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MOLECULAR MODELING STUDY OF TERNARY COMPLEXES OF HYDROXYPROPYL-β-CYCLODEXTRIN WITH NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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ABSTRACT. In this study, ternary complexes of four nonsteroidal anti-inflammatory drugs (ibuprofen, ketoprofen, flufenamic acid and mefenamic acid) with hydroxypropyl-β-cyclodextrin were studied using a molecular modeling technique. As third component in the complexes, different types of small molecules were chosen – regarding the acid-base character, molecular volume, etc. The binding energy, surface area contraction and volume contraction of these binary and ternary complexes were calculated. The results show that, by adding auxiliary substance to the active ingredient-cyclodextrin binary system, more stable complexes are formed. The ternary complexes are stabilised by hydrogen bonds and van der Waals interactions.

Keywords: *cyclodextrin, ternary complex, molecular modeling, NSAID*

INTRODUCTION

Cyclodextrins (CD) are cyclic oligosaccharides, consisting of 6-8 glucopyranose units (α-, β-, and γ-CD). They are amphiphilic molecules, shaped like a truncated cone, with a hydrophilic exterior and a hydrophobic interior surface. Cyclodextrin complexes are often used in the pharmaceutical industry to optimize biopharmaceutical properties, especially to increase aqueous solubility of poorly water-soluble drugs [1,2].

The most common are the binary, so-called 'host-guest complexes', when the drug molecule is inserted into the cyclodextrin cavity. Guest molecules are usually drugs with low solubility and molecular size that allow integral or partial fitting in the cavity of cyclodextrin. These complexes have better solubility than the original drug molecule [1,3,4].

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By adding a third auxiliary substance, a ternary complex is formed with a higher complexation efficiency associated with a better water solubility compared to the binary complex. The most commonly used third molecules are polymers (polyvinylpyrrolidone, hydroxypropyl methylcellulose, polyethylene glycols) and small molecules (amino acids, lecithin, carboxylic acids, ethanol, Fe ions, Mg ions, etc) [5–8]. The aim of this study was to investigate the geometry and structure of some ternary complexes in order to assess the variations in the complexation efficiency in the presence of a third component using molecular modeling technique. Four non-steroidal anti-inflammatory drugs (NSAIDs) were chosen as model guest molecules, because their binary cyclodextrin complexes are widely studied due to their low water solubility and gastro-intestinal adverse effects.

The examples reported in the scientific literature show that association of an organic acid or base as third component to the cyclodextrin-guest molecule complex has positive influence on complexation energy, regardless of the acid-base character of the guest molecule. Therefore, seven auxiliary substances were used as third component: three low molecular weight organic acids (citric acid, CA; malic acid, MA and fumaric acid, FA), two amino acids (glutamic acid, Glu and thyrosine, Tyr) and two organic amines (diethanolamine, DEA and triethanolamine, TEA). As host molecule, a modified cyclodextrin (hydroxypropyl-β-cyclodextrin, HP-β-CD (DS = 3)) was used.

RESULTS AND DISCUSSION

In this study, the energy difference between the minimum energy before and after complexation (named binding energy, BE), and some QSAR properties (logP, volume and the van der Waals or solvent-accessible surface area) of the binary and ternary complexes of the HP-β-CD were investigated. The most stable complexes indicated by the highest negative values of the BE and the energy with the highest contribution to the BE (the van de Waals energies, E_{vdW}), are shown in the Table 1.

As can be seen, by adding a third component to the binary system, the binding energy increases in all cases (the lowest BE value corresponds to the most stable complex), meaning that the auxiliary substances stabilise the guest-host interaction. By analysing the partial energies of the BE (bond energy, angle energy, dihedral energy, stretch-bond energy and the energy from van der Waals interactions), it was observed that the van der Waals forces have the largest contribution. The positive value of the dihedral energy (resulting in a higher value of de E_{vdW} compared to the BE value) in the case of the ternary complexes show a dihedral “freezing” when complex is formed. Beside van der Waals forces, in some cases, hydrogen bonds also play a part in stabilizing the complexes (Table 2).

Table 1. The binding energy of the binary and ternary complexes

Binding Energy (kJ/mol)

IBU	**binary**	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
BE	-113.5	-163.7	-156.3	-148.1	-148.1	***-194.5***	-141.3	-153.8
E_{vdW}	-117.6	-162.6	-152.5	-147.7	-154.8	-193.3	-144.7	-141.1
KETO	**binary**	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
BE	-129.0	-180.4	-179.4	-166.4	-173.2	***-190.7***	-158.8	-158.7
E_{vdW}	-117.9	-163.4	-174.8	-177.8	-182.0	-178.9	-176.2	-154.2
FLU	**binary**	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
BE	-122.4	-169.4	-157.4	-156.9	-164.1	***-183.7***	-154.2	-159.1
E_{vdW}	-111.5	-183.5	-174.7	-164.1	-147.3	-190.6	-159.3	-159.1
MEF	**binary**	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
BE	-114.7	-160.7	-149.7	-152.6	-159.8	***-192.8***	-155.3	-154.2
E_{vdW}	-110.0	-150.8	-145.4	-138.7	-160.7	-185.5	-142.3	-148.3

Abbreviations: IBU - ibuprofen, KETO - ketoprofen, FLU - flufenamic acid, MEF - mefenamic acid, CA - citric acid, MA - malic acid, FA - fumaric acid, GLU - glutamic acid, Tyr - thyrosine, DEA - diethanolamine, TEA - triethanolamine

Table 2. The presence of hydrogen bonds in the complexes

	binary	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
IBU	+	+	-	+	-	+	-	+
KETO	-	-	+	+	+	-	-	-
FLU	-	+	+	-	-	-	+	**+**
MEF	-	+	-	+	-	-	+	-

A statistically significant difference between the sum of the minimum energies of the components and the energies of the optimized system exists in all cases ($p < 0.05$; Kruskal-Wallis one-way test). In the case of the binary systems, the KETO forms the most stable complex with HP-β-CD. The Tyr-containing complexes are the most thermodynamically stable in all four cases, followed by the CA-containing complexes, which can be explained by the fact that, due to their lesser volume they fit better between the CD and the active ingredient.

The formation of inclusion complexes can manifest by the contraction of the surface area (BSA, binding surface area) and, to a lesser extent, the volume (BV, binding volume) of the system. The highest BSAs and highest BVs are presented in Table 3 and Table 4, respectively.

Table 3. The binding surface area (Å^2) of the binary and the ternary complexes

	binary	ternary						
		CA	MA	FA	GLU	TYR	DEA	TEA
IBU	-476.0	-757.0	-729.2	-715.2	-745.9	***-778.7***	-744.4	-762.2
KETO	-546.3	-846.5	-784.5	-789.7	-780.7	***-893.6***	-796.5	-804.8
FLU	-478.2	-788.5	-761.2	-769.6	-809.9	-808.9	-763.1	***-827.4***
MEF	-484.9	-772.0	-742.3	-726.7	-764.1	***-803.3***	-757.1	-786.6

Table 4. The binding volume (Å^3) of the binary and the ternary complexes

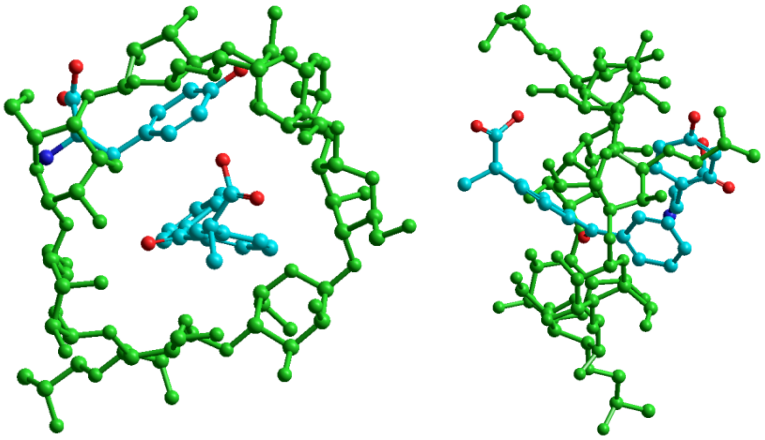
	binary	ternary						
		CA	MA	FA	GLU	TYR	DEA	TEA
IBU	-458.3	***-701.2***	-669.9	-676.6	-676.3	-700.3	-674.7	-688.3
KETO	-513.3	-746.0	-730.9	-724.4	-703.6	***-803.2***	-767.8	-732.0
FLU	-445.8	-709.9	-688.8	-712.4	-729.4	-751.8	-722.5	***-758.9***
MEF	-347.9	***-707.4***	-574.8	-539.3	-658.4	-543.7	-587.3	-640.8

The surface area decreased by 32.14 ± 0.61%, 33.86 ± 0.39%, 33.43 ± 0.29% and 32.87 ± 0.72% in the case of ternary complexes of IBU, KETO, FLU and MEF, respectively. In the case of the binary complexes, the average surface area decreased by 24 ± 0.93%, the difference being statistically significant ($p<0.05$, Wilcoxon Signed-Rank test). The decrease of the solvent-accessible surface area in the case of the ternary systems can be explained by the formation of the re-entrant surfaces.

The volume of the ternary complexes of IBU, KETO, FLU and MEF decreased by 14.56 ± 0.29%, 15.46 ± 0.14%, 15.35 ± 0.13% and 15.10 ± 0.31%, respectively. In the case of the binary complexes the average surface area decreased by 10.95 ± 0.52%. The difference between the BV of the binary complexes and BV of the ternary complexes is statistically significant ($p<0.05$, Wilcoxon Signed-Rank test). These surface area and volume contractions were evaluated by comparing the sum of the individual values to the geometrically optimized complexes.

Each ternary complex has a distinct molecular geometry; the difference is attributed to the extent to which the studied NSAIDs and the auxiliary substances are inserted into the CD cavity.

The topology of the binary complexes was similar to that observed in our previous work: all active ingredients enter the cavity of CD [9]. Regarding the ternary complexes, in the case of the most stable KETO-complex, the whole molecule enters in the cyclodextrin's cavity and the auxiliary component is situated between the secondary hydroxyl groups of the HP-β-CD and the benzoyl group of the KETO (Figure 1a and 1b, hydrogen depleted structure).



a *b*

Figure 1. The 3D structure of the HP-β-CD:KETO:Tyr complex (*a* top view; *b* side view)

In the case of the most stable HP-β-CD:IBU:Tyr complex, the active ingredient is fully included inside the CD's cavity and the Tyr is situated close to the isobutyl moiety of the IBU. The IBU complexes, those with MA, FA and GLU content, have similar molecular geometry to the Tyr containing complex. In the case of those complexes in which the third component is the MA, DEA and TEA, both the active ingredient and the third component are situated bound to one of the edges of the CD.

Regarding the conformation of the ternary complexes of the FLU, the active ingredient is totally inserted into the CD's cavity in the case of the CA-, MA-, Tyr-, DEA-, TEA-complexes; the third component is situated close to the trifluoromethyl moiety of the FLU, oriented to the secondary hydroxyls of the CD.

In the case of the HP-β-CD:MEF:MA/Glu/TEA complexes, both substances are located on the edges of the CD. In the other cases the MEF is included in the cavity of the CD and the auxiliary substance is located on the wider edge of the CD.

In the cases of MEF and FLU, the FA containing complexes have a different topology, compared to the others; the auxiliary substance is included in the cavity of the CD, and the active ingredient is situated at the edge of secondary hydroxyl groups.

Regarding the correlation between the average values of BSA and BV parameters, the positive association between the two variables is statistically significant ($p<0.01$, Spearman correlation, two-tailed) in all four cases. Between the BE and BSA a statistically significant, positive correlation exists in the case of the KETO and MEF ($p<0.05$, Spearman correlation, two-tailed).

The molecular dynamics simulation results show that the entropy (TS) of the ternary complexes is slightly increased (7.3-14%) compared to the binary complexes in all four cases; similarly to the calculated Helmholtz free energy.

The change in free energy (ΔF) and the entropy change (TΔS) of the complexes are presented in Table 5.

Table 5. Changes in the free energy (ΔF, kJ/mol) and entropy (TΔS, kJ/mol) of the complexes

IBU	**binary**	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
ΔF	-85.4	-88.0	-59.6	-89.3	-88.7	***-139.7***	-40.8	-73.8
TΔS	-69.7	-106.1	-106.2	-92.8	-132.9	-92.1	-116.9	-99.3
KETO	**binary**	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
ΔF	-92.4	-129.9	-88.0	-122.6	-139.6	-131.7	-61.9	***-155.3***
TΔS	-44.7	-101.0	-126.4	-111.0	-125.7	-141.9	-98.4	-110.9
FLU	**binary**	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
ΔF	-56.0	-117.9	-112.1	***-143.4***	-143.4	-104.5	-103.6	-106.3
TΔS	-101.0	-138.9	-150.4	-130.0	-144.0	-146.8	-229.1	153.9
MEF	**binary**	**ternary**						
		CA	**MA**	**FA**	**GLU**	**TYR**	**DEA**	**TEA**
ΔF	-73.0	-71.2	-170.7	-114.1	-85.3	***-132.2***	-118.6	-80.8
TΔS	-88.2	-158.4	-192.4	-104.4	-126.7	-138.2	-127.5	-117.3

CONCLUSIONS

Our results show that the ternary complexes of the studied active ingredients have higher binding energy compared to the binary complexes: therefore, by adding a third component to the system, higher stability constants can be achieved. The surface area and volume contraction, observed in the case of the ternary complexes, suggest that the auxiliary substances significantly increase the active ingredient affinity for HP-β-CD, compared to the binary complex. The mechanism of which the third components help the complexation of the active ingredient is complex: one can assume that they favour the formation of the van der Waals bonds between the CD and the guest molecule.

In the case of IBU, FLU and MEF exists a positive correlation ($p<0.05$, Spearman correlation, two-tailed) between the molecular mass of the third component and the binding energy. Other parameters (pKa, logP, number of H donating and accepting groups) do not show correlation with the binding energies.

In this "in vacuum" model, the volume and surface contraction of the complexes suggest an entropy decrease, supported by the molecular dynamics simulation results. The results show that adding a second guest molecule to the binary complex the process is accompanied by a large loss of entropy. The negative value of the ΔF demonstrates the spontaneity of the complexation process, which could be explained by the enthalpy-entropy compensation; the complexation process is a mainly enthalpy driven mechanism: therefore, the van der Waals forces have a major role in stabilising the complex. This interpretation agrees with the scientific literature data [10].

In the case of the ternary complexes, additional mechanisms help the complexation process (e.g. salt formation, pH adjustment, etc.) In aqueous medium the role of the water molecules is important; the release of the water from the cyclodextrin cavity can result in a positive entropy change, favouring the reduction of the free energy.

Zhang et al. got similar result: -85.91 kJ/mol as the binding energy of the binary complexes of the doxycycline with HP-β-CD [11]. In a research made by Huang et al. [12], the interval of the binding energy of the binary complexes of the βCD with salsolinol, N-methyl-salsolinol and 1-benzyl-tetrahydroisoquinoline is between -57.64 and -108.72 kJ/mol, which is similar to our result. Tan et al. also measured a similar value: -86.14 kJ/mol as the binding energy of the binary complex of the β-CD with rifaldazine [13]. The findings of Mendez et al. are close to our results, they show that amino acids can enhance the affinity of the benzoic acid for β-CD [14].

In 2014 Barbosa et al. made a research in which the binary and the ternary complexes of the βCD were examined, they got -81.62 kJ/mol as the binding energy of the binary complex and -135.75 kJ/mol as the binding energy of the ternary complex with TEA; results which are close to our findings [15].

EXPERIMENTAL SECTION

Energy minimisation

In this study the formation energy, the volume and the surface area of the binary (1:1 molar ratio) and ternary (1:1:1 molar ratio) inclusion complexes of HP-β-CD with four NSAIDs (IBU, KETO, FLU, MEF) and seven auxiliary

substances (CA, MA, FA, Glu, Tyr, DEA, TEA) were measured. In the first step, the structures of the NSAIDs, auxiliary substances and the HP-β-CD were geometrically optimised using the molecular mechanics method with MM+ force in HyperChem Professional software, version 8.0 [16]. The NSAIDs were manually inserted in the HP-β-CD's cavity, and then the auxiliary substances were placed between the active ingredient and the edge of the cyclodextrin. The adduct structures were optimized using the Polak-Ribiere algorithm until 0,01 RMS gradient was achieved. Optimization of the complexes was repeated forty times, starting with forty different layouts (n=40). The calculations were performed in vacuum phase.

The binding energy (BE) of the binary and the ternary complexes was calculated by the following formula:

$$BE = E_{binary} - (E_{CD} + E_{NSAID})$$
$$BE = E_{ternary} - (E_{CD} + E_{NSAID} + E_{III})$$

E_{binary}, $E_{ternary}$ represent the average minimum energy of the binary complexes and ternary complexes; E_{CD}, E_{NSAID} and E_{III} represent the minimum energy of HP-β-CD, NSAIDs and the third auxiliary component, respectively. Negative formation energy shows a thermodynamically favoured complex.

The binding surface area (BSA) and the binding volume (BV) of the binary and the ternary complexes were calculated using the formulas:

$$BSA = SA_{binary} - (SA_{CD} + SA_{NSAID})$$
$$BSA = SA_{ternary} - (SA_{CD} + SA_{NSAID} + SA_{III})$$

SA_{binary}, $SA_{ternary}$, SA_{CD}, SA_{NSAID} and SA_{III} represent the surface area of the binary complex, ternary complex, HP-β-CD, NSAIDs and the third component, respectively.

$$BV = V_{binary} - (V_{CD} + V_{NSAID})$$
$$BV = V_{ternary} - (V_{CD} + V_{NSAID} + V_{III})$$

V_{binary}, $V_{ternary}$, V_{CD}, V_{NSAID} and V_{III} represent the volume of the binary complex, ternary complex, cyclodextrin, NSAID and the third component, respectively.

In all cases forty conformations were tested; the results were statistically analysed using Kruskal-Wallis one-way test, Wilcoxon Signed-Rank tests and Spearman rank correlation test.

Molecular dynamics

Molecular dynamics simulations of the most stable complexes were performed at constant temperature of 298.15 K for 10 ps, with a time step of 0.001 ps, using the HyperChem Professional software, version 8.0 [16].

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