Molecular recognition patterns between vitamin B12 and different animal and plant based proteins explored through STD–NMR and docking tool

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Introduction

Caseins in bovine milk bind high quantities of CBL, due to the formation of Casein–Cbl coordination bonds[1]. The casein–Cbl bond is sufficiently stable to cross through the stomach's acidic medium, indicating that casein and its peptides play a role in Cbl absorption by intestinal cells[1]. This indicates that the vitamin B12 binding proteins are well absorbed, which would otherwise limit absorption[2]. The Saturation Transfer Difference–NMR (STD–NMR) technique has been recently used to gain qualitative and quantitative information about physiological interactions at atomic-resolution. The molecular recognition patterns between cyanocobalamin (CNBL) and Different plant and animal proteins were investigated by STD–NMR supplemented by molecular docking.
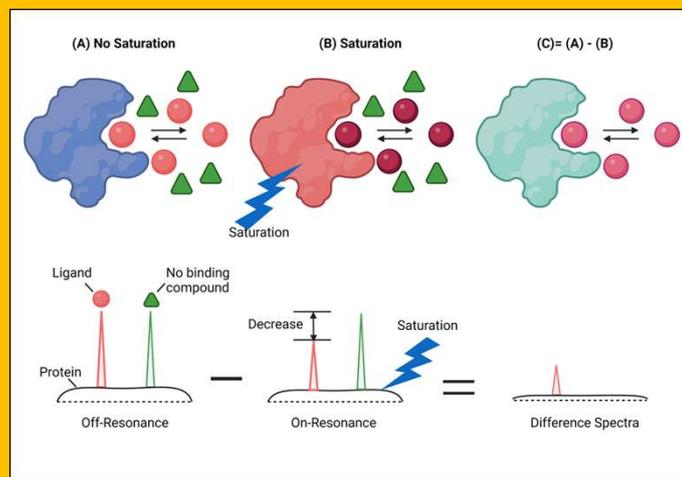

Fig.1 : Schematic diagram of STD-NMR principle

Methods

80 mM Milk protein casein, Meat protein myoglobin, Rice protein and pea protein and cyanocobalamin of varying concentration and D2O were used to prepare the sample for STD-NMR. CB-Dock and Discovery studio visualiser was used to understand exact amino acids in those proteins that interact with vitamin B12.

Results

STD-NMR spectra was generated using 80mM proteins and varying conc, of vitamin b12. Fig.2 shows that with the increase of vitamin b12 concentration, the interaction is also increasing. Similar spectra was generated for every protein and K_D and $K\alpha$ was calculated. The smaller the K_D value, the greater the binding affinity of the ligand for its target. The larger the K_D value, the more weakly the target molecule and ligand are attracted to and bind to one another. $K\alpha$ is directly proportional to binding affinity. Compared between different proteins, myoglobin protein shows highest and Pea protein shows lowest binding affinity (fig.3).
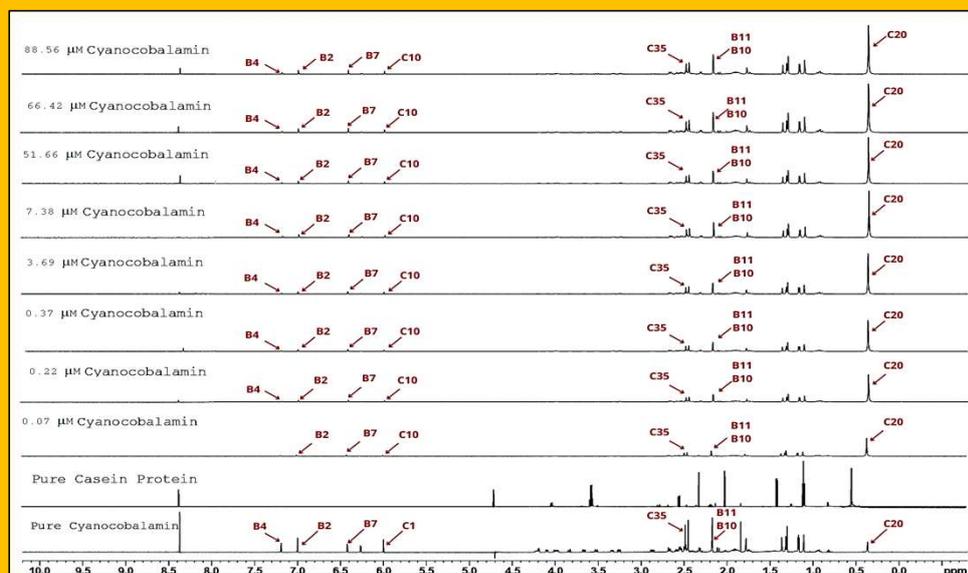

Fig.2: vitamin B_{12} and casein interaction visualised by STD-NMR (an example for all interaction)

Results

Table.1: Kd and Kα of vitamin B₁₂ and proteins interaction

	Kd (mM)	Kα (L/mol)
Myoglobin	0.02911	34353.06
Casein	0.03401	29399.73
Pea	0.10247	9758.70
Rice	0.09200	10869.40

The main binding forces of cyanocobalamin to Casein were hydrogen bond, π-sigma, π-alkyl and π-π interaction. The easily dissociated groups of cyanocobalamin played a major role in the formation of hydrogen bonds, and the substituent on the tetra pyridine ring of cyanocobalamin was important in the binding with proteins[3]. The binding conformation predicted by computer simulation(Fig.3) confirmed the experimental results. Casein and myoglobin shows more hydrogen bond with cobalamin compares to rice and pea protein and also the interaction distance is also lower in animal protein.
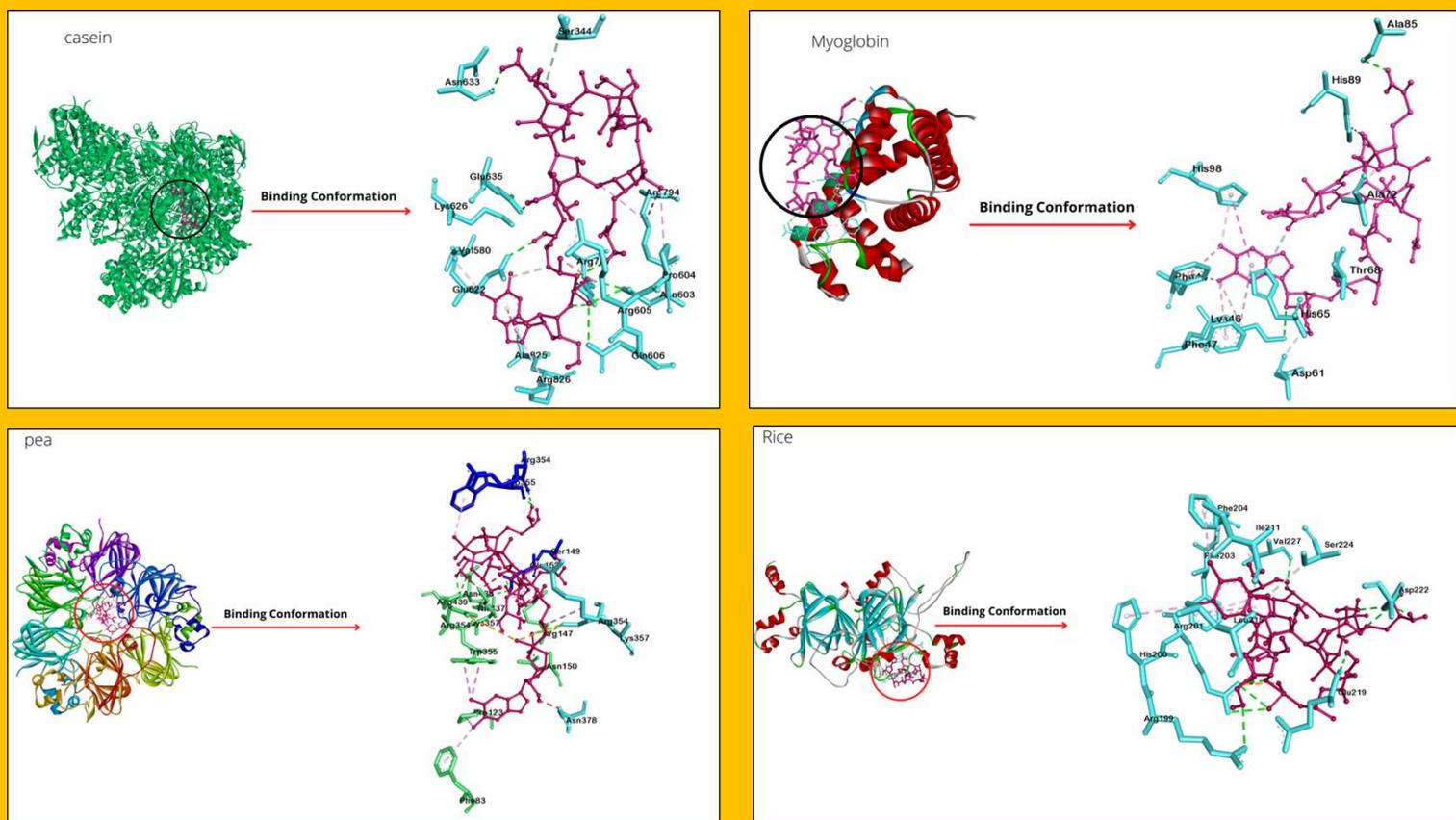

Fig.3: vitamin B₁₂ and casein interaction visualised Molecular Docking Using CB- Dock

Conclusion

❖ Myoglobin shows highest binding affinity, whereas Gluten shows lowest affinity.
❖ Casein also shows higher binding affinity to cyanocobalamin when compared with plant-based proteins.
❖ The mechanism of cyanocobalamin-proteins was a static binding mechanism with a moderate binding capability sustained by hydrogen bond, hydrophobic force, and π-π interaction.

References

1. Fedosov SN, Nexo E, Heegaard CW. Binding of aquocobalamin to bovine casein and its peptides via coordination to histidine residues. Int Dairy J. 2018;76:30-39.
2. Fedosov SN, Nexo E, Heegaard CW. Vitamin B12 and its binding proteins in milk from cow and buffalo in relation to bioavailability of B12. J Dairy Sci. 2019;102(6):4891-4905.
3. Sun Q, Zhai Y, Wang W, et al. Molecular recognition patterns between vitamin B12 and human serum albumin explored through STD–NMR and spectroscopic methods. Spectro Acta Part A Mol Biomol Spectrosc. 2021;258:119828.