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# Rutin-saturated fatty acid bioconjugate/cyclodextrin supramolecular systems: molecular modeling and docking studies

**Iulia Andreea Pînzaru[1], Daniel I. Hădărugă[1*], Nicoleta G. Hădărugă[2], B. Rădoi[2] Francisc Peter[1]**

[1] *"Politehnica" University of Timişoara, Faculty of Industrial Chemistry and Environmental Engineering, Applied Chemistry and Organic-Natural Compounds Engineering, 300006-Timişoara, P-ţa Victoriei 2, Romania*
[2] *Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology, Food Quality Department, 300645-Timişoara, C. Aradului 119, Romania*

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***Abstract***

The paper presents a theoretical study on the possibility to obtain stable rutin-fatty acid (palmitic, stearic) bioconjugate/cyclodextrin supramolecular systems by means of molecular modeling and docking experiments. Bioconjugates and cyclodextrins (α-, β-, and γ-cyclodextrin) were builded and conformationally analyzed by using HyperChem 5.1 package (molecular mechanics, MM+). The bioconjugate and cyclodextrin molecules, in minimal energy conformations, were oriented along the symmetry axis with fatty acid moiety to the primary or secondary face of cyclodextrin, at a distance of ~8Å between the gravity centres of the molecules; docking experiments were performed by using the same molecular mechanics program, and the most stable bioconjugate/cyclodextrin assemblies established from the interaction energy. The best results were obtained with the fatty acid moiety oriented to the secondary OH-groups face of cyclodextrins, with interaction energies between 18-35 kcal/mole, higher in the case of γ-cyclodextrin.

***Keywords***: rutin-fatty acid bioconjugates, cyclodextrins, molecular encapsulation, molecular modeling, docking

## 1. Introduction

Flavonoids are widely distributed in raw products as well as in many foods; they are biosynthesized from phenylalanine and are the main functional components of many herbal and insect preparations for medical use (anticancer, antioxidant, antimicrobial, and anti-inflammatory activities) [1-4], *e.g.* propolis and honey which have been used since ancient times [5,6]. Among these, rutin (known as rutoside) is a flavonoside found in different part plants such as leaves and seeds (buckwheat), fruits (orange, grapefruit, lemon, lime), and berries (cranberries) [2,7].

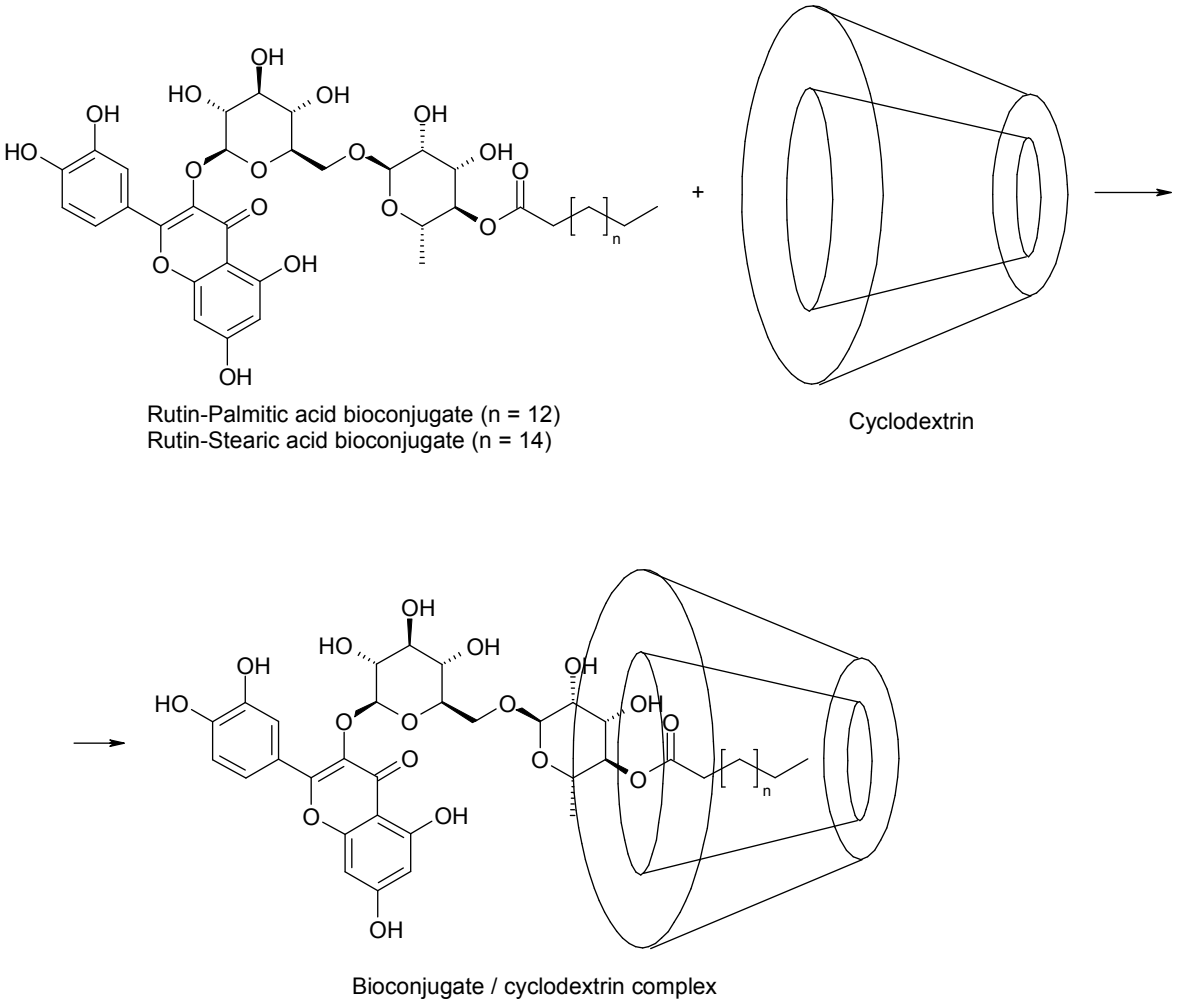
Rutin has significant scavenging properties on oxidizing species (OH radical, superoxide and peroxyl radicals), which conduct to pharmacological activities such as antiallergic, anti-inflammatory, antitumor, antibacterial, antiviral, and anti-protozoal properties [7].

Some recent studies were performed in order to obtain flavonoid bioconjugates. Thus, flavonoids-fatty acids bioconjugates were obtained by biosynthesis and have antioxidant, antimicrobial, and antiviral activities [8-13] or antitumoral properties [14,15].

Corresponding author: *e-mail:* daniel.hadaruga@chim.upt.ro

In order to enhance the bioactive properties of these compounds, nanoencapsulation methods could be used. Naturally occurring cyclodextrins (α-, β-, and γ-cyclodextrin), which are cyclic oligosaccharides with 6, 7, and 8 glucopyranose moieties, are proper to use for nanoencapsulation of these bioconjugates in order to obtain powdery formulations with higher water solubility, protecting capacity (against air, light, humidity), and controlled release properties [16-18].

In this study the possibility of molecular encapsulation of rutin-fatty acid (palmitic and stearic acid) bioconjugates in natural cyclodextrins by means of molecular modeling and docking experiments was evaluated (Scheme 1).




**Scheme 1.** Schematic rutin fatty acid bioconjugate/cyclodextrin encapsulation process

## 2. Materials and methods

*Molecular modeling.* Molecular modeling of rutin-fatty acid bioconjugate molecules as well as α-, β-, and γ-cyclodextrins was performed by using the molecular mechanics MM+ program from the HyperChem 5.1; a RMS of 0.005 kcal/mole and a Polak-Ribiere algorithm were used in the molecular modeling process.

*Conformational analysis.* In order to find the most stable conformation even for bioconjugates or cyclodextrins, a conformational analysis by using *Conformational Search* program (HyperChem 5.1) was performed. In the case of bioconjugates, the great numbers of flexible bonds were due to the fatty acid and disaccharide moieties; the flexible rings were pyranone ring from aglicon moiety and the two pyranose rings from disaccharide moiety. On the other hand, the flexible bonds in cyclodextrins were only those corresponding to the hydroxymethyl from $C^5$ position of glucopyranose unit; the flexible rings were all glucopyranose rings and the corresponding macrocyclic ring. The following conditions were set up for conformational search: variation of the flexible torsion angles ±60º ÷ ±180º, energy criterion for acceptance of the conformation 4 kcal/mole above minimum, all conformations with atomic distances lower than 0.5 Å and differences between torsion angles lower than 15º were not considered as well as conformations with energy differences lower than 0.05 kcal/mole (duplicates); the maximum number of optimization and iterative calculations was 1000 and maximum 100 conformations were retained. The hydrogen atoms were neglected.

*Docking of bioconjugates in cyclodextrins.* The docking of the more stable conformations of studied bioconjugates in α-, β-, and γ-cyclodextrin was realized by using the molecular mechanics interactions of the host-guest molecules in vacuum. The bioconjugate and cyclodextrin structures in minimal energy conformations were set up at distances of ~8Å between the gravity centres of the host-guest molecules, and the bionjugate structure was oriented with fatty acid moiety in front of the primary (A) or secondary (B) face of cyclodextrin (the principal axis corresponding to the biocompound was perpendicular to the A or B plan of cyclodextrin).

The complex was modeled in absence of water molecules by using the same MM+ program and the interaction was stopped when the RMS gradient was lower than 0.005 kcal/mole. The biocompound-cyclodextrin interaction energy was evaluated as the difference between the the overall energies of these two molecules and the complex energy.

## 3. Results and Discussion

Molecular modeling and conformational analysis of rutin-fatty acid bioconjugates indicate that the fatty acid flexible moieties are in a spiral-like conformation, which interact with benzopyranone moiety by hydrophobic bonds. The number of conformations with energies lower than 4 kcal/mole than the energy of the most stable conformation was 42 for rutin-palmitic acid bioconjugate (Figure 1) and 30 for rutin-stearic acid bioconjugate. All conformations retained were in spiral-like form, but for docking experiments only the most stable conformation from each case was used.

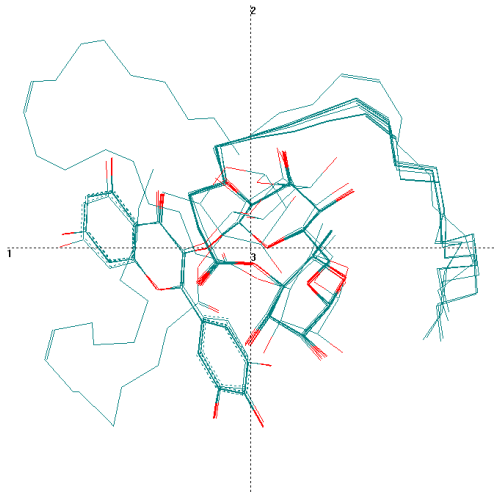


**Figure 1.** Superimposed most stable conformations (up to 0.5 kcal/mole above best) for rutin-palmitic acid bioconjugate

The cyclodextrin conformations were very close in the range up to 0.5 kcal/mole above best, almost all hydroxymethyl moieties being oriented close to the main axis of molecules (Figure 2). The structures are stabilized by the hydrogen bonds formed between these groups. The mobility of cyclodextrin structure is observed only in the γ-cyclodextrin (right of the Figure 2).

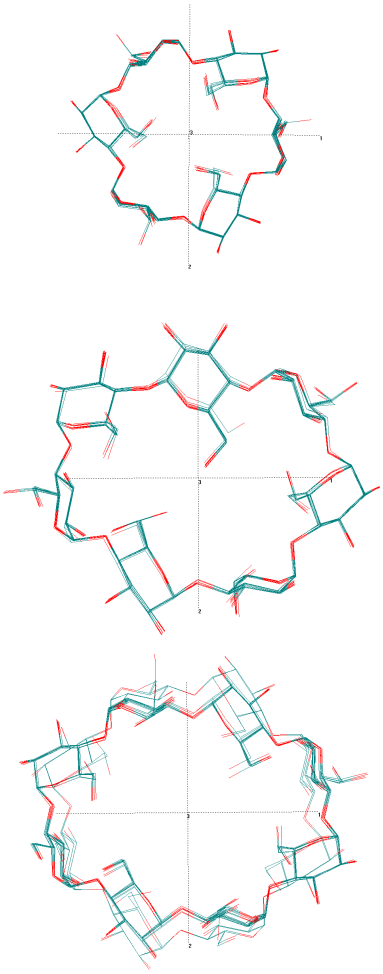


**Figure 2.** Superimposed most stable conformations (up to 0.5 kcal/mole above best) for α-cyclodextrin (left), β-cyclodextrin (middle), and γ-cyclodextrin (right)

In order to evaluate the bioconjugates encapsulation capacity of cyclodextrins the molecular mechanics MM+ program was used. The bioconjugate structure (in minimum energy conformation) was oriented even to the A or B face of cyclodextrin (also in the minimum energy conformation) with both structures on the symmetry axis of cyclodextrin, at a start distance of ~8 Å (Figure 3).

The best interaction was obtained when the fatty acid hydrophobic moiety of bioconjugate was oriented to the secondary face (B face). In the case of α-cyclodextrin the molecular encapsulation of hydrophobic moiety is not complete, the interaction energy being 19.2 kcal/mole for rutin-palmitic acid bioconjugate and little bit lower (16.5 kcal/mole) in the case of rutin-stearic acid bioconjugate (Table 1). The interaction of rutin-fatty acid bioconjugates with β- and γ-cyclodextrin was more efficient because the fatty acid chain is almost completely encapsulated in the cyclodextrin cavity, especially in the case of γ-cyclodextrin. Interaction energies are relatively close in the case of rutin-palmitic acid bioconjugate/β- and γ-cyclodextrin complex (28 kcal/mole and 32.3 kcal/mole, respectively; Figures 4 and 5, Table 1). In the case of rutin-stearic acid bioconjugate/β- and γ-cyclodextrin complexes the interaction energies were 18 kcal/mole and 35.3 kcal/mole (Figure 6, Table 1).

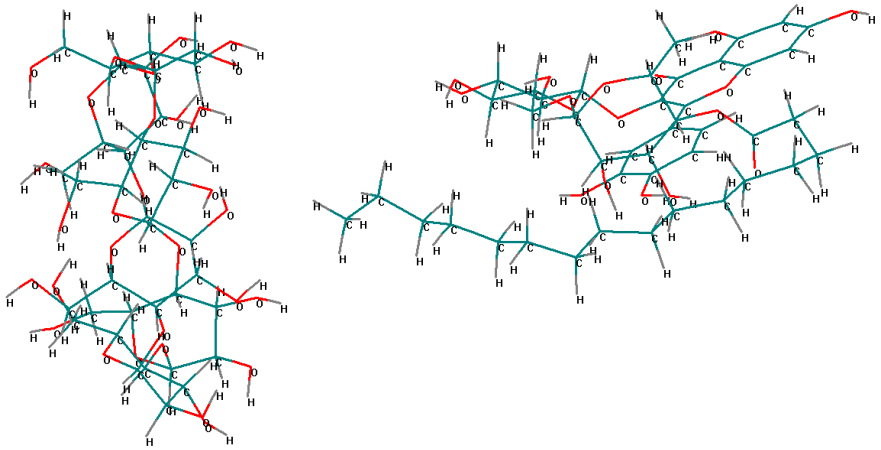


**Figure 3.** Starting position for modeling the interaction between rutin-palmitic acid bioconjugate and α-cyclodextrin

**Table 1.** Interaction energies of rutin-saturated fatty acid bioconjugates / cyclodextrins, obtained by docking experiments

| No | Code | E (CD) (kcal/mole) | E (bioconj.) (kcal/mole) | E (CD+Bioconj.) (kcal/mole) | E (complex) (kcal/mole) | E (interact.) (kcal/mole) |
|---|---|---|---|---|---|---|
| 1 | R_P_aCD | 69.35 | 28.58 | 97.93 | 78.72 | 19.21 |
| 2 | R_P_bCD | 79.80 | 28.58 | 108.39 | 80.40 | 27.99 |
| 3 | R_P_gCD | 91.29 | 28.58 | 119.87 | 87.60 | 32.27 |
| 4 | R_S_aCD | 69.35 | 30.22 | 99.57 | 83.07 | 16.50 |
| 5 | R_S_bCD | 79.80 | 30.22 | 110.03 | 92.11 | 17.92 |
| 6 | R_S_gCD | 91.29 | 30.22 | 121.51 | 86.19 | 35.32 |

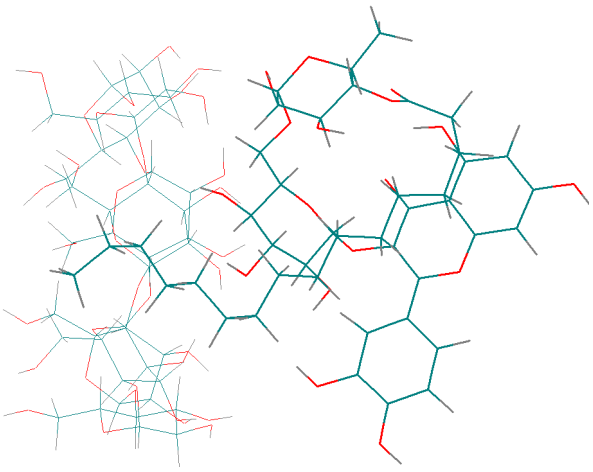


**Figure 4.** Optimized rutin-palmitic acid bioconjugate/β-cyclodextrin complex, obtained by docking experiments

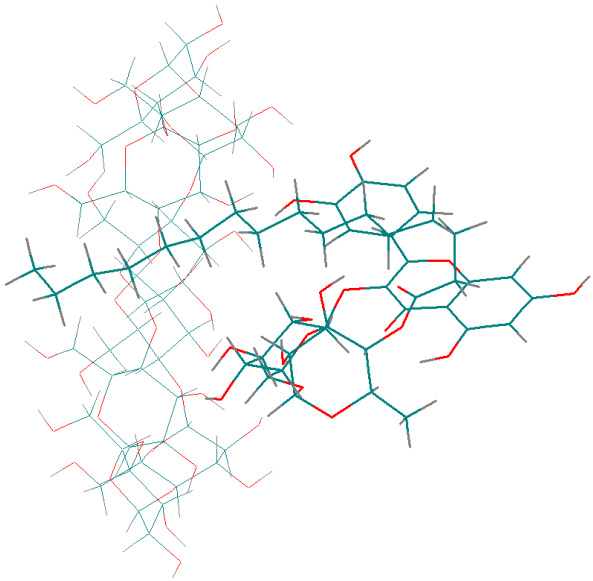


**Figure 5.** Optimized rutin-palmitic acid bioconjugate/γ-cyclodextrin complex, obtained by docking experiments

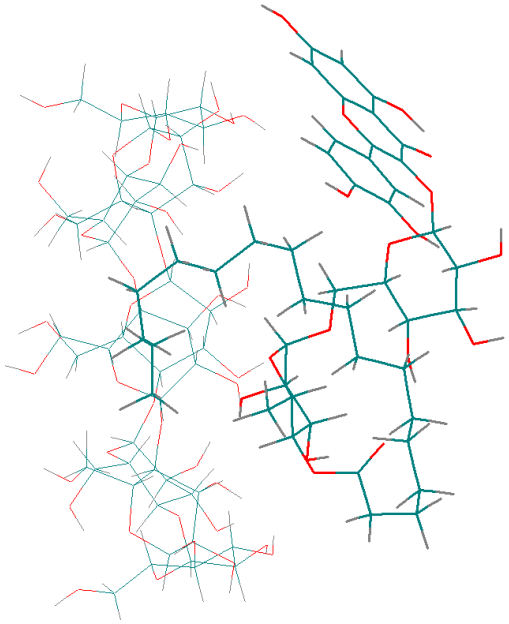


**Figure 6.** Optimized rutin-stearic acid bioconjugate/γ-cyclodextrin complex, obtained by docking experiments

The evolution of interaction energy between bioconjugates and cyclodextrins can be observed in the Figure 7. After the "accommodation" period of the pair molecules (up to 400 calculation cycles), the molecular encapsulation process are relatively rapid (up to 1000-1200 calculation cycles), followed by a "finishing" process which conduct to the final complex. The molecular encapsulation of biomolecule in α-cyclodextrin take a long time (great number of calculation cycles) and is relatively slower, compared with those corresponding to β- and γ-cyclodextrin, where the equilibrium is reached in a short time (Figure 7).

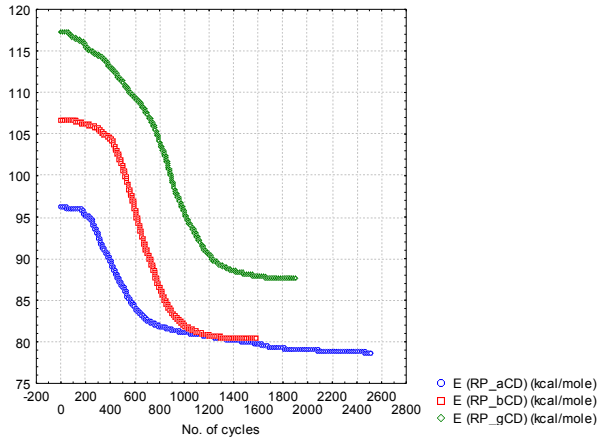



**Figura 7.** Interaction energy variation (kcal/mole) for rutin-palmitic acid bioconjugate/α-, β-, and γ-cyclodextrin supramolecular systems in the molecular encapsulation process (form docking experiments)

## 4. Conclusion

The following conclusions can be drawn among the molecular modeling and docking experiments on molecular encapsulation of rutin-saturated fatty acid bioconjugates in α-, β-, and γ-cyclodextrin: (1) due to the hydrophilic character of rutin, it cannot be proper encapsulated in natural cyclodextrins; the "hydrophobization" of rutin with long fatty acid chains could furnish bioconjugates with enhanced molecular encapsulation properties; (2) the proposed rutin-saturated fatty acid bioconjugates conduct to a more efficient encapsulation possibility, even the stable conformations have relatively "hindered" hydrophobic moieties; (3) bioconjugate-cyclodextrin complexes are more stable in the case of β- and γ-cyclodextrin, compared with the α-cyclodextrin case, and these complexes could be practically obtained as biologically active formulations with enhanced bioavailability.

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