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Studies on the interaction between linamarin and human serum albumin

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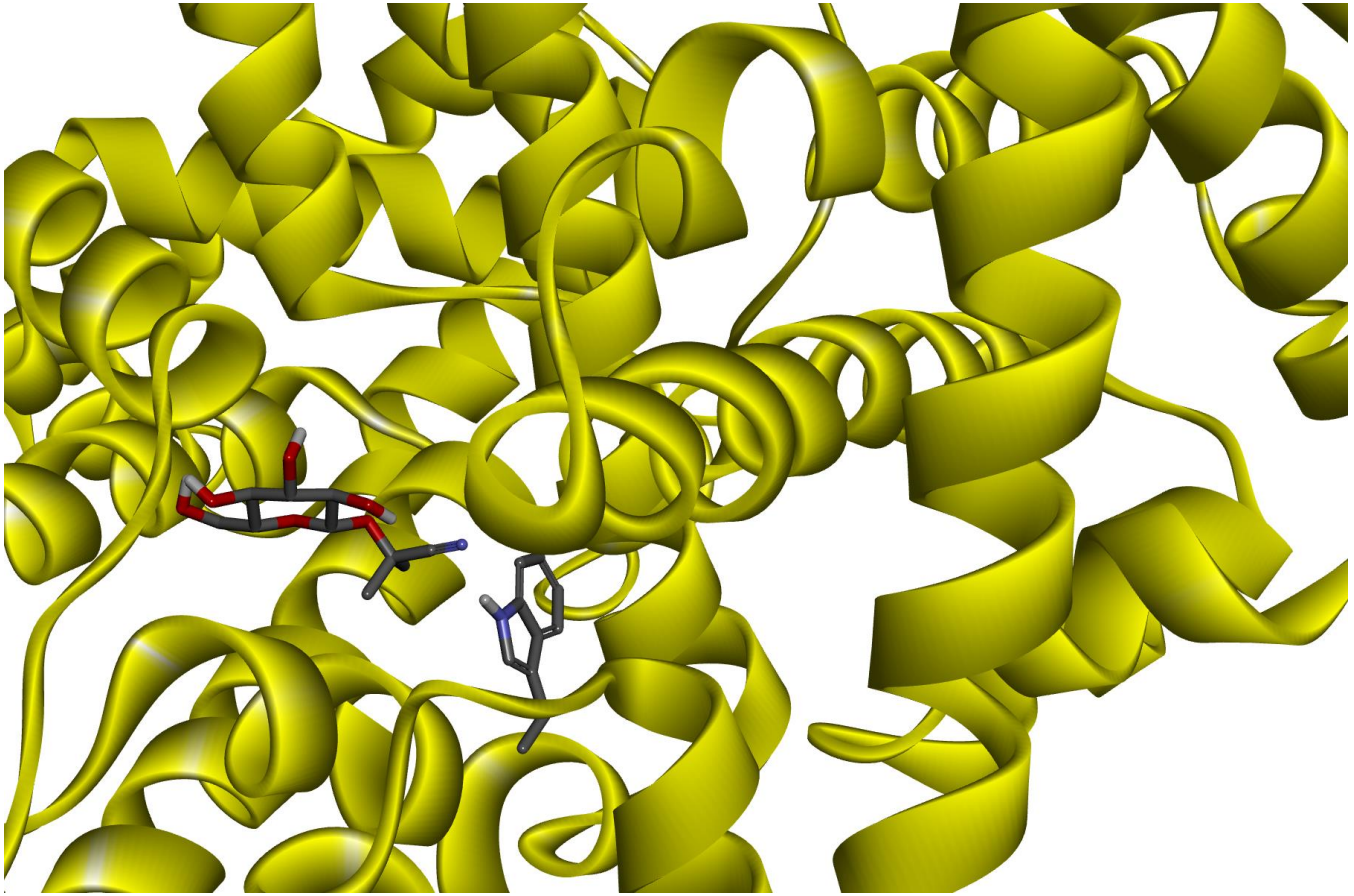

Abstract:

Linamarin (2-β-D-glucopyranosyloxy-2-methylpropanenitrile) is a cyanogenic glucoside found in Cassava, *Manihot esculenta* Crantz. In recent years it has been examined as potential antitumor therapy. Results has been shown that it has promising effects on breast cancer, colon adenocarcinoma and acute myeloid leukemia. The aim of this study was to evaluate the interaction between linamarin and human serum albumin, as the most common protein in circulatory system, using fluorescence spectroscopy and molecular simulation under simulated physiological conditions. In our experimental results we have found that linamarin binds to human serum albumin (K_a~10^3). Also, experimental and theoretical investigations are in good agreement.

Keywords: Linamarin; human serum albumin; fluorescence spectroscopy

Introduction

Human serum albumin (HSA)

- ✓ is a protein which makes up to 60% of the human plasma
- ✓ is the most common protein in circulation[1,2]
- ✓ the protein is synthetized in the liver and possesses an array of functions

Role of human serum albumin

- ✓ reversibly bind a huge number of compounds thereby acting as a transporter
- ✓ the binding increases the compounds solubility
- ✓ helps in its distribution and to achieve the desired effect

[1] Y. Wang, S. Wang, M. Huang, Structure and enzymatic activities of human serum albumin, Curr Pharm Des. (2015) 21(14) 1831-1836.

[2] Q. Wu, C.H. Li, Y.J. Hu, Y. Liu, Study of caffeine binding to human serum albumin using optical spectroscopic methods, Science in China Series B: Chemistry, (2009) 52 2205-2212.

Introduction

Linamarin (LIN)

- Is a cyanogenic glucoside found in Cassava, *Manihot esculenta* Crantz, a plant widely used as a food source in many developing countries[3]
- Upon ingestion it undergoes hydrolysis by a type of β-glucosidase called linamarase, produced by intestinal bacteria
- The reaction yields glucose and acetonecyanohydrin, which later deteriorates into acetone and hydrogen cyanide[4,5]
- Since HCN is toxic to normal cells and the fact that these cells do not possess the gene for linamarase but has been discovered on some types of cancer cells, linamarin has been researched as a potential antitumor therapy[6]

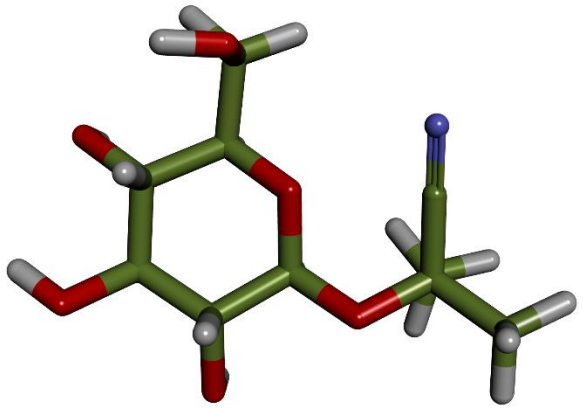

Figure 1. Linamarin

[3] C.A. Idibie, H. Davids, S.E. Iyuke, Cytotoxicity of purified cassava linamarin to a selected cancer cell lines, Bioprocess Biosyst Eng. (2007) 30(4) 261-269.

[4] B.P. Kamalu, The adverse effects of long-term cassava (*Manihot esculenta* Crantz) consumption, Int. J. Food Sci. Nutr. (1995) 46(1) 65-93.

[5] P. Cressey, J. Reeve, Metabolism of cyanogenic glycosides: A review, Food Chem Toxicol, (2019) 125 225-232.

[6] D. Sutiningsih, M.A. Wuryanto, H.S. Susanto, Hariyadi S, Mustofa, Anticancer Activity of Linamarin from Cassava Leaves (*Manihot esculenta* Cranz) on Raji Cells, International Journal of Cancer Research, (2020) 16(1) 18-27.

Results and discussion

Florescence spectroscopy

- was used to investigate the drug protein binding
- The intrinsic fluorescence of proteins is due to the aromatic amino acids tryptophan (Trp), Tyrosine (Tyr) and Phenylalanine (Phe).
- The fluorescence spectra for HSA recorded at an excitation wavelength of 295 nm at room temperature (298K) under physiological conditions (pH=7.4)
- Concentrations of linamarin varied from 0 to 2 x 10^{-5} M (Figure 2)

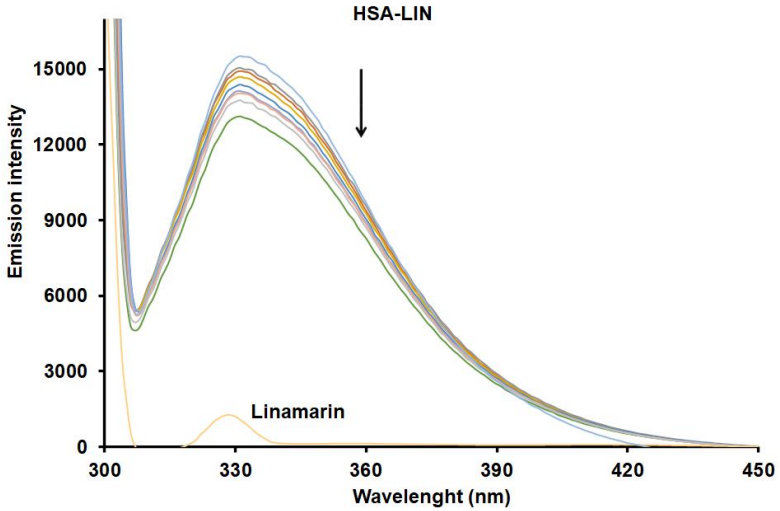

Figure 2. Fluorescence emission spectra of HSA-LIN

Fluorescence quenching

The quenching mechanism between HSA and linamarin is based on the K_{sv} values obtained from Stern-Volmer plot[7] (Figure 3) and the following equation:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q]$$

Where:
F_0 - the fluorescence intensity of HSA
F - the fluorescence intensity of HSA-Linamarin complex
Ksv - Stern–Volmer quenching constant
[Q] - quencher concentration
Kq - bimolecular quenching constant
τ_0 fluorophore lifetime without quencher.

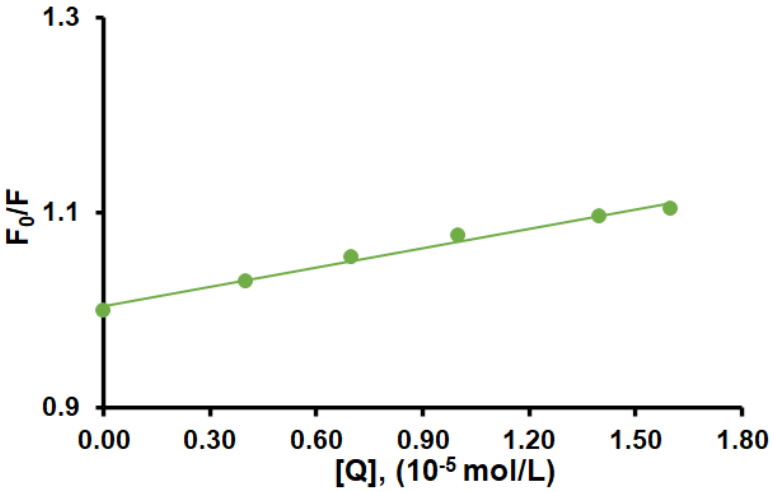

Figure 3. Stern-Volmer plots of the fluorescence quenching of HSA-LIN system[7]

[7] J.R. Lakowicz, Principles of fluorescence spectroscopy, 3rd ed. Springer, New York, (2006)

Binding constant

The binding constant and the binding stoichiometry for the HSA - limamarin interaction were obtained with the double logarithmic regression curve (Figure 4) represented by the equation:

$$\log\frac{F_0 - F}{F} = \log K_a + n\log[Q]$$

Where, K_a and n represent the binding constant and binding stoichiometry. The obtained results are given in Table 1.

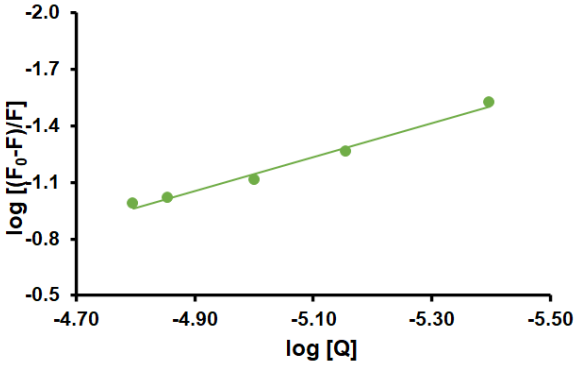

Table 1. The interaction parameters of the binary (HSA-TGC) and ternary (HSA-TGC-CAT, HSA : CAT = 1 : 1) systems

System [a]	K_{SV} x 10^{-3} [b]	k_q x 10^{-11} [c]	R^2 [d]	K_a x 10^{-3} [b]	n	R^2
HSA-Linamarin	6.56	6.56	0.9848	2.25	0.9	0.9872

[a] 298 K; [b] M^{-1}; [c] $M^{-1}s^{-1}$; [d] R is the correlation coefficient

Figure 4. Logarithmic plots of the fluorescence quenching of HSA by linamarin[7]

[7] J.R. Lakowicz, Principles of fluorescence spectroscopy, 3rd ed. Springer, New York, (2006)

Synchronous fluorescence spectroscopic studies

The conformational change of the HSA was analyzed using synchronous fluorescence spectroscopy (Figure 5).

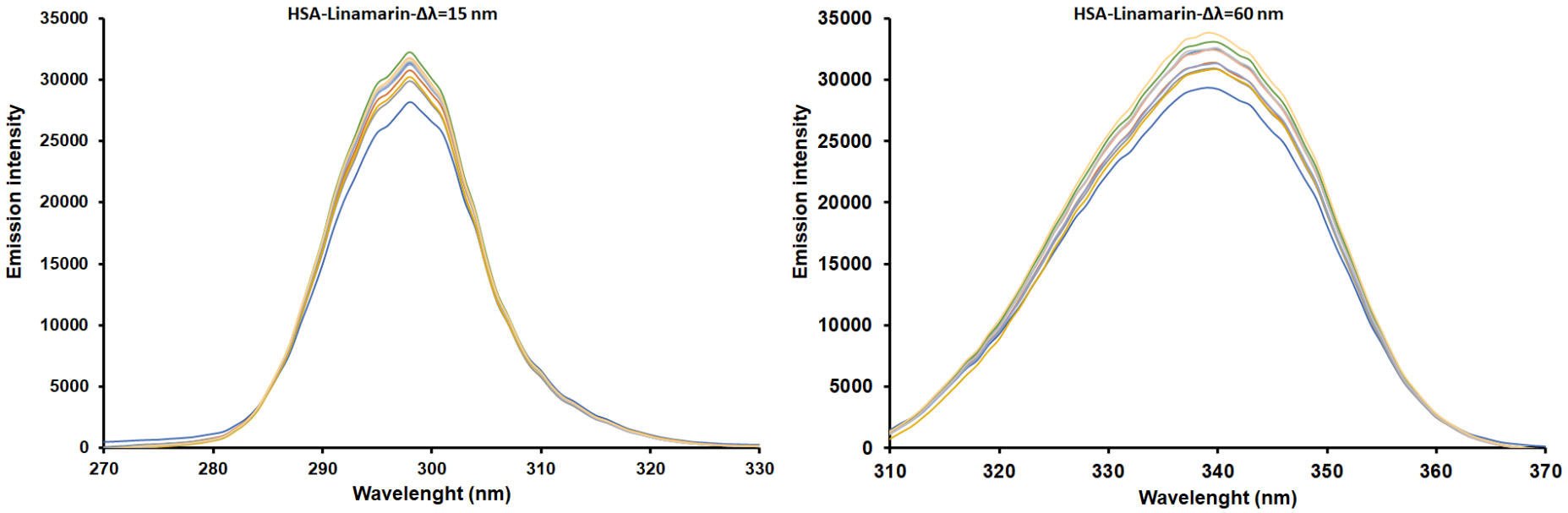

Figure 5. The effect of Linamarin on the synchronous fluorescence emission spectra of HSA (T = 298 K, pH = 7.4). [HSA] = 2 μM and [Linamarin] = 0 to 2 x 10^{-5} M.

Docking expriments

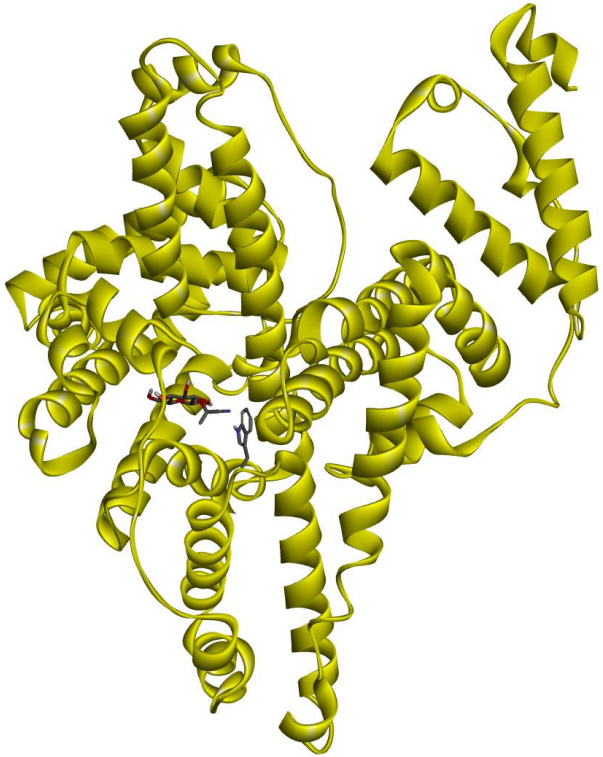

Figure 6. HSA-Linamarin system by molecular docking simulations

Conclusions

- ✓ Linamarin binding to HSA in simulated physiological conditions
- ✓ According to fluorescence measurements, it is obvious that the internal emission fluorescence of the HSA gradually decreased, which indicated the formation of the HSA-linamarin complex
- ✓ The binding constant of linamarin to human serum albumin is K_a~10^3
- ✓ Obtained values of *n* indicate the existence of only one binding site for linamarin toward HSA
- ✓ Results of synchronous fluorescence spectra show that linamarin does not change the microenvironments around the tryptophan (Trp) and tyrosine (Tyr) residues in the HSA
- ✓ Docking experiments toward HSA protein have been done, indicating a good correlation with experimental results.

Acknowledgments