Research article

Ibuprofen-tyrosine (Val-Tyr, Val-Tyr-Val) interactions. Theoretical and experimental studies

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Abstract: *In this work, the interactions between ibuprofen and tyrosine as well selected tyrosine-containing oligopeptides (Val-Tyr, Val-Tyr-Val) have been studied using both experimental (absorption and fluorescence spectroscopy) and theoretical (the PM3 method) techniques. The experimentally obtained values of association constant together with free Gibbs energy of association for the studied tyrosine-ibuprofen and Val-Tyr-; Val-Tyr-Val-ibuprofen complexes have been determined. The obtained results indicated that the mechanism of action of ibuprofen is most probably based on the hydrogen bonding interaction which involves hydroxyl group of tyrosine.*

Keywords: *ibuprofen; hydrogen bonding interactions; PM3 calculations*

Introduction

The influence of drugs on human body is measured by their pharmacokinetics and pharmacodynamics. The former is the study of drug processing in the body which includes: absorption, distribution, metabolism and excretion of the drug in the human body. The latter is a study about mechanism of action of the drug on the human body [1]. Ibuprofen, 2-[4-(2-methylpropyl)phenyl] propanoic acid, (Figure 1), is a drug from the group of non-steroidal anti-inflammatory drugs (NSAID). This compound is usually used as analgesic, antipyretic and anti-

rheumatic [2]. Ibuprofen is used to reduce fever, inflammation and sensation pain, as most probably, this drug works by inhibiting the action of prostaglandins [1].

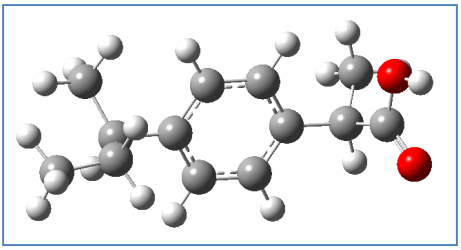

Figure 1. Molecular structure of ibuprofen

Prostaglandins are chemicals that cause inflammation and contribute to the brains perception of pain [3]. Ibuprofen reduces fever by blocking prostaglandin synthesis in the hypothalamus, a structure in the brain that regulates body temperature. The accurately mechanism of action of ibuprofen and other NSAID is not completely known. It is very likely that limitation of prostaglandin synthesis due to cyclooxygenase (COX) inhibition is involved in it.

Cyclooxygenases (COX) are enzymes which could be found in three isoforms of COX1, COX2 and COX3, have an active role in the production of prostaglandins. COX1 catalyzes the normal production of prostaglandins in the body. The second isoform has a role in production of prostaglandins in inflammatory cells. COX 3 has been found to be selectively inhibited by paracetamol, phenacetin and antipyrine. There are two active sites on COX1: cyclooxygenase and a peroxidase. COX1 catalyzes the production of prostaglandins by removing a hydrogen atom from arachidonic acid and transferring it to Tyr-385 in the active site of COX1. A hydrogen bond between Tyr-348-Tyr385 is crucial for this activity [3]. Any interruption has an influence in changing prostaglandins synthesis. Due to the role that tyrosine residues have in the biological activity of COX1, tyrosine and some tyrosine-containing oligopeptides (dipeptide (Val-Tyr), tripeptide (Val-Tyr-Val)) became also main object in this study.

Tyrosine is one of the 20 basic amino acids. It is extremely reactive because of its hydrophilic side chain. Tyrosine is also an essential amino acid. It has a role in the intercellular transport, synthesis of hormones, and biologically active substances such as adrenaline, noradrenaline and dopamine [3]. Additionally, tyrosine is the largest constituent of all residues at the active site of cyclooxygenase.

The knowledge of the intermolecular interactions between tyrosine residue and ibuprofen might shed light into the complex mechanism of action of ibuprofen. Therefore, the aim of this study is to investigate the molecular interactions between ibuprofen and tyrosine residue in aqueous solution. This

aim will be obtained using both experimental (absorption and fluorescence spectroscopy) and theoretical (semi-empirical PM3) methods.

Experimental

Materials

Ibuprofen, L-valine-L-tyrosine, L-valine-L-tyrosine-L-valine and L-tyrosine used in experimental part of this project were commercial products produced by Sigma-Aldrich. Dipeptide, tyrosine and tripeptide were dissolved in distilled water. Because of very poor solubility of ibuprofen in water, the stock solution of ibuprofen (0,05 M) was prepared in ethanol. All reagents were least analytical grade and were used without further purification.

Experimental methods

Nicolet Evolution 300 UV-Vis spectrophotometer from Thermo Electron Corporation with resolution 0,5 nm and range 0-6 was used in absorption measurements. Spectrofluorometer Generic Fluoromax-4P from Horiba Jobin Yvon was used in steady state measurements. Solutions were placed into a 10 mm quartz cuvette. During the measurements, temperature was constant (25 °C). In titration experiments the concentration of tyrosine, dipeptide and tripeptide was kept constant ($5*10^{-5}$ M) while varying the concentration of ibuprofen.

Computational methods

The geometries of tyrosine, dipeptide, tripeptide (in zwitterionic form) and ibuprofen (anion) as well as tyrosine (dipeptide, tripeptide)-ibuprofen complexes have been optimized applying the PM3 method implemented in Gaussian 03 program. We have also performed frequency calculations of the above mentioned systems at the same level of theory. The analysis of the PM3 calculated frequencies have been performed to verify whether the optimized structures correspond to the minima. Harmonic oscillator approximation has been used in the vibration calculations.

The ground state interaction energies (ΔE) of the interacting systems have been calculated using the supermolecule approach [4] as the electronic energy difference between the complex and the isolated molecules (tyrosine, di- and tri-peptide, ibuprofen):

$$\Delta E = E_{complex} - (E_{Tyr(Val-Tyr,Val-Tyr-Val)} + E_{ibuprofen})$$

The entalphy of association was calculated using the following equation from the data listed in the output of Gaussian calculation without scaling their values:

$$\Delta H = H_{complex} - (H_{Tyr(Val-Tyr,Val-Tyr-Val} + H_{ibuprofen})$$

All calculations have been performed in the gas phase with the use of Gaussian 03 program [5].

Results and Discussion

UV-Vis spectroscopy

The absorption spectra of tyrosine, dipeptide, tripeptide and ibuprofen in water are shown in Figure 2. The UV-Vis spectra of dipeptide and tripeptide similarly to the UV-Vis spectrum of tyrosine, consist of two maxima at about 220 and 275 nm. It is not surprising, because in the above mentioned systems tyrosine is the only chromophore. This spectral characteristics is consistent with previously reported data [6].

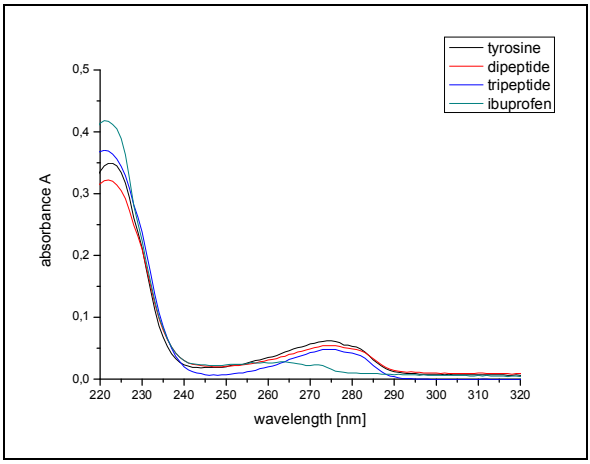

Figure 2. Absorption spectra of tyrosine, dipeptide, tripeptide and ibuprofen ($4*10^{-5}$ M) in water

The determined (from the Beer-Lambert law) molar extinction coefficient of tyrosine, dipeptide and tripeptide in water at 275 nm is equal to $1,4*10^3$ $M^{-1}cm^{-1,}$ $1,4*10^3$ $M^{-1}cm^{-1}$ and $1,3*10^3$ $M^{-1}cm^{-1}$, respectively. This value is very similar to the value of 1480 $M^{-1}cm^{-1}$ obtained by Mach H. and coworkers for tyrosine in water [7].

The absorption spectrum of ibuprofen (Figure 2) is characterized by an intensive peak with maximum absorption wavelength of 222 nm and molar extinction coefficient of $9,9*10^3$ $cm^{-1}M^{-1}$. This value is close to the data of $1,3*10^4$ $cm^{-1}M^{-1}$ (222 nm) reported by Du L. and coworkers for sodium salt of ibuprofen [8]. The longest wavelength region of ibuprofen spectrum is dominated by maximum which consists of two peaks at 264 and 272 nm, respectively. This spectral characteristic of ibuprofen is consistent with previously reported data [2].

In order to study the interactions between ibuprofen and the studied tyrosine derivatives, absorption titration experiment have been performed. No considerable changes in the longest wavelength region (250-300 nm) of absorption spectra of tyrosine, dipeptide and tripeptide have been observed, which indicate low sensitivity of absorption techniques in this study. Indeed, the emission signal is

directly observed, enhanced and registered, while in absorption spectroscopy the difference between the incident and transmitted light intensity is measured. Due to low sensitivity of absorption technique in the study of intermolecular interactions between tyrosine derivatives and ibuprofen, the fluorescence spectroscopy will be used to determine association constant of tyrosine (dipeptide, tripeptide)-ibuprofen complexes.

Fluorescence spectroscopy

The steady-state fluorescence spectrum of tyrosine (excited at 280 nm) in water (not shown) shows the maximum at 303 nm, which is consistent with previously reported data [9]. Fluorescence spectra of dipeptide (Figure 3) and tripeptide (not shown) in water show maximum at 309 nm. This red shift of 6 nm with respect to tyrosine fluorescence maximum may suggest that tyrosine emission is very sensitive to the surrounding medium (the presence of valine residue in the studied di- and tripeptide). Fluorescence intensity of ibuprofen ($5*10^{-6}$ M-$1*10^{-4}$ M) in water (not shown) is very low with the maximum at about 340 nm, which is consistent with previously reported data [2].

Complex formation between Tyr (dipeptide, tripeptide) (represented by M) and ibuprofen (represented by L) proceeds according to:

$$[M] + [L] \leftrightarrows [ML]$$

The association constant (K_a) of the complex can be expressed as:

$$K_a = \frac{[M]}{[M][L]} \qquad \text{eq. 2}$$

[M] and [L] is the concentration of free (non-bonded in complex), tyrosine (dipeptide, tripeptide) and ibuprofen; respectively and can be expressed as:

$$[M] = [M]^0 - [ML]$$
$$[L] = [L]^0 - [ML] \qquad \text{eq. 3}$$

where $[M]^0$ and $[L]^0$ is the analytical concentration of tyrosine (dipeptide, tripeptide) and ibuprofen, respectively; [ML] is the concentration of the complex. Assuming that $[L]^0 \ggg [L]$ ($[L]^0 \to \infty$) $[L] = [L]^0$, and inserting equation 3 to 2 we obtain:

$$[ML] = \frac{K_a.[M]^0[L]^0}{1 + K_a.[L]^0} \qquad \text{eq. 4}$$

It is known that the fluorescence can be expressed as:

$$F = \alpha \,.\, \phi_i \,.\, \varepsilon_i \,.\, c_i \qquad \text{eq. 5}$$

where:
α – apparatus factor
φ_i – fluorescence quantum yield of i
ε_i – molar extinction coefficient of i
c_i – concentration of i
Under the condition that $A \leq 0{,}1$, the fluorescence intensity of the mixture of tyrosine (dipeptide, tripeptide) and ibuprofen (F_{mix}) can be written as:

$$F_{mix} = \alpha \,.\, \phi_M \,.\, \varepsilon_M [M] + \alpha \,.\, \phi_L \,.\, \varepsilon_L [L] + \alpha \,.\, \phi_{ML} \,.\, \varepsilon_{ML} [ML] \qquad \text{eq. 6}$$

Inserting equation 3 to equation 6 and taking into consideration that $\phi_L \cong 0$ we obtain:

$$F_{mix} = \alpha \,.\, \{ \phi_M \,.\, \varepsilon_M [M]^0 + (\phi_{ML} \,.\, \varepsilon_{ML} - \phi_M \,.\, \varepsilon_M)[ML] \} \qquad \text{eq. 7}$$

In the absence of ibuprofen, the fluorescence intensity is equal to:

$$F^0 = \alpha \,.\, \phi_M \,.\, \varepsilon_M [M]^0 \qquad \text{eq. 8}$$

If $[L]^0 \rightarrow \infty$, all tyrosine is incorporated in complexes formation, therefore we can write:

$$F^\infty = \alpha \,.\, \phi_{ML} \,.\, \varepsilon_{ML} [M]^0 \qquad \text{eq. 9}$$

Inserting eq. 8 and eq. 9 to eq. 7 we obtain:

$$F_{mix} = F^0 + [ML] \left(\frac{F^\infty}{[M]^0} - \frac{F^0}{[M]^0} \right) \qquad \text{eq. 10}$$

Inserting eq. 4 to eq. 10 and converting, we finally come to the following formula:

$$\frac{F_{mix} - F^0}{F^\infty - F_{mix}} = K_a [L]^0 \qquad \text{eq. 11}$$

In this way the slope of :

$$\frac{F_{mix} - F^0}{F^\infty - F_{mix}} = f[L]^0$$

enables us to determine K_a of tyrosine–ibuprofen, dipeptide–ibuprofen and tripeptide–ibuprofen complexes.

In order to determine K_a, fluorescence titration experiment (excitation at 280 nm) have been performed in which the concentration of tyrosine, dipeptide and tripeptide were kept constant ($5*10^{-5}$ M), while varying the concentration of ibuprofen. The maximum possible concentration of ibuprofen in the sample of $6*10^{-4}$ M could only be achieved, because above this limit value, the crystals of ibuprofen in aqueous solutions are formed. Upon adding increasing amounts of ibuprofen in ethanol (0,05 M), the decrease in fluorescence intensity of tyrosine, di- and tripeptide was observed. The most significant changes in fluorescence intensity upon increasing concentration of ibuprofen have been observed for the studied tripeptide (Figure 3).

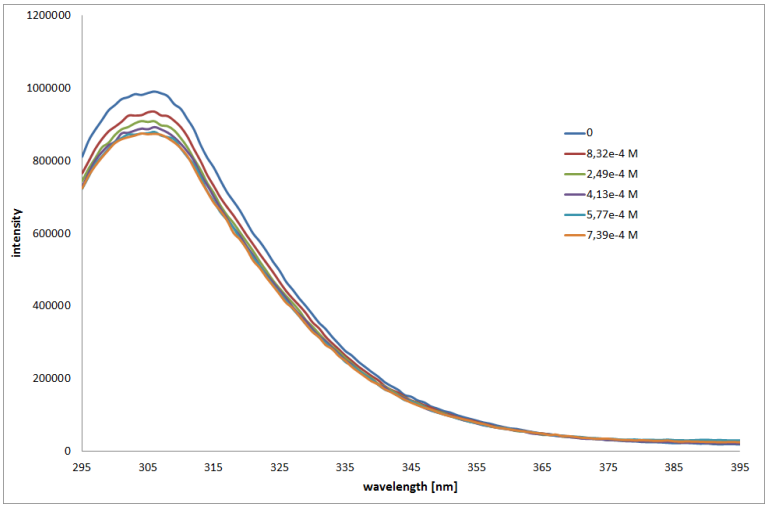

Figure 3. Fluorescence spectra of tripeptide in water ($5*10^{-5}$ M) and its changes upon increasing concentration of ibuprofen

The plots of function $y = (F_{mix}-F_0)/(F_\infty-F_{mix})$ versus ibuprofen concentration for titration of tyrosine (dipeptide, tripeptide) ($5*10^{-5}$ M) have been made. The example of the plot for ibuprofen-tyrosine system is shown in Figure 4. Based on the equation 11, the association constant for Tyr-ibuprofen complexes is determined to be $2,1*10^3$ M^{-1}, whereas for Val-Tyr-ibuprofen and Val-Tyr-Val-ibuprofen is equal to $6,4*10^3$ $M^{-1}$and $1,9*10^4$ M^{-1}, respectively.

The free Gibbs energy of association for the studied complexes can be estimated using equation 12:

$$\Delta G = -RTlnK_a \qquad \text{(eq. 12)}$$

The calculated free Gibbs energy of association is equal to -4,542 kcal/mol, -5,191 kcal/mol and -5,864 kcal/mol for tyrosine-ibuprofen, dipeptide-ibuprofen and tripeptide-ibuprofen complexes, respectively. This value is typical for hydrogen-bonded interactions.

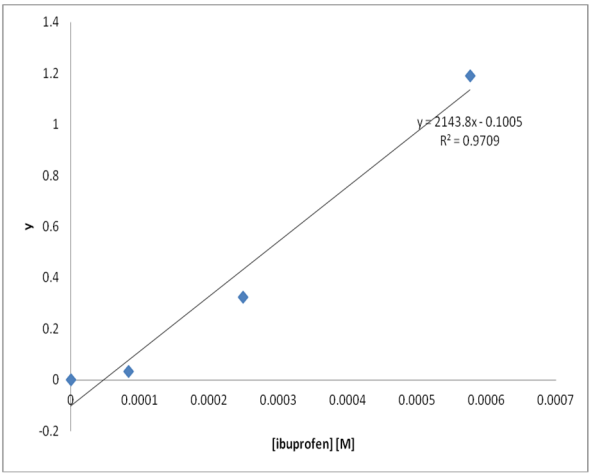

Figure 4. The plot of function $y = (F^{303}_{mix}-F^{303}_{0})/(F^{303}_{\infty}-F^{303}_{mix})$ versus ibuprofen concentration for titration of tyrosine ($5*10^{-5}$ M) in water with ibuprofen

Theoretical results

The PM3 optimized geometries of the studied complexes are shown in Figure 5. The analysis of the calculated vibrational frequencies indicated that all calculated geometries correspond to the minimum on potential energy hypersurface.

Inspection of the geometry of tyrosine-ibuprofen complex (Figure 5a) reveals the presence of two strong hydrogen bonds: the former is formed between hydroxyl group of tyrosine and the carbonyl oxygen atom of ibuprofen, the latter hydrogen bond interaction involves the CH group of tyrosine and carbonyl oxygen atom of ibuprofen. The calculated geometrical parameters of the above mentioned interactions (O…HO = 2,68Å, <OH…O = 168,2°; CH…O = 2,88Å, < CH…O = 169,1°) suggest that the hydrogen bonds are strong. Additionally, closer investigation of the geometry of the studied complex reveals that the proton from CH group of tyrosine has migrated towards the carbonyl oxygen of ibuprofen. This proton migration may lead to the greater stabilization of the studied complex *in vacuo*; so very large, artificial value of interaction energy is expected in the gas phase. Indeed, as can be seen from Table 1, the calculated interaction energy of tyrosine-ibuprofen complex is largely overestimated.

The presence of two strong (on the basis of geometrical criteria of hydrogen bonds) interactions: 1) between hydroxyl group of tyrosine residue and carbonyl oxygen atom of ibuprofen (OH…O = ~2,7Å, < OH…O = ~167°) and 2) between the tyrosine CH group and the other oxygen atom of ibuprofen (CH…O = ~2,9Å, < CH…O = ~172°) can also be noticed in the PM3 optimized geometries of the studied dipeptide-ibuprofen and tripeptide-ibuprofen complexes (Figure 5b and 5c).

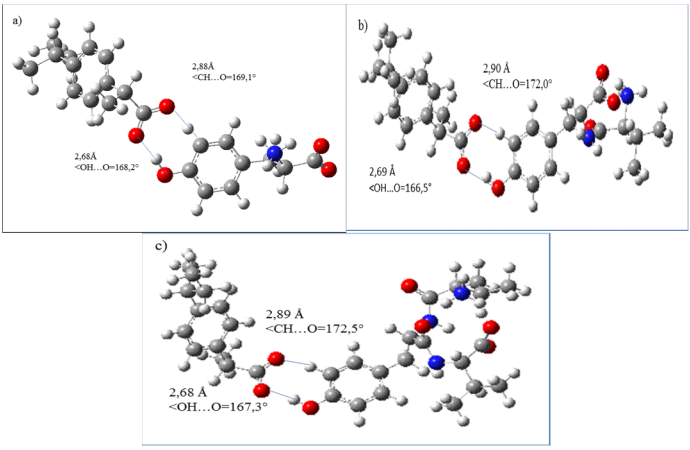

Figure 5. The PM3 optimized structure of a) tyrosine-ibuprofen, b) dipeptide-ibuprofen and c) tripeptide-ibuprofen complexes. Tyrosine, di-and tripeptide are in the zwitterionic form, ibuprofen is in the anionic form

The calculated (applying supermolecular approach) values of interaction energy, enthalpy and free Gibbs energy of association for the studied complexes are gathered in Table 1. The results of calculations indicate that the interactions between tyrosine and ibuprofen are the strongest. However, this increase in interaction energy is the consequence of already mentioned PM3 prediction of proton transfer from tyrosine CH group towards oxygen atom of ibuprofen in tyrosine-ibuprofen complex in the gas phase. It seems that inclusion of solvent effects into the model may prevent from the occurrence of the proton transfer in this complex.

Table 1. The PM3 calculated values of interaction energy (ΔE [kcal/mol]), enthalpy of association (ΔH [kcal/mol]) together with experimental and calculated values of free Gibbs energy of association ΔG [kcal/mol]) for the studied complexes

	ΔE [kcal/mol]	ΔH [kcal/mol]	ΔG [kcal/mol]	ΔG^{exp}[kcal/mol]
tyrosine-ibuprofen	-35,895	-33,865	-23,848	-4,542
dipeptide-ibuprofen	-17,009	-14,873	-3,862	-5,191
tripeptide-ibuprofen	-24,636	-23,015	-12,432	-5,864

The PM3 calculated values of Gibbs free energy of association differ significantly from the experimentally obtained values (Table 1). This discrepancy between theoretical and experimental values may be explained by low level of calculation (semi-empirical), negligence of solvent effects and the use of harmonic approximation in the calculations of low frequency vibrations. It is known, that for the latter vibrations applied harmonic oscillator approximation is expected to give very poor description. The poor performance of the harmonic oscillator approximation results in significant errors in estimation of low frequency vibrations,

which leads to very large errors in calculated entropies, which in turn means large errors in free energy. Therefore, the calculated ΔG of the association is not reliable to predict reasonable stabilities of the system studied [10].

It is well known that the proof for the existence of typical hydrogen bonds in the interacting systems is that vibrations which involve the proton-donating groups show shifts in their frequencies towards lower energy, whereas the intensities of corresponding vibrations are enhanced largely. The magnitude of the above mentioned red shifts and changes of vibrational intensities of proton donating groups depends on the strength of the hydrogen bonded interactions [11]. In order to take insight into the nature of interactions involved in the studied complexes, the PM3 calculated frequencies of the vibrations involving the proton donating groups (tyrosine OH and CH groups) have been analyzing in isolated molecules of tyrosine, dipeptide and tripeptide as well as in the studied complexes. The results of this analysis are gathered in Table 2.

From Table 2 it may be noticed that hydrogen bonding interactions between tyrosine hydroxylic OH group and carbonyl atom of ibuprofen in the studied interacting systems are accompanied by the red shift of the calculated OH stretching vibration frequencies. Moreover, the intensities of the above mentioned OH stretching vibrations are enhanced largely. The decrease of the calculated frequency of CH tyrosine stretching vibration together with the enhancement of the calculated intensity of CH stretching vibration are also noticed in dipeptide-ibuprofen and tripeptide-complexes, but these changes are less evident comparing to the changes in spectroscopic parameters involving OH group.

Table 2. The PM3 calculated spectroscopic parameters (frequency (f) [cm^{-1}], intensity (I)) of NH and CH stretching vibrations in tyrosine, dipeptide, tripeptide and their changes due to the complex formation

	f^{OH} [cm^{-1}]	I^{OH}	Δf^{OH}	$I^{OH}_{complex} / I^{OH}_{monomer}$	f^{CH} [cm^{-1}]	I^{CH}	Δf^{CH}	$I^{CH}_{complex} / I^{CH}_{monomer}$
tyrosine	3888,2	19,300	-	-	3049,7	5,0517	-	-
dipeptide	3889,6	18,522	-	-	3060,1	25,267	-	-
tripeptide	3887,0	23,319	-	-	3069,0	22,588	-	-
tyrosine-ibuprofen	3587,8	2356,6	-300,40	122,1	2831,0	1472,0	43	291,4
dipeptide-ibuprofen	3608,4	2160,8	-281,20	116,7	2854,2	1212,6	-206,0	48,0
tripeptide-ibuprofen	3592,3	2331,3	-294,7	100,0	2845,3	1380,6	-223,7	61,1

This proves the existence of hydrogen bonds in the studied interactions systems and indicates that the interaction between tyrosine hydroxyl group and carbonyl oxygen atom of ibuprofen is stronger in comparison to the interaction involving tyrosine CH group and oxygen atom of ibuprofen.

Additionally, the vibrational analysis reveal that the interaction involving CH group of tyrosine and ibuprofen oxygen atom in the tyrosine-ibuprofen complex *in vacuo* is not a typical hydrogen bond (as the result of complex formation blue shift instead of red shift of tyrosine CH stretching frequency has been predicted by the PM3 method).

In conclusions, the interactions between ibuprofen and tyrosine as well selected tyrosine-containing oligopeptides (Val-Tyr, Val-Tyr-Val) have been studied using both experimental (absorption and fluorescence spectroscopy) and theoretical (the PM3 method) techniques. The experimentally obtained values of association constant together with free Gibbs energy of association for the studied tyrosine-ibuprofen and Val-Tyr-; Val-Tyr-Val-ibuprofen complexes have been determined as the results of fluorescence titration experiment of the studied tyrosine derivatives with ibuprofen. The obtained values suggest that, assuming that the complexes of 1:1 stoichiometry are only formed, the intermolecular interactions involved in ibuprofen/tripeptide and ibuprofen/dipeptide are stronger in comparison to the interactions between tyrosine and ibuprofen.

Theoretical calculations failed to provide reasonable values of free Gibbs energy of association. The reason for this is the poor performance of applied harmonic oscillator approximation in the calculations of low-frequency vibrations. However, the results of PM3 calculations revealed the nature of interactions involved in ibuprofen/Tyr (Val-Tyr, Val-Tyr-Val) systems. The above mentioned interactions are mainly based on the formation of strong hydrogen bond between the tyrosine hydroxyl group and carbonyl oxygen atom of ibuprofen. Additionally, the presence of the interaction incorporating tyrosine ring CH-group, which is close to tyrosine hydroxyl group, enhanced the stability of the studied interacting systems. The results obtained in this study may lead to the conclusion that the mechanism of action of ibuprofen is most probably based on the hydrogen bonding interaction which involves hydroxyl group of tyrosine.

Acknowledgements

The authors would like to thank Prof. S. Wysocki, head of the Institute of General Food Chemistry, who funded the project and provided the equipment. We acknowledge IAESTE for the opportunity to gain invaluable work experience.

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