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EMPIRICAL MODELLING OF NONHEMINIC IRON-TANNINS COMPLEXES BY SPASIBA FORCES FIELD : CASE OF $Fe^{3+/2+}$-PROCYANIDIN B2 COMPLEXES.

Kicho Denis YAPO [1,*] **, Philippe LAGANT** [2] **et Gerard VERGOTEN** [2]

[1] *Laboratoire de Chimie Organique Structurale (LCOS) UFR-SSMT,*
Université de Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2] *UMR 8576 CNRS « Glycobiologie Structurale et Fonctionnelle »*
Université des Sciences et Technologies de Lille, Bât. C9
59655 Villeneuve D'Ascq France.

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* Correspondance et tirés à part, e-mail : *denis.yapo@univ-cocody.ci*

ABSTRACT

SPASIBA empirical forces field allowed theoretical study of mechanisms of complexes formation between nonheminic iron (Fe^{3+}, Fe^{2+}) and Procyanidin B2 (molecule of condensed tannin or proanthocyanidines) into organism.
With this intention, models of complexes Procyanidine B2-$Fe^{3+/2+}$ and Procyanidine B2-$Fe^{3+/2+}$-Procyanidine B2, were built.
Energy and conformational characteristics resulting from Molecular Dynamic of these models gave us results more or less functions of nonheminic iron engaged in complexes. Thus, for ferric complexes, Mon2-Fe^{3+}, Mon1-Fe^{3+}-Mon2 and Mon2-Fe^{3+}-Mon2 models could be possibly formed more easily in organism. As for ferrous complexes, Mon2-Fe^{3+} and Mon2-Fe^{3+}-Mon2 models would have a greater probability to be formed in organism.

Keywords : *Non heminic iron, Condensed Tannins, Procyanidin B2, Iron-Tannins Complexes, SPASIBA Forces Field, Molecular Dynamic.*

RÉSUMÉ

Modélisation empirique des complexes Fer non héminique-Tannins condensés par le champ de forces SPASIBA : cas des complexes $Fe^{3+/2+}$-Procyanidine B2.

Le champ de forces empirique SPASIBA a permis l'étude théorique des mécanismes de formation des complexes entre le fer non héminique (Fe^{3+},

Fe^{2+}) et le Procyanidine B2 (molécule de tannins condensés ou proanthocyanidines) au niveau de l'organisme.
Pour ce faire, des modèles de complexes de type Procyanidine B2-$Fe^{3+/2+}$ et Procyanidine B2-$Fe^{3+/2+}$-Procyanidine B2, par l'intermédiaire des monomères, ont été construits.
Les caractéristiques énergétiques et conformationnelles résultantes des Dynamiques Moléculaires de ces modèles nous ont donné des résultats plus ou moins fonction du fer non héminique engagé dans les complexes. Ainsi, pour les complexes ferriques, les modèles Mon2-Fe^{3+}, Mon1-Fe^{3+}-Mon2 et Mon2-Fe^{3+}-Mon2 pourraient éventuellement se former plus facilement dans l'organisme. Quant aux complexes ferreux, les modèles Mon2-Fe^{3+} et Mon2-Fe^{3+}-Mon2 auraient une plus grande probabilité à se former au niveau de l'organisme

Mots-clés : *Fer non héminique, Tannins condensés, Procyanidine B2, Complexes Fer-Tannins, Champ de forces SPASIBA, Dynamique Moléculaire.*

I - INTRODUCTION

Iron deficit in organism, prevalent cause in ferriprive anaemia, mainly finds it explanation in insufficiency of it absorption partly caused by vegetal molecules contained in foods (tannins) [1-4].
Polyphenols in general and tannins in particular are known for their inhibiting effects of nonheminic iron intestinal absorption resulting from foods. These effects were identified for the first time by Disler et al [5-8]. These studies showed a drastic reduction from approximately 60% of iron absorption resulting from food consumed such as meat after the catch of 200 to 250 mL of a tea cup [6-11]. Absorption of iron resulting from bread or a vegetables soup is respectively reduced third or quarter when they are been useful with tea instead of water [12-17]. Absorption of Western countries breakfast iron is reduced of 56% when they are accompanied by a cup of 2,5 g of tea dissolves in 150 mL of water [10]. Reduction is 64% for a hamburger consumed with a cup of 1,75 g of tea dissolves in 200 mL of water [11]. It's 45% for cornflakes consumed with a cup of 5 g of tea dissolves in 150 mL of water [12].
These inhibition effects are due to formation of insolubles complexes between phenolic hydroxyls of tannins and nonheminic iron brought by foods [5]. This complexation generally occurs in the duodenum [13].

Inhibition is proportional to quantity of tannins and nonheminic iron contained in foods consumed [12, 14]. This phenomena concern all types of tannins contained in the vegetal foods [15,16].

These nutriments (nonheminic iron, tannins) are very significant for human organism [17-23]. Optimization of their bioavailability requires the search for solutions to formation of these complexes.

Although complexation phenomena of nonheminic iron with condensed tannin molecules are established, we have little information on mechanisms governing them [5-8, 15]. What can be geometries, arrangements of these complexes?

To try to answer these questions in order to elucidate the mechanisms, we decided, during this work, to model complexation of nonheminic (Fe^{3+}, Fe^{2+}) iron with one of the most abundant molecules of proanthocyanidines in foods : Procyanidin B2 [24].

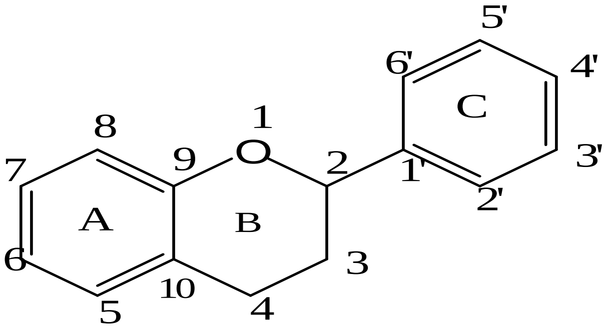
Empirical potential energy function or SPASIBA forces field defines will enable us to model these complexes [25-27].This theoretical study will contribute to knowledge of these mechanisms in sights of possible solutions.

II- MATERIALS AND METHODS

II-1. MATERIALS: MODELS OF COMPLEXES

Chemically, formation of complexes by coordination bonds of tannin molecules with nonheminic iron (Fe^{3+}, Fe^{2+}) is explained by existence of an incomplete Atomic Orbital (d) concerning this transition metal [28]. Ideal complex of iron is in octahedral form, but only its inorganic forms Fe^{2+} ($Z=24$, $3d^6\,4s^0$) and Fe^{3+} ($Z=23$, $3d^5\,4s^0$) are hexacoordinated [28-29]. It leads us to consider an octahedral symmetry for models during this study.

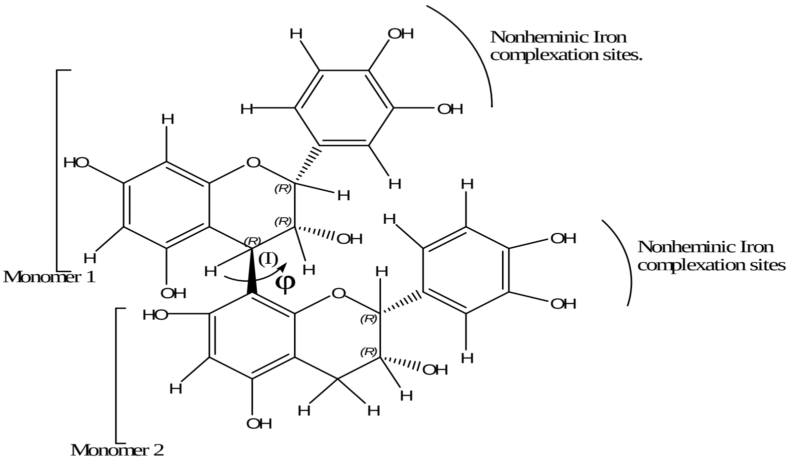
This coordination takes place by local hydroxyls groups in position 3' and 4' of proanthocyanidines.***Figure 1*** below illustrated the basic structural skeleton of proanthocyanidines monomers [5-7, 24, 30 and 31].



A: Phloroglucinol cycle.
B: Catechol cycle for procyanidines or pyrogalloyl cycles in prodelphinidines.
C: Pyranne cycle.

Figure 1 : *Basic structural skeleton of Proanthocyanidines monomers.*

Figure 2 below shows the sites of coordination for nonheminic iron in study molecule (Procyanidin B2).



(I): Interflavanic junction between monomer 1 and monomer 2.

φ: Interflavanic dihedral angle between monomer 1 and monomer 2.

Figure 2 : *Molecule of Procyanidin B2 (epicatechin (4β→8) epicatechin) or (2R, 2'R, 3R, 3'R, 4R)-2, 2'-bis (3, 4-dihydroxyphenyl)-4, 8'-bichroman-3, 3', 5, 5', 7, 7' hexaol.*

These various sites of coordination give us, in the case of monometallic or mononuclear complexes between nonheminic iron in octahedral symmetry and a Procyanidin B2 molecule, two possibles compounds of coordination:

- An octahedral complex between one Fe^{2+}/Fe^{3+} cation, one molecule of Procyanidin B2 via it monomer 1 and four water molecules.

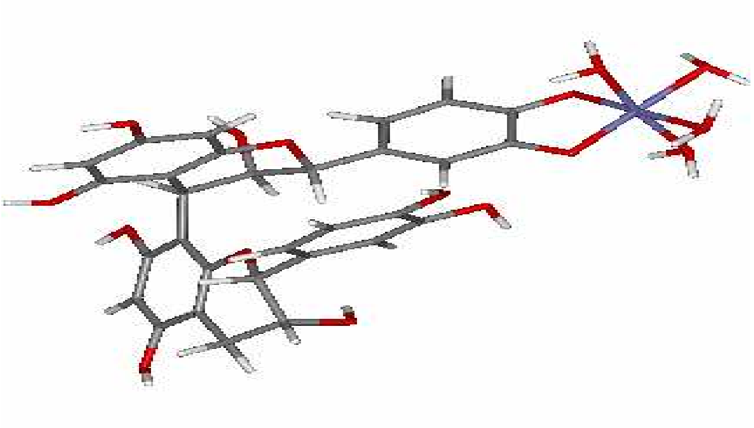


Figure 3 : *Monomer 1-Nonheminic iron (Mon1-$Fe^{2+/3+}$).*

- An octahedral complex between one Fe^{2+}/Fe^{3+} cation, one molecule of Procyanidin B2 via it monomer 2 and four water molecules.

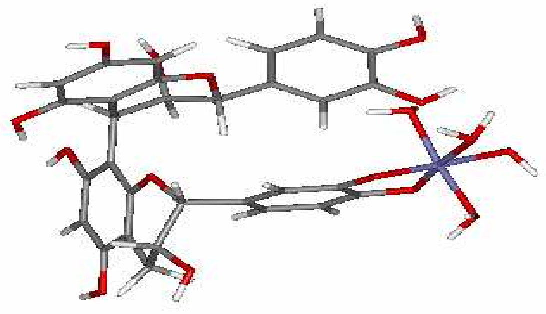


Figure 4 : *Monomer 2-Nonheminic iron (Mon2-$Fe^{2+/3+}$).*

In the case of monometallic complexes between nonheminic iron in octahedral symmetry and two molecules of Procyanidin B2, three compounds of coordination are possibles:

-An octahedral complex between one molecule of Procyanidin B2 via it monomer 1, one Fe^{2+}/Fe^{3+} cation, one molecule of Procyanidin B2 via it monomer 1 and two water molecules.

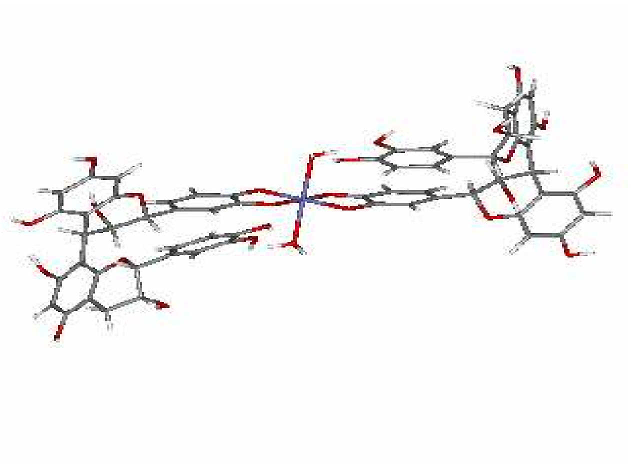


Figure 5 : *Monomer 1-Nonheminic iron -Monomer 1 (Mon1-Fe$^{2+/3+}$-Mon1).*

- An octahedral complex between one molecule of Procyanidin B2 via it monomer 2, one Fe^{2+}/Fe^{3+} cation, one molecule of Procyanidin B2 via it monomer 2 and two water molecules.

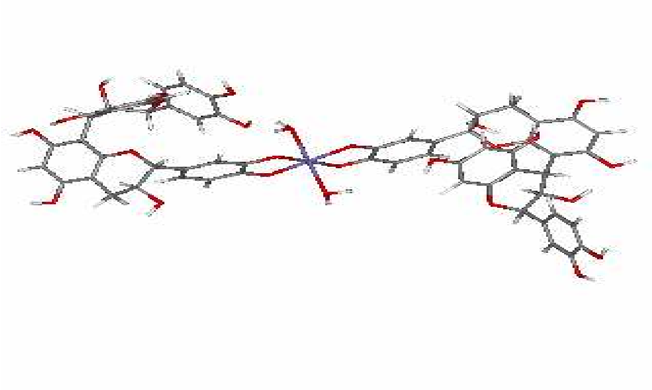


Figure 6 : *Monomer 2-Nonheminic iron -Monomer 2 (Mon2-Fe$^{2+/3+}$-Mon2).*

- An octahedral complex between one molecule of Procyanidin B2 via it monomer 1, one Fe^{2+}/Fe^{3+} cation, one molecule of Procyanidin B2 via it monomer 2 and two water molecules.

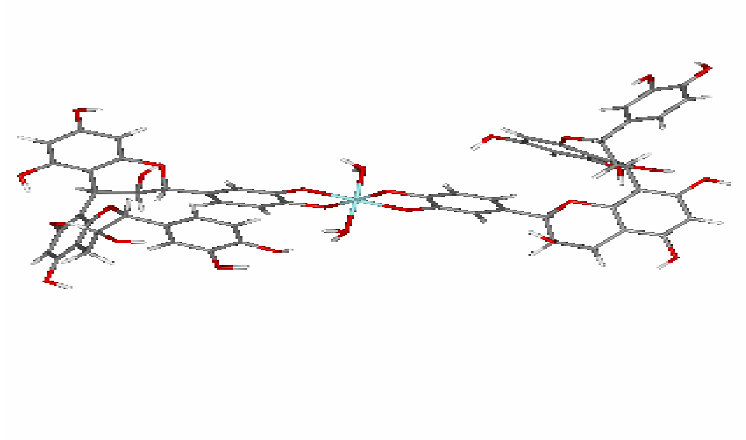


Figure 7 : *Monomer 1-Nonheminic iron -Monomer 2 (Mon1-$Fe^{2+/3+}$-Mon2).*

Presence of water molecules in models indicates hydration of nonheminic iron in the duodenum, place of complexation, where most of it absorption takes place [32, 33].

These various models of complexation will result in defining the preferential sites, i.e., the monomers allowing or not formation of complexes. This phenomen being proportional to quantity of tannins and nonheminic iron contents in foods consumed, the study of the monoatomic complexes will inform us about the models likely to be used as basis for formation of polyatomic buildings [22, 24].

This definition of preferential sites will be done by using empirical molecular modelling (SPASIBA forces field). All resulting calculations of simulation were carried out on a SGI (Silicone Graphics Incorporated) octane workstation.

II-2. METHODS

II-2-1. Empirical method of calculation: SPASIBA forces field

SPASIBA (Spectroscopic Potential Algorithm for SImulating Biomolecular conformational Adaptability) is an empirical potential energy function. It combines AMBER (V_{AMBER}) potential energy function of molecular mechanics adapted to conformational studies and Urey-Bradley-Shimanouchi (V_{UBS}) function adapted to vibrationnal Infrared and Raman spectroscopy studies [24-27].

$$V_{SPASIBA} = V_{AMBER} + V_{UBS} \qquad (1)$$

SPASIBA forces field makes it possible to reproduce at the same time the geometrical and vibrationnal characteristics. The forces constants from SPASIBA can be transferred to molecular systems carrying the same chemical functions. This transferability property constitutes one of qualities of this forces field.

II-2-2. Methodology

Molecular Dynamics using SPASIBA forces field will make it possible to judge disposition of Procyanidin B2 monomers to facilitate or not formation of complexes with nonheminic iron. This one will be used for qualification of complexes behaviours during a defined time by energetic cost and conformational variation of models in simulated biological environment. These factors will determine the susceptibility of formation of complexes in organism [34].

Molecular Dynamic allows conformational space exploration of complexes models according to time. Simulation in implicit biological environment leads us to use a continuous dielectric field (ε=4). This field acts like a continuous perturbation of solute in gas phase. Molecular Dynamic itself is carried out with constant volume and temperature. The time of simulation is fixed at one nanosecond (1ns). The temperature of simulation, after phase of thermalisation, is 300 K. Structural and energetic characteristics are safeguarded every 0.1 picoseconds (0.1 ps). Analysis of these data, energies and conformations, will make it possible to know more about viability of complexes models. Energies do not have any physical signification in absolute term but absence of experimental data leads us to use them to compare the complexes between them as for their stability. On the other hand, analysis of resulting conformations will be done by comparison to observable structural data of Procyanidin B2. More especially, a variation of octahedral building of inorganic iron in complexes is summarized by a more or less significant possible distortion being able to be explained by Jahn Teller effect [34-37]. Indeed, Jahn Teller in 1937 postulates about electronic state of complexes, that: ‘’ *any non-linear molecular system in a degenerated electronic state, either by orbital, or as for spin, becomes deformed in order to decrease it symmetry, with raising the degeneration and minimizing it energy*’’.

Procyanidin B2 conformation is determined by interflavanic dihedral angle φ between its two monomers (***Figure 2***). This interflavanic angle is defined precisely between carbon atoms 3 and 4 of monomer 1 and carbons 8 and 9 of monomer 2 (***Figure 1***).

Observation indicates that molecule of Procyanidin B2 adopts two conformations in solution with interflavanics dihedrals angles φ being located around 90° and -90° [34]. We left during our study, dihedral angle of 98.2°, corresponding to a local minimum of Procyanidin B2 experimental structure. So, a greater probability of a complex model formation compared to another in the same type refers to a lower energy and dihedral(s) variation(s) closer to conformational observation of Procyanidin B2 molecule.

III - RESULTS AND DISCUSSION

III-1. COMPLEXES MODELS OF PROCYANIDIN B2 WITH FERRIC IRON (Fe^{3+})

III-1-1. Energetic characteristics

Although not having any physical signification in absolute term, energies resulting from molecular dynamic simulations will be used to compare models of complexes between them as for their relative stability.
Table 1 below gives those of Fe^{3+} complexes.

Table 2 : *Potential energy (E_P) in kcal.mol^{-1} of Procyanidin B2-Fe(3+) and Procyanidin B2-Fe(3+)-Procyanidin B2 models in implicit biological environment.*

Models	Procyanidin B2-Fe(3+) type		Procyanidin B2-Fe(3+)-Procyanidin B2 type		
	Monomer 1-Fe(3+)	Monomer 2-Fe(3+)	Monomer 1-Fe(3+)-Monomer 1	Monomer 2-Fe(3+)-Monomer 2	Monomer 1-Fe(3+)-Monomer 2
E_P ± RMSD	67.32±5.25	45.46 ± 5.22	97.76± 7.06	86.51 ± 7.17	83.55 ± 6.98

Average energetic fluctuation during the time (1 ns) of Molecular Dynamics is characterized by potential Energy (E_P) expressed in Kcal.mol^{-1}. Root Mean Square Deviation or average quadratic deviation (RMSD) indicates the standard deviation.

- <u>Complexes type</u>: Procyanidin B2-Fe(3+)

Potential energies indicate that Monomer 2-Fe(3+) complex, with 45.46 kcal.mol^{-1}, is more stable than Monomer 1-Fe(3+) complex, with 67.32

kcal.mol^{-1}. RMSD show little energetic fluctuations around average value for each model. We could say that monomer 2 local hydroxyls groups of Procyanidin B2 molecule would more support formation of complex with ferric iron that in the case of monomer 1.

- <u>Complexes type</u>: Procyanidin B2-Fe(3+)-Procyanidin B2

Complexes models Monomer 2-Fe(3+)-Monomer 2 and Monomer 1-Fe(3+)-Monomer 2, with respectively 86.51 kcal.mol^{-1} and 83.55 kcal.mol^{-1}, are relatively more stable than complex Monomère 1-Fe(3+)-Monomer 1 with 97.76 kcal.mol^{-1}. It means that for this type of complexes, formation of coordination compounds between two Procyanidin B2 molecules and ferric iron would be done more with respective local hydroxyls groups of monomers 2 or with those of monomer 1 and monomer 2. This formation would take place with depends on complexation between ferric iron and two Procyanidin B2 molecules by intermediary of respective monomers 1, which would be less favoured.

III-1-2. Conformational characteristics

Table 2 : *Average variation of dihedral angle φ of Procyanidin B2-Fe(3+) and Procyanidin B2-Fe(3+)-Procyanidin B2 models in implicit biological environment.*

Models	Procyanidin B2-Fe(3+) type		Procyanidin B2-Fe(3+)-Procyanidin B2 type					
	Monomer 1-Fe(3+)	Monomer 2-Fe(3+)	Monomer 1-Fe(3+) - Monomer 1		Monomer 2-Fe(3+) - Monomer 2		Monomer 1-Fe(3+) - Monomer 2	
Dihedral angle φ average in degree (°) ± RMSD	92.99 ± 12.00	95.69 ± 11.58	89.45(1) ± 10.91	88.82(2) ± 10.56	93.26(1) ± 11.56	74.15(2) ± 10.43	88.90(1) ± 10.38	72.24(2) ± 10.52

(1) : Dihedral angle φ of complex molecule 1   (2) : Dihedral angle φ of complex molecule 2

Average dihedral fluctuation during Molecular Dynamics time (1 ns) is expressed in degree (°). RMSD indicates standard deviation of dihedral angle φ average.

- <u>Complexes type</u>: Procyanidin B2-Fe(3+)

Dihedral angle φ has an average which is established around 93° for monomer 1-Fe(3+) model and 96° for monomer 2-Fe(3+) model. These data join those derived from observation [34]. Significant RMSD are explained by a great fluctuation around average value for two models.

- <u>Complexes type</u>: Procyanidin B2-Fe(3+)-Procyanidin B2

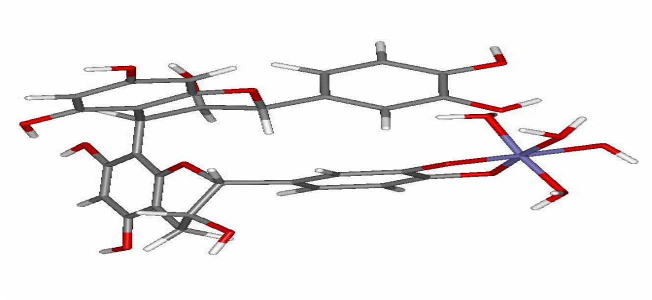
Monomer 1-Fe(3+)-Monomer 1 model has its two dihedrals angles φ which vary approximately around 89° and are to be brought closer values obtained by observation [34]. On the other hand, the two other complexes have only one molecule whose dihedral angle φ varies around 93.26° and 88.9° in agreement with experimentation. The second respective molecules, have each one a dihedral angle φ which varies around 74.15° and 72.24° and presenting variations from 15 to 20° compared to observation [34]. Their energetic costs being less significant than the first complex, we would be tempted to say that an increased stability would involve more or less significant distance of dihedrals angles φ of complexes molecules (Procyanidin B2) compared to observable value.

- Partial conclusion : Probable models of ferric complexes in organism.

By coupling energetic results with those of conformations, following observations could be made:

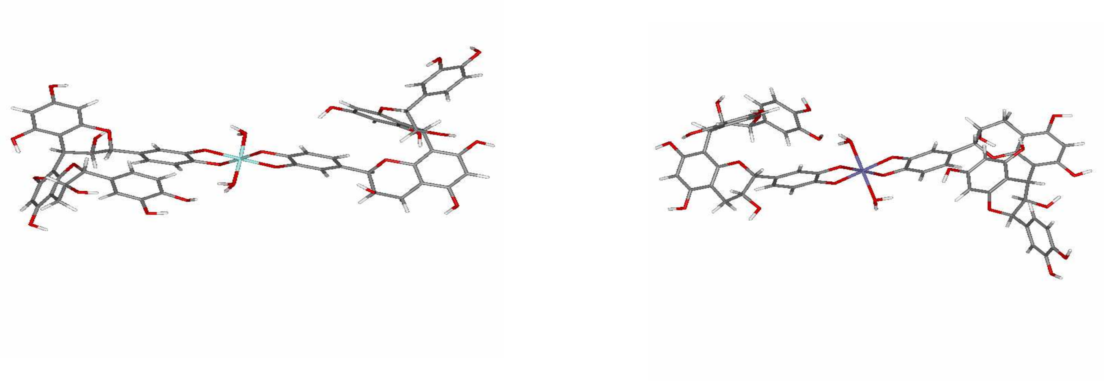
- A Procyanidin B2 molecule would formed easily a monoatomic complex with ferric iron and its monomer 2 local hydroxyls groups in organism.

-Two molecules of procyanidin B2 would more easily form a monoatomic complex with ferric iron via monomer 1 local hydroxyls groups of the first molecule and those of the second molecule monomer 2. It would be formed in the same way, a monoatomic complex with ferric iron and monomers 2 local hydroxyls groups’ of the two Procyanidin B2 molecules in organism. Possible formation of polyatomic complexes would be done starting from two preceding monoatomic complexes. I.e. buildings being established either only with local hydroxyls groups of monomers 2 of Procyanidin B2 molecules engaged in complexes with ferric iron cations or alternatively with local hydroxyls groups of monomer 1 of a first molecule and those of a second Procyanidine B2 molecule monomer 2.



Complex of Mon2-Fe^{3+}

Figure 8 : *Probable model of Procyanidin B2-Fe^{3+} ferric complex in organism.*



Complex of Mon1-Fe^{3+}- Mon2 Complex of Mon2-Fe^{3+}-Mon2

Figure 9 : *Probable models of Procyanidin B2-Fe^{3+}-Procyanidin B2 ferric complexes in organism.*

III-2. COMPLEXES MODELS OF PROCYANIDIN B2 WITH FERROUS IRON (Fe^{2+})

III-2-1. Energetic characteristics

Table 3 here after gives those of Fe^{2+} complexes.

Table 3 : *Potential energy (E_P) in kcal.mol^{-1} of Procyanidin B2-Fe(2+) and Procyanidin B2-Fe(2+)-Procyanidin B2 models in implicit biological environment.*

Models	Procyanidin B2-Fe(2+) type		Procyanidin B2-Fe(2+)-Procyanidin B2 type		
	Monomer 1-Fe(2+)	Monomer 2-Fe(2+)	Monomer 1-Fe(2+)-Monomer 1	Monomer 2-Fe(2+)-Monomer 2	Monomer 1-Fe(2+)-Monomer 2
E_P ± RMSD	49.79 ± 5.14	38.98 ± 5.24	102.90 ± 6.91	83.41 ± 7.02	95.18 ± 7.37

- Complexes type: Procyanidin B2-Fe(2+)

Potential energies indicate that complex of Monomer 2-Fe(2+), with 38.98 kcal.mol^{-1}, is more stable than Monomer 1-Fe(2+) complex, with 49.79 Kcal.mol^{-1}. RMSD show that energy fluctuates little around its average value for each model. With a difference in energetic cost of 10,81 kcal.mol^{-1}, we could say that monomer 2 local hydroxyls groups of Procyanidin B2 molecule would more support complex formation with ferrous iron that those of monomer 1 compared to energetic criteria.

- Complexes type: Procyanidin B2-Fe(2+)-Procyanidin B2

Potential energies values show that Monomer 2-Fe(2+)-Monomer 2 complex model, with 83.41 kcal.mol^{-1}, is relatively more stable than Monomer 1-Fe(2+)-Monomer 2 and Monomer 1-Fe(2+)-Monomer 1 models with respectively 95.18 kcal.mol^{-1} and 102.9 kcal.mol^{-1}. It would like to say that for this type of complexes in implicit biological environment, formation of coordination compounds between two Procyanidin B2 molecules and ferrous iron would be done more with respective monomer 2 local hydroxyls groups.

III-2-2. Conformational characteristics

Table 4 : *Average variation of dihedral angle φ of Procyanidin B2-Fe(2+) and Procyanidin B2-Fe(2+)-Procyanidin B2 models in implicit biological environment.*

Models	Procyanidin B2-Fe(2+) type		Procyanidin B2-Fe(2+)-Procyanidin B2 type					
	Monomer 1-Fe(2+)	Monomer 2-Fe(2+)	Monomer 1-Fe(2+)-Monomer 1		Monomer 2-Fe(2+)-Monomer 2		Monomer 1-Fe(2+)-Monomer 2	
Dihedral angle φ average in degree (°) ± RMSD	92.93 ± 12.55	91.30 ± 12.36	96.60(1) ± 12.77	89.69(2) ± 10.06	94.27(1) ± 11.12	88.07(2) ± 11.30	94.28(1) ± 14.05(1)	71.62(2) ± 10.81

(1) : Dihedral angle φ of complex molecule 1 (2) : Dihedral angle φ of complex molecule 2

- Complexes type: Procyanidin B2-Fe(2+)

Dihedrals angles φ have averages which are established around 93° for monomer 1-Fe(2+) model and 91° for monomer 2-Fe(2+) model. RMSD show great fluctuations around average values for the two models. We can, nevertheless, say that these data are in agreement with experimentation [34].

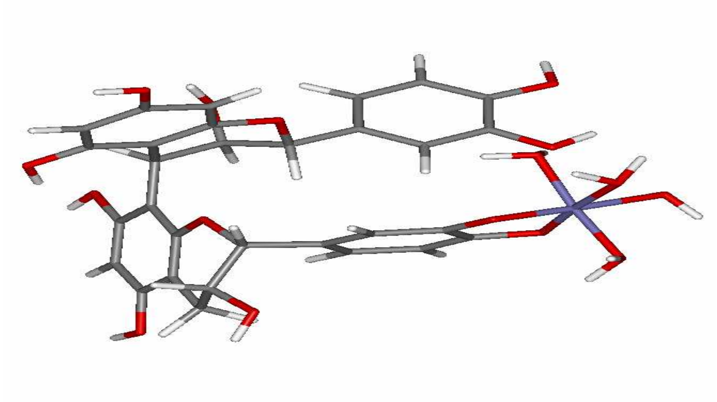
- Complexes type: Procyanidin B2-Fe(2+)-Procyanidin B2

Monomer 1-Fe(2+)-Monomer 1 and Monomer 2-Fe(2+)-Monomer 2 models have their respective dihedrals angles φ oscillating to 96°, 90° and 94°, 88° in correspondence with observation [34]. On the other hand, Monomer 1-Fe(2+)-Monomer 2 complex has only one molecule whose dihedral angle φ varies around an average (94.28°), in agreement with experimentation. The second molecule has a dihedral angle φ which varies around 74.62°, presenting a variation of 20° compared to observation.

- Partial conclusion : Probable models of ferrous complexes in organism.

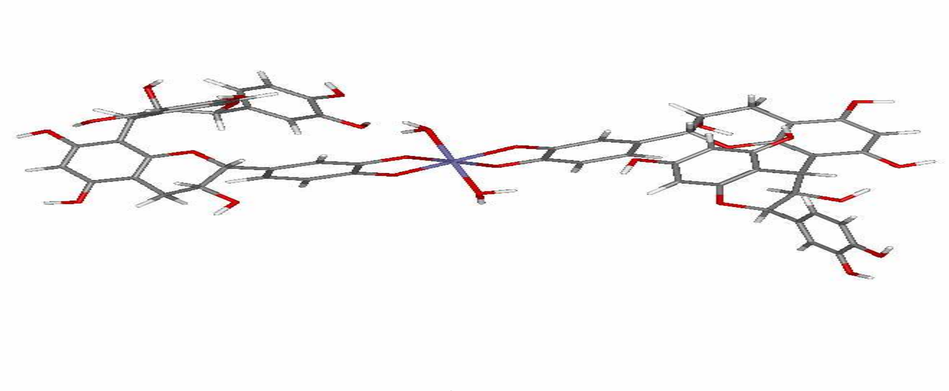
Energetic results associated to those of conformations, could give following remarks :

- A Procyanidin B2 molecule would formed easily a monoatomic complex with ferrous iron and its monomer 2 local hydroxyls groups in organism.
- Two molecules of procyanidin B2 would more easily formed a monoatomic complex with ferrous iron via monomer 2 local hydroxyls groups of the two Procyanidin B2 molecules in organism. Possible formation of polyatomic complexes would be done starting from preceding monoatomic complex, i.e. buildings being established only with monomer 2 local hydroxyls groups of Procyanidine B2 molecules engaged in complexes with ferrous iron cations.



Complex of Mon2-Fe^{2+}

Figure 10 : *Probable model of Procyanidin B2-Fe^{2+} ferrous complex in organism.*



Complex of Mon2-Fe^{2+}- Mon2

Figure 11 : *Probable model of Procyanidin B2-Fe^{2+}-Procyanidin B2 ferrous complexes in organism.*

IV - CONCLUSION

Molecular modelling, by empirical method (molecular dynamic), made us possible to have an outline of complexation modes between condensed tannin molecules and nonheminic iron in simulated biological environment (application of continuous dielectric field). Specific study of Procyanidine B2-$Fe^{3+/2+}$ interactions, by using SPASIBA forces field, enabled us to understand that inorganic iron would be able to form complexes with monomer 1 and monomer 2 local hydroxyls groups of Procyanidin B2 molecules in organism.

Although Procyanidin B2 is representative of condensed tannins class, nothing says that any other molecule of this category will have the same behaviour with respect to nonheminic iron. This uncertainty encourages us to regard this work as an indication in probable mechanisms of formation of tannins condensed with nonheminic iron complexes in biological environment.

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