimizations are performed by applying the Gaussian 98 program [18]. The most stable Ac-Phe-OMe/pyrazole clusters contain two intermolecular hydrogen bonds between the N(1)H(1)

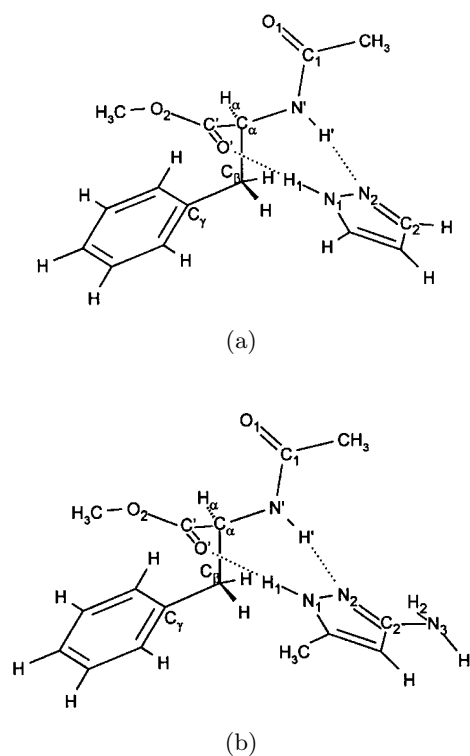


Fig. 4. Structural arrangement of (a) the ($\beta_L(a)$ Ac-Phe-OMe)/pyrazole cluster and (b) the ($\beta_L(a)$ Ac-Phe-OMe)/MAP(1) cluster as obtained from DFT calculations (B3LYP/6-31G(d) level). The geometrical parameters are given in Table 2.

Table 1. Structural parameters of pyrazole and the two tautomers of 5-methyl-3-aminopyrazole MAP(1) and MAP(2). The values are obtained from B3LYP/6-31G(d) calculations. The labelling of the atoms refer to the one given in Figure 2. The angle ρ describes the non-planarity of the NH_2 group with respect to the five-membered pyrazole ring.

	pyrazole	MAP(1)	MAP(2)
bond lengths [\AA]			
H_1-N_1	1.009	1.008	1.008
H_2-N_3	—	1.017	1.015
H_3-N_3	—	1.014	1.014
N_2-N_1	1.351	1.364	1.364
bond angles [$^\circ$]			
$\text{H}_1-\text{N}_1-\text{N}_2$	118.73	119.23	118.56
$\text{H}_3-\text{N}_3-\text{H}_2$	—	108.73	110.02
ρ	—	49.68	49.39

and $\text{C}'=\text{O}'$ groups as well as the $\text{N}'\text{H}'$ group and the N(2) atom of pyrazole (*cf.* Fig. 4a). The geometrical parameters of the pyrazole monomer and the Ac-Phe-OMe/pyrazole cluster are listed in Tables 1 and 2. Similar to the (Ac-Phe-OMe) $_2$ dimer [9] the bond lengths of the hydrogen bonded $\text{C}'=\text{O}'$, N(1)–H(1) and $\text{N}'\text{H}'$ groups are about $0.01\text{--}0.02\text{ \AA}$ longer compared to the corresponding bond

Table 2. Structural parameters of the ($\beta_L(a)$ Ac-Phe-OMe)/pyrazole, ($\beta_L(g^+)$ Ac-Phe-OMe)/pyrazole, ($\beta_L(a)$ Ac-Phe-OMe)/MAP(1), and ($\beta_L(g^+)$ Ac-Phe-OMe)/MAP(1) clusters. The values are obtained from B3LYP/6-31G(d) calculations. The labelling of the atoms refer to the one given in Figure 4. The angle ρ describes the non-planarity of the NH₂ group.

	$\beta_L(a)$ + pyrazole	$\beta_L(g^+)$ + pyrazole	$\beta_L(a)$ + MAP(1)	$\beta_L(g^+)$ + MAP(1)
bond lengths [\AA]				
N'-H'	1.023	1.022	1.025	1.025
C'-O'	1.223	1.218	1.222	1.216
C $^\alpha$ -N'	1.449	1.444	1.449	1.441
C $^\alpha$ -C'	1.531	1.526	1.531	1.529
C $^\alpha$ -C $^\beta$	1.552	1.560	1.552	1.557
C $^\beta$ -C $^\gamma$	1.514	1.514	1.514	1.514
N'-C ₁	1.370	1.372	1.369	1.368
C ₁ -O ₁	1.226	1.228	1.227	1.229
H'-N ₂	2.013	2.060	1.987	1.977
O'-H ₁	1.879	1.898	1.908	1.986
H ₁ -N ₁	1.022	1.019	1.019	1.016
N ₃ -H ₂	—	—	1.015	1.016
N ₃ -H ₃	—	—	1.014	1.014
N ₃ -C ₂	—	—	1.398	1.398
C ₂ -N ₂	1.336	1.336	1.335	1.333
N ₂ -N ₁	1.351	1.349	1.365	1.365
bond angles [$^\circ$]				
N-C $^\alpha$ -C'	110.63	111.67	110.65	111.11
C $^\alpha$ -N'-H'	118.61	120.77	118.41	119.39
C $^\alpha$ -C'-O'	125.11	126.86	125.14	126.47
C $^\alpha$ -C $^\beta$ -C $^\gamma$	113.77	115.12	113.48	114.96
N'-C $^\alpha$ -C $^\beta$	111.05	113.90	110.78	113.15
C'-C $^\alpha$ -C $^\beta$	109.73	111.29	109.75	113.77
N'-H'-N ₂	168.75	167.05	169.28	165.61
C'-O'-H ₁	132.77	138.91	132.54	137.19
H ₁ -N ₁ -N ₂	117.70	117.47	117.51	116.69
N ₁ -N ₂ -H'	106.45	103.22	106.10	105.50
H ₂ -N ₃ -C ₂	—	—	111.99	111.23
dihedral angles [$^\circ$]				
ϕ	-110.64	-124.10	-108.06	-123.79
ψ	127.87	169.75	127.94	-176.12
χ_1	-179.61	68.68	179.43	56.75
χ_2	78.69	97.43	79.29	85.73
ω	-177.96	179.91	-179.08	179.28
ρ	—	—	47.84	49.35

lengths of the monomers (the C'=O' and N'-H' bond-lengths of Ac-Phe-OMe obtained at the B3LYP/6-31G(d) level are 1.209 \AA and 1.009 \AA , respectively).

The calculated relative stabilities of the ($\beta_L(a)$ Ac-Phe-OMe)/pyrazole and ($\beta_L(g^+)$ Ac-Phe-OMe)/pyrazole clusters differ only by 350 cm^{-1} including BSSE [19] and zero point energy corrections (*cf.* Tab. 3). Due to the size of the system no higher level calculations (including normal vibrational analyses) can be performed in order to verify the energy difference predicted at the B3LYP/6-31G(d) level.

The calculated NH stretching frequencies of the two clusters are scaled with a factor of 0.9587. This factor is obtained from scaling the calculated NH stretching frequency of Ac-Phe-OMe (B3LYP functional, 6-31G(d) basis set) with respect to the experimental value of 3458 cm^{-1} [9]. The scaled NH stretching frequencies of the most stable ($\beta_L(a)$ Ac-Phe-OMe)/pyrazole cluster (3265 and 3323 cm^{-1} , *cf.* Tab. 3) are in excellent agreement with the experimentally observed vibrational transitions at 3259 and 3312 cm^{-1} . In the case of the ($\beta_L(g^+)$ Ac-Phe-OMe)/pyrazole structure both the calculated NH(Ac-Phe-OMe) frequency and the NH(pyrazole) frequency

Table 3. Calculated and scaled NH stretching frequencies (B3LYP/6-31G(d) level) for the ($\beta_L(a)$ Ac-Phe-OMe)/pyrazole, ($\beta_L(g^+)$ Ac-Phe-OMe)/pyrazole, ($\beta_L(a)$ Ac-Phe-OMe)/MAP and ($\beta_L(g^+)$ Ac-Phe-OMe)/MAP clusters (MAP refers either to tautomer MAP(1) or tautomer MAP(2)). The scaling factor of 0.9587 is obtained from experimentally observed NH stretching mode of the Ac-Phe-OMe monomer [9] and the frequency calculated at the B3LYP/6-31G(d) level. All values in cm^{-1} .

	NH (Ac-Phe-OMe)	NH (pyrazole/MAP)	NH (sym.)	NH (asym.)	rel. energies
$\beta_L(a)$ + pyrazole	3 265	3 323	—	—	0
$\beta_L(g^+)$ + pyrazole	3 298	3 370	—	—	344
$\beta_L(a)$ + MAP(1)	3 242	3 352	3 386	3 479	0
$\beta_L(a)$ + MAP(2)	3 242	3 296	3 389	3 484	549
$\beta_L(g^+)$ + MAP(1)	3 232	3 417	3 375	3 470	419
$\beta_L(g^+)$ + MAP(2)	3 262	3 372	3 384	3 479	966

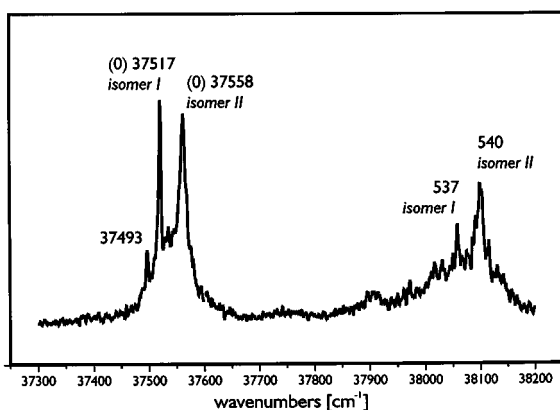


Fig. 5. R2PI spectrum of Ac-Phe-OMe/MAP. The assignment of the transitions at 37517 cm^{-1} and 37558 cm^{-1} as origins of two different isomers result from the IR/R2PI spectra (see Fig. 6).

exhibit a stronger deviation from the experimental values, *i.e.* the cluster observed in the R2PI spectrum can be assigned to the doubly hydrogen bonded ($\beta_L(a)$ Ac-Phe-OMe)/pyrazole arrangement.

It should be mentioned that the calculated and observed red-shifts of the NH stretching vibrations are larger than in the case of the (Ac-Phe-OMe)₂ dimer (NH stretching vibrations at about 3380 cm^{-1} [9]). This is a result of the strong hydrogen bonding between pyrazole and Ac-Phe-OMe. DFT calculations at the B3LYP/6-31G(d) level including zero point energy and BSSE corrections yield stabilization (binding) energies of the dimer and the ($\beta_L(a)$ Ac-Phe-OMe)/pyrazole cluster of 1950 and 2930 cm^{-1} , respectively; *i.e.* the relative stability of the ($\beta_L(a)$ Ac-Phe-OMe)/pyrazole cluster is 980 cm^{-1} higher than the stability obtained for the (Ac-Phe-OMe)₂ dimer.

3.2 Ac-Phe-OMe/MAP cluster

The R2PI spectrum of the Ac-Phe-OMe/MAP cluster is shown in Figure 5. Two strong transitions are observed at 37517 and 37558 cm^{-1} . The corresponding IR/R2PI spectra obtained *via* these two transitions are given in Figure 6. The IR/R2PI spectra are not identical, *i.e.* the transitions at 37517 (isomer I) and 37558 cm^{-1} (isomer II)

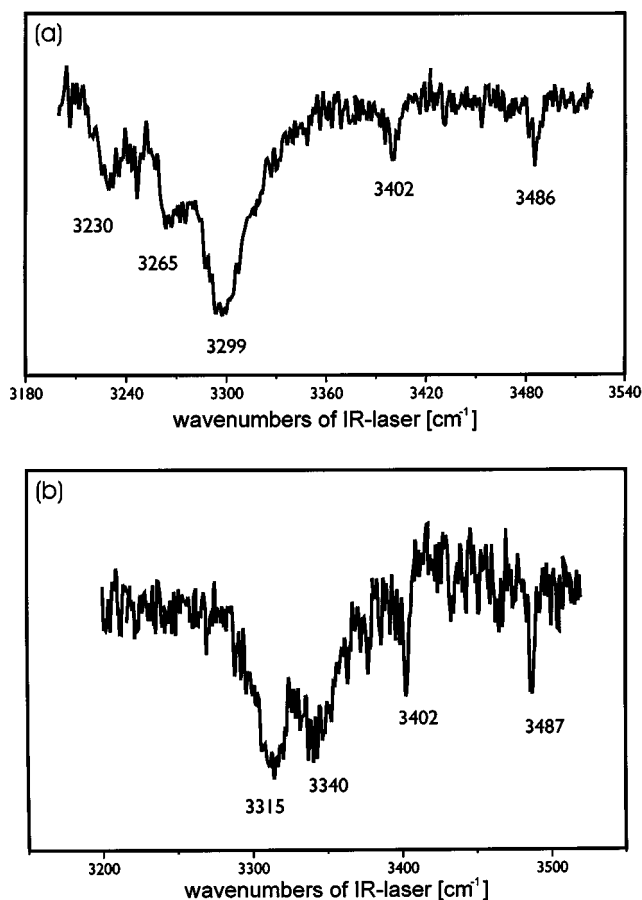


Fig. 6. IR/R2PI spectra of the Ac-Phe-OMe/MAP cluster in the range from 3200 to 3525 cm^{-1} obtained *via* the electronic $S_1 \leftarrow S_0$ transition at (a) 37517 cm^{-1} and (b) 37558 cm^{-1} of the R2PI spectra (see Fig. 5).

of the R2PI spectra belong to different isomers. The appearance of a hot band can be excluded since the intensity ratio of both transitions does not depend on the expansion conditions of the molecular beam. The transition at 537 cm^{-1} (540 cm^{-1}) and the electronic origin of isomer I (isomer II) belong to the same isomer since both transitions exhibit the same IR/R2PI spectra. A vibrational transition of about 540 cm^{-1} is also typical for the Ac-Phe-OMe monomer (537 cm^{-1} [9]) and its dimer

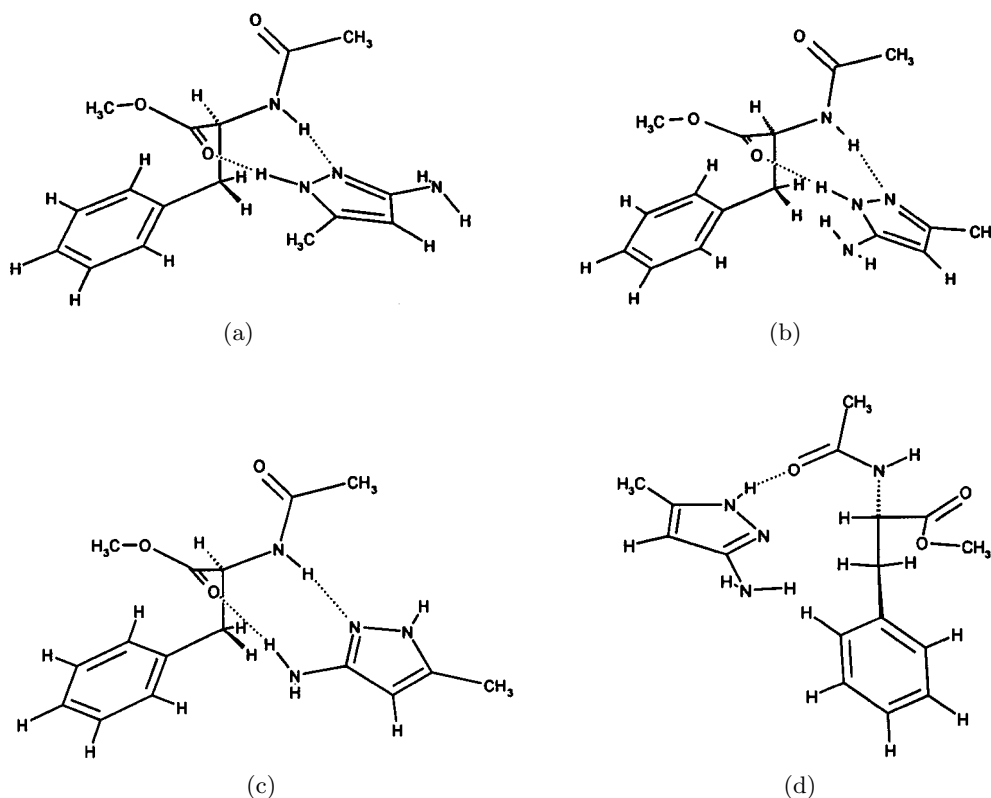


Fig. 7. Possible structural arrangements of the Ac-Phe-OMe/MAP cluster as obtained from DFT calculations (B3LYP functional, 6-31G(d) basis set). Structures (a) and (c) belong to double hydrogen bonded structures of tautomer MAP(1) (see also Fig. 4b). Structure (b) is a doubly hydrogen bonded structure between Ac-Phe-OMe and tautomer MAP(2) (see Fig. 2c). Structure (d) represents a singly hydrogen bonded structure between the C(1)–O(1) group of Ac-Phe-OMe (see Fig. 4) and the N(1)–H(1) bond of MAP(1) (*cf.* Fig. 2b).

(533 cm^{-1} [9]). According to *ab initio* and DFT calculations (see below) this vibration can be assigned to the 6a mode of the aromatic ring of phenylalanine.

The electronic origins of both isomers of Ac-Phe-OMe/MAP are red-shifted with respect to the origin of the monomer (37579 cm^{-1} [9]). Similar to the (Ac-Phe-OMe) $_2$ dimer (37537 cm^{-1} [9]) and the Ac-Phe-OMe/pyrazole cluster (37536 cm^{-1}) the observed red-shifts for the Ac-Phe-OMe/MAP clusters indicate a stabilization resulting from intermolecular hydrogen bonds. It has to be taken into account that in the case of the protected amino acid Ac-Phe-OMe not more than two hydrogen bonds (between C=O and N–H) can be formed simultaneously with one MAP molecule. The MAP molecule itself offers the possibility to form three hydrogen bonds. This can be realized in a cluster of tautomer MAP(1) (see Fig. 2b) with a (protected) dipeptide [12, 13, 20].

For the Ac-Phe-OMe/MAP cluster three different configurational isomers are possible (see Fig. 7) which contain a double hydrogen bonding. In the first two clusters shown in Figure 7 the five-membered pyrazole ring of the two MAP tautomers form two hydrogen bonds with the C=O and N–H group of Ac-Phe-OMe. The third isomer contains hydrogen bonds involving the NH $_2$ group of

tautomer MAP(1). A corresponding structural arrangement cannot be formed with tautomer MAP(2). Except for the cluster structures containing two intermolecular hydrogen bonds, several structures can be formed which contain only a single hydrogen bond (see *e.g.* Fig. 7d).

The two IR/R2PI spectra shown in Figure 6 exhibit (nearly) the same transitions in the region from 3360 to 3520 cm^{-1} . In agreement with DFT calculations (see below and Tab. 3) the transitions at 3402 (3402) cm^{-1} and 3486 (3487) cm^{-1} can be assigned as symmetric and antisymmetric NH stretching modes of a non hydrogen bonded NH $_2$ group of MAP. Thus a structural arrangement like the one given in Figure 7c including a hydrogen bonded NH $_2$ group should be excluded. A fully optimized structural arrangement including a hydrogen bonded NH $_2$ group (see Fig. 7c) and Ac-Phe-OMe with a $\beta_L(a)$ conformation would lead to NH stretching frequencies (B3LYP/6-31G(d) level) of 3332 cm^{-1} (hydrogen bonded N(3)–H(2) bond) and 3471 cm^{-1} (free N(3)–H(3) bond, see nomenclature in Fig. 2). A NH stretching frequency at about 3400 cm^{-1} would not be observed in the IR/R2PI spectrum, *i.e.* structures which contain a hydrogen bonded NH $_2$ group (Fig. 7c) can be excluded.

According to calculations (B3LYP/6-31G(d) level) on singly hydrogen bonded structures (see Fig. 7d) only a

very small red-shift of the NH stretching frequency of Ac-Phe-OMe (3458 cm^{-1}) can be expected, *i.e.* in the region from 3400 to 3500 cm^{-1} three vibrational transitions (symmetric and antisymmetric NH stretching modes of the NH_2 group and the $\text{NH}(\text{Ac-Phe-OMe})$ stretching mode) must be observed. In the IR/R2PI spectra of isomers I and II (Fig. 6) only 2 vibrations appear in the region from 3400 to 3500 cm^{-1} . A vibrational transition of about 3450 cm^{-1} resulting from the $\text{NH}(\text{Ac-Phe-OMe})$ cannot be observed. From these considerations the existence of singly hydrogen bonded structures as displayed in Figure 7d can be excluded. Thus only the doubly hydrogen bonded structures (Figs. 7a and 7b) have to be discussed.

In order to find out whether the configurational isomer given in Figure 7a or the one shown in Figure 7b is more stable the conformational isomerism of the Ac-Phe-OMe moiety has also to be considered. The most stable arrangement of Ac-Phe-OMe contains a $\beta_{\text{L}}(a)$ or a $\beta_{\text{L}}(g^+)$ structure. Starting from one of these beta-sheet related structures full geometry optimizations at the HF/3-21G(d) and B3LYP/6-31G(d) level have been performed for the four possible clusters, *i.e.* the clusters of ($\beta_{\text{L}}(a)$ Ac-Phe-OMe) and ($\beta_{\text{L}}(g^+)$ Ac-Phe-OMe) with tautomers MAP(1) and MAP(2) are considered.

Both *ab initio* and DFT calculations exhibit the clusters containing tautomer MAP(1) to be more stable than the clusters with tautomer MAP(2). Due to the size of the Ac-Phe-OMe/MAP cluster, no higher level calculations including full optimizations and normal vibrational analyses can be performed in order to get a further proof for the calculated energy differences.

The structural parameters obtained for the ($\beta_{\text{L}}(a)$ Ac-Phe-OMe)/MAP(1) and ($\beta_{\text{L}}(g^+)$ Ac-Phe-OMe)/MAP(1) clusters at the B3LYP/6-31G(d) level are listed in Table 2, the corresponding parameters of the MAP monomer (see Figs. 2b and 2c) are given in Table 1. As in the case of the (Ac-Phe-OMe)₂ dimer and the (Ac-Phe-OMe)/pyrazole cluster the $\text{C}'=\text{O}'$, $\text{N}'-\text{H}'$ and $\text{N}(1)-\text{H}(1)$ bond lengths are about $0.01\text{--}0.02\text{ \AA}$ longer than the corresponding bond lengths of the monomers (*cf.* Sect. 3.1).

The NH stretching frequencies calculated at the B3LYP/6-31G(d) level are listed in Table 3. Since the NH_2 group of MAP is not hydrogen bonded the calculated symmetric and antisymmetric NH stretching frequencies of the NH_2 group of all clusters are nearly identical. Only the frequencies of the ($\beta_{\text{L}}(g^+)$ Ac-Phe-OMe)/MAP(1) cluster are slightly lower than the frequencies of the other clusters. The calculated values are in good agreement with the experimental frequencies of 3402 (3402) cm^{-1} and 3486 (3487) cm^{-1} observed for the symmetric and antisymmetric NH stretching mode of the two isomers (see Figs. 6a and 6b).

The frequencies calculated for the NH stretching vibration of the Ac-Phe-OMe moiety in the different clusters turn out to be very similar, only the vibration of the ($\beta_{\text{L}}(g^+)$ Ac-Phe-OMe)/MAP(2) cluster has a frequency which is about $20\text{--}30\text{ cm}^{-1}$ higher than the one of the other isomers. The calculated values can be correlated

with the transition at 3265 cm^{-1} in the IR/R2PI spectrum of isomer I (see Fig. 6a). Furthermore the transition at 3265 cm^{-1} is in very good agreement with the corresponding $\text{NH}(\text{Ac-Phe-OMe})$ vibration of the Ac-Phe-OMe/pyrazole cluster (see Sect. 3.1 and Fig. 3b). It should be mentioned that the weaker transition at 3230 cm^{-1} in the IR/R2PI spectrum of isomer I can be correlated with the calculated overtone of the NH_2 bending mode.

The frequencies of the last remaining $\text{NH}(\text{MAP})$ stretching mode of the MAP ring ($\text{N}(1)-\text{H}(1)$ stretching mode) in the (Ac-Phe-OMe)/MAP clusters depends significantly on the MAP tautomer and the back-bone conformation of Ac-Phe-OMe. With respect to the IR/R2PI spectrum of isomer I the frequencies calculated for the ($\beta_{\text{L}}(a)$ Ac-Phe-OMe)/MAP(2) cluster show the best agreement, but the calculated relative stability of this cluster is by more than 500 cm^{-1} lower than the one of the ($\beta_{\text{L}}(a)$ Ac-Phe-OMe)/MAP(1) cluster (*cf.* Tab. 3).

In contrast to the IR/R2PI spectrum of isomer I ($\text{NH}(\text{Ac-Phe-OMe})$: 3265 cm^{-1} ; $\text{NH}(\text{MAP})$: 3299 cm^{-1}) the vibrational frequencies observed in the corresponding spectrum of isomer II are shifted to higher frequencies (3315 and 3340 cm^{-1}). No $\text{NH}(\text{Ac-Phe-OMe})$ transition at about 3265 cm^{-1} can be observed. A tentative assignment of this spectrum to the spectrum calculated for the ($\beta_{\text{L}}(g^+)$ Ac-Phe-OMe)/MAP(2) cluster (with a higher $\text{NH}(\text{Ac-Phe-OMe})$ frequency) is questionable since this cluster is by more than 900 cm^{-1} less stable than the most stable ($\beta_{\text{L}}(a)$ Ac-Phe-OMe)/MAP(1) cluster.

It should be noted that the experimentally observed $\text{NH}(\text{MAP})$ and $\text{NH}(\text{Ac-Phe-OMe})$ stretching frequencies of isomers I and II are in agreement with the scaled frequencies obtained from *ab initio* HF/3-21G(d) calculations performed on ($\beta_{\text{L}}(a)$ Ac-Phe-OMe)/MAP (isomer I) and ($\beta_{\text{L}}(g^+)$ Ac-Phe-OMe)/MAP (isomer II) whereas MAP is the same tautomer in both clusters (either MAP(1) or MAP(2)). At least for the $\text{NH}(\text{MAP})$ stretching modes calculated at the B3LYP/6-31G(d) level the shift to a higher frequency can also be observed by comparing clusters containing the same MAP tautomer but different conformers of Ac-Phe-OMe ($\beta_{\text{L}}(a)$ or $\beta_{\text{L}}(g^+)$) (see Tab. 3).

It can be concluded that the calculated NH stretching frequencies lead to no final decision which two clusters are observed in the R2PI spectrum. But the predicted frequency shifts for clusters with different back-bone conformations of Ac-Phe-OMe and the higher stability calculated for clusters containing tautomer MAP(1) may indicate that the different experimentally observed isomers result from a change of the back-bone configuration in the (Ac-Phe-OMe)/MAP(1) clusters and not from clusters containing different MAP tautomers.

By comparing the doubly hydrogen bonded (Ac-Phe-OMe)₂ dimer, (Ac-Phe-OMe)/pyrazole cluster, and (Ac-Phe-OMe)/MAP clusters an increasing stability can be observed. The (ZPE and BSSE corrected) binding energy of ($\beta_{\text{L}}(a)$ Ac-Phe-OMe)/MAP(1) is 4020 cm^{-1} , *i.e.* the stability is about 1100 cm^{-1} higher than the one of the ($\beta_{\text{L}}(a)$ Ac-Phe-OMe)/pyrazole cluster and about

2100 cm^{-1} higher than the one of the $(\text{Ac-Phe-OMe})_2$ dimer. This increasing stability supports the efficiency of amino pyrazoles to form stable clusters with β -sheet related structures, *i.e.* they may be good candidates to prevent pathogenic β -sheet aggregation of larger peptide molecules.

4 Conclusions

In this paper clusters are discussed which contain a β -sheet model system (Ac-Phe-OMe) and pyrazole or the pyrazole derivative 5-methyl-3-aminopyrazole (MAP). The pyrazole derivatives are designed to inhibit the growth of a β -sheet structure. An IR spectroscopical investigation of the isolated species as given in this paper yield information about the efficiency to form clusters of different pyrazoles with peptides. According to our R2PI and IR/R2PI spectra as well as our *ab initio* and DFT calculations on the (Ac-Phe-OMe)/(pyrazole, MAP) clusters both pyrazole and MAP form doubly hydrogen bonded structures with Ac-Phe-OMe. This is the maximum number of intermolecular hydrogen bonds which can be formed between Ac-Phe-OMe and the pyrazoles. Furthermore the clusters of Ac-Phe-OMe and pyrazoles are more stable than the corresponding structure of the doubly hydrogen bonded $(\text{Ac-Phe-OMe})_2$ dimer. The stability increases from the dimer to the (Ac-Phe-OMe)/pyrazole cluster and the (Ac-Phe-OMe)/MAP cluster. This is in agreement with the results obtained for investigations in the liquid phase [13].

In the case of the (Ac-Phe-OMe)/MAP cluster two stable isomers are observed in our gas phase spectra. From the experimentally observed NH stretching vibrations it can be concluded that both isomers are doubly hydrogen bonded and contain a free NH_2 group. According to the calculated relative energies (HF and DFT level) of different isomers it can be assumed that the two experimentally observed clusters have different back-bone configurations of Ac-Phe-OMe. Further investigations on clusters of aminopyrazole derivatives with dipeptides will show if a very effective three point connection between the peptide and the aminopyrazole can be formed in the gas phase.

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