6th International Electronic Conference on Medicinal Chemistry

1-30 November 2020

sciforum.net/conference/ECMC2020

sponsored by pharmaceuticals

Structural bases for rational design of new biomaterials based on metallodrug/β-lactoglobulin adducts

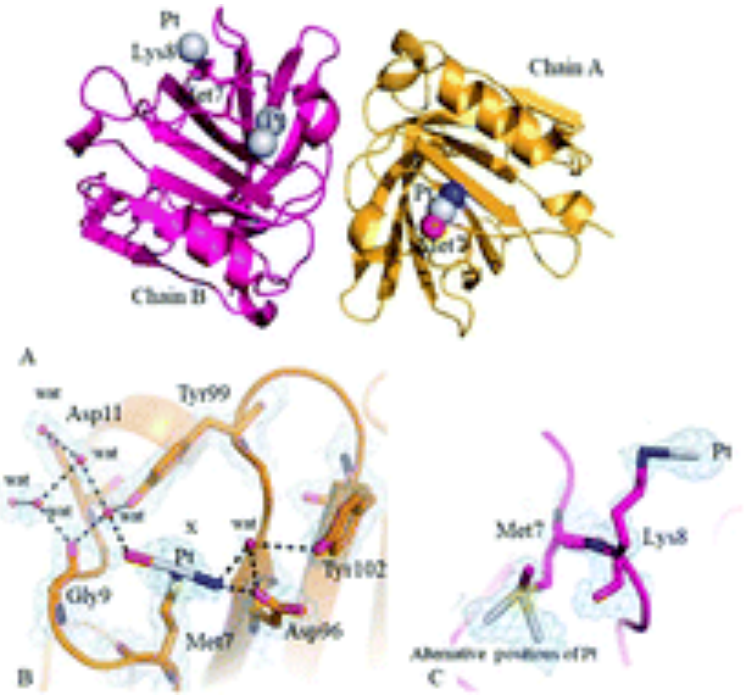
Domenico Loreto[1] , Giarita Ferraro[2], Nicole Balasco[3], Ilaria Iacobucci[3,4], Maria Monti[3,4] and Antonello Merlino[1,*]

[1] Department of Chemical Science, Univeristy of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia, I-80126, Napoli, Italy;
[2] Department of Chemistry, University of Florence, Via della Lastruccia, 50019, Sesto Fiorentino, Firenze, Italy;
[3] Institute of Biostructures and Bioimaging, CNR, Via Mezzocannone 16, 80134 Napoli, Italy;
[4] CEINGE Advanced Biotechnologies, Via G. Salvatore 486, 80145 Naples, Italy

* Corresponding author: antonello.merlino@unina.it

Structural bases for rational design of new biomaterials based on metallodrug/β-lactoglobulin adducts

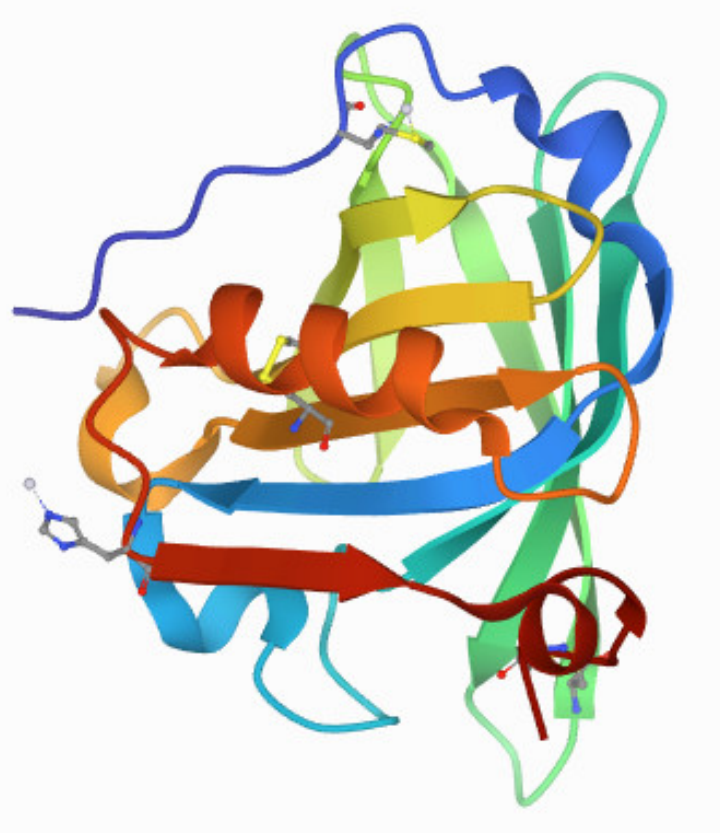
Graphical Abstract



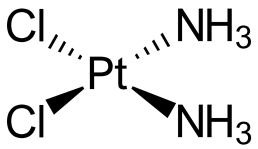
Abstract:

β-lactoglobulin is a whey carrier protein of 18.4 kDa. Due to its high solubility, safe status, biodegradable nature, gel forming ability, abundance, stability at acidic pH and stability against gastric pepsin, β-lactoglobulin can be considered as a good system for the preparation of micro- or nanoparticles for pharmaceutical industry. It has been demonstrated that β-lactoglobulin–pectin nanoparticles are able to transfer cytotoxic Pt compounds to cancer cells. With the aim to unveil the molecular basis of the metallodrug recognition by β-lactoglobulin, we are analyzing the interactions between this protein and several metallodrugs. The interaction between cisplatin (CDDP), the most used Pt-based anticancer agent, and β-lactoglobulin have been investigated both in solution and at solid state. UV-vis absorption spectroscopy and circular dichroism data indicate that the protein retains its conformation upon CDDP binding. X-ray crystallography analysis reveals that β-lactoglobulin interacts with CDDP through coordination of Pt fragments to the side chains of Met7, His146 and Lys8, with the number of binding sites increasing over time. ESI-MS data indicate that $[Pt(NH_3)_2Cl^+]$, $[Pt(NH_3)_2OH^{2+}]$ and $[Pt(NH_3)_2^{2+}]$ fragments interact with the protein. These results open the way for a rational design of new biomaterials based on metallodrug/β-lactoglobulin adduct nanoparticles.

Introduction



- **β-lactoglobulin** is the major whey protein in cow's milk of 18.4 kDa (162 amino acids)
- It is a globular protein composed by **8-stranded antiparallel β-barrel** with a **3-turn α-helix** on the outer surface.



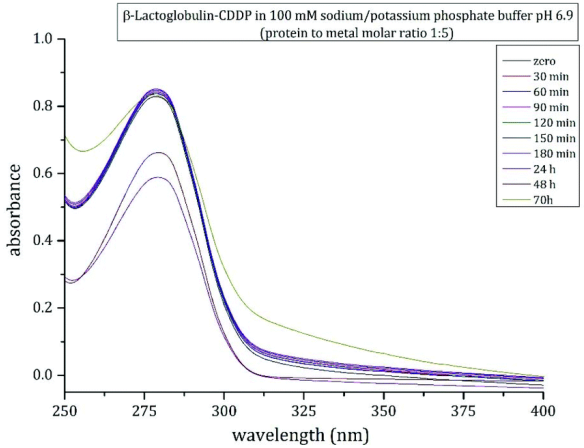
Cisplatin: the most used Pt-based anticancer agent

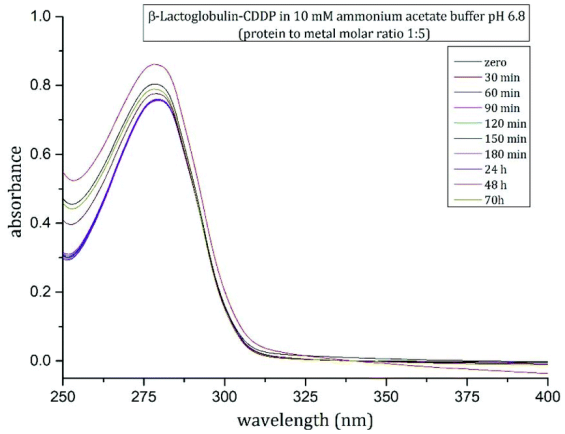
The interactions between β-lactoglobulin and cisplatin have been investigated both in solution and at solid state

E. Dufour *et al. Biochimica et Biophysica Acta,* 1994, **1205**(1), 105. Z. Teng *et al. Food Chemistry*, 2016, **204**, 391. Z. Teng *et al. Food Chemistry*, 2014, **159**, 333. Z. Izadi *et al. Chem Biol and Drug Design* 2016, **88**(2), 209. Z.H. Siddik**,** *Oncogene* 2003, **22**, 7265. L. Kelland, *Nature Reviews Cancer* 2007, **7**, 573. M.H. Green, *J. Nat Cancer Inst*. 1992, **84**, 306.

Results and discussion

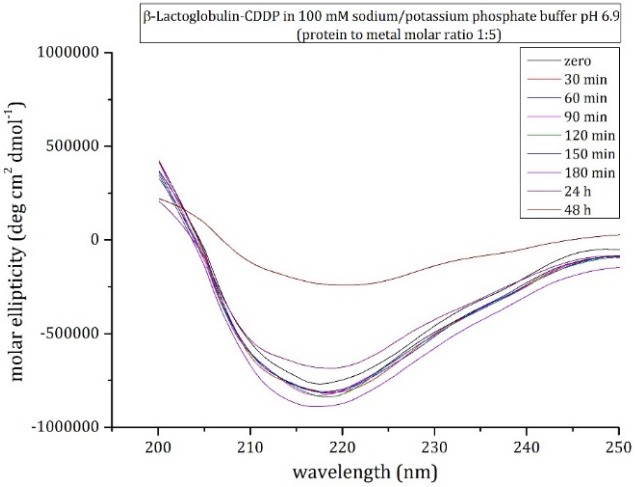
In solution characterization of CDDP binding to β-lactoglobulin

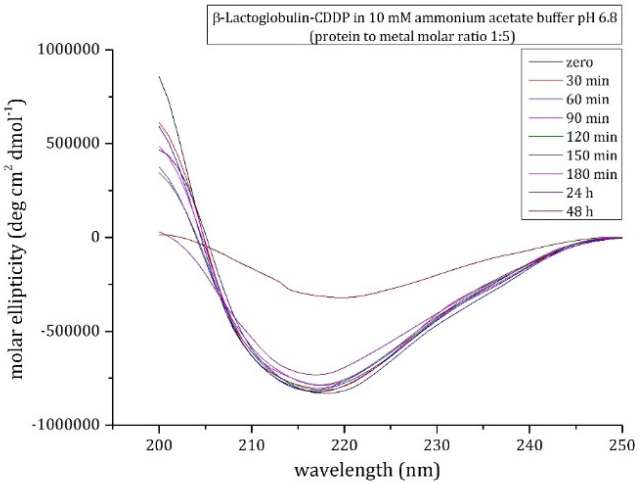




- Absorption intensity decreases in the first two hours
- After two hours, an increase of absorption intensity occurs, due to CDDP binding

Despite a slight precipitation of the sample, **β-lactoglobulin** conserves its secondary structure in the presence of the metal compound



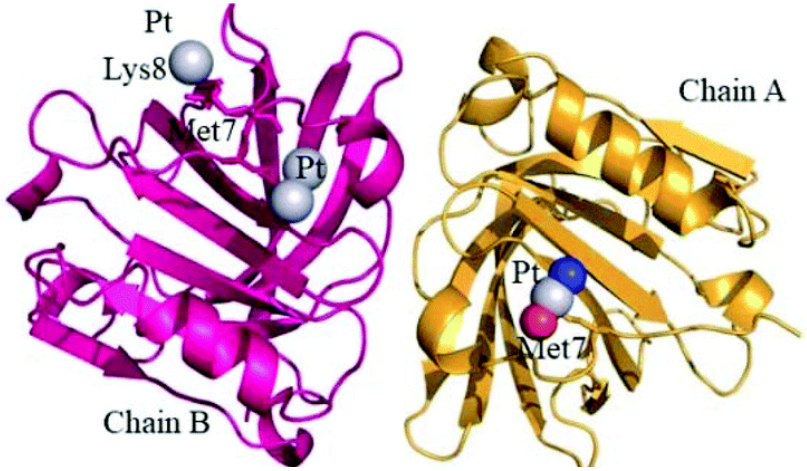


Results and discussion

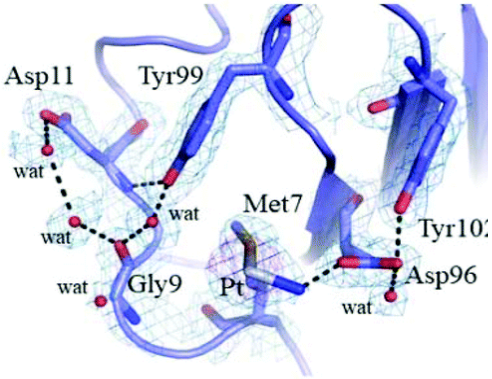
Crystallographic studies

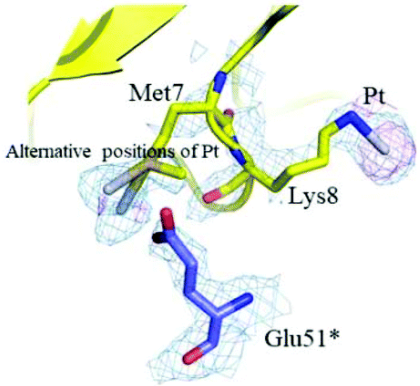
The structure of the CDDP/β-lactoglobulin adducts contain two molecules in the asymmetric unit

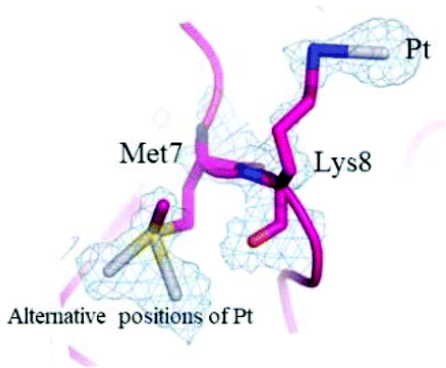
CDDP soaking for 18h

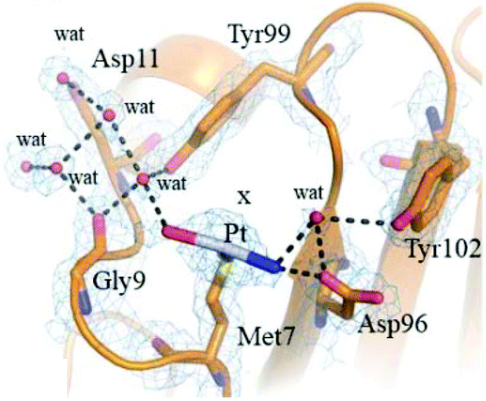


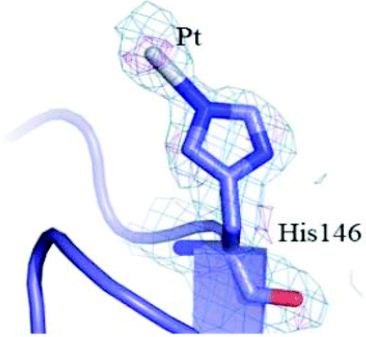
CDDP soaking for 72h

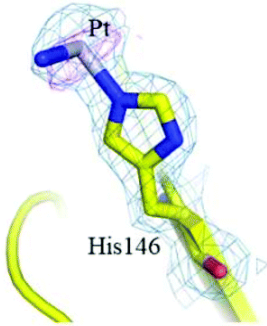








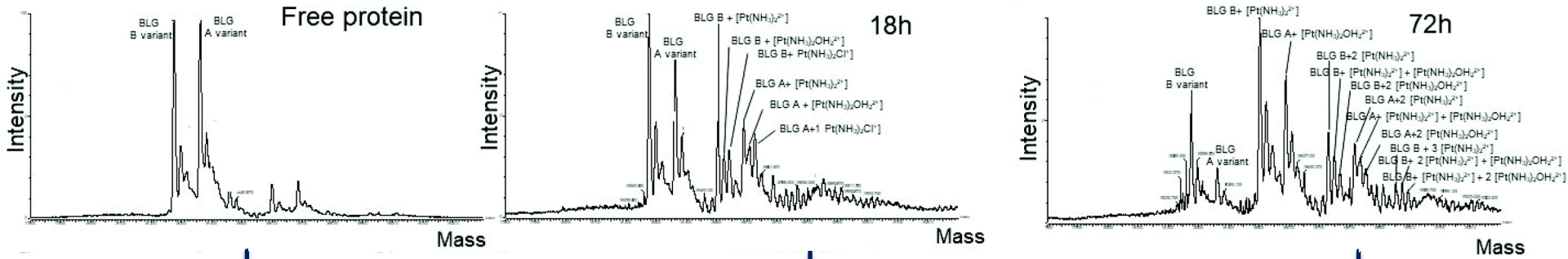


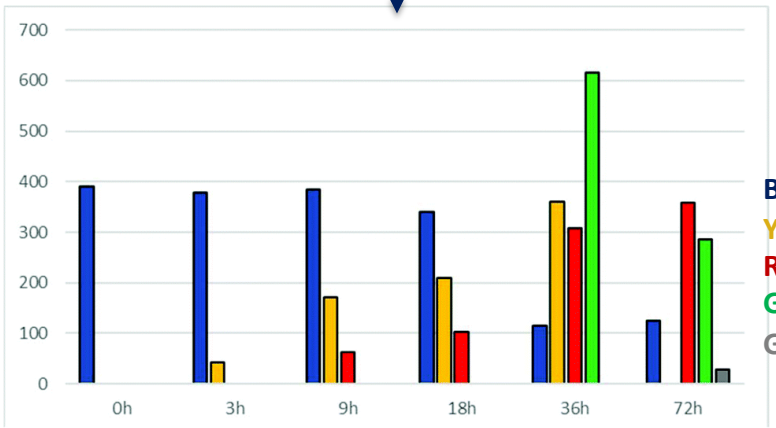


Results and discussion

Mass spectrometry analysis

Electrospray ionization mass spectra of the CDDP/β-lactoglobulin are collected as a function of time





Blue: Metal-free protein;
Yellow: **$[Pt(NH_3)_2Cl^+]$** or **$[Pt(NH_3)_2OH_2^+]$**;
Red: **$Pt(NH_3)_2^{2+}$**;
Green: Adduct with **2 CDDP** fragments;
Grey: Adduct with **3 CDDP** fragments.

Bar graph summarizes the results of mass spectrometry course experiments. Two forms of native protein are observed for A and B variants of the protein. After three hours the first Pt/β-lactoglobulin is formed. Longer reaction times (9h) give rise to another protein-metal fragment. Only after 36h the presence of two binding Pt sites is observed, while 72h are needed for observing adducts of the protein with three CDDP molecules

Conclusions

- The first structural study on the interaction of **β-lactoglobulin** with an anticancer metallodrug is reported;
- **In solution data** demonstrate that the protein binds CDDP without affect its overall conformation;
- **Crystallographic studies** underline the presence of binding sites of CDDP close to the side chain of **Met7**, **Lys8** and **His146**. β-lactoglobulin can form adducts with one Pt-containing fragment coordinating protein residues side chains;
- **ESI-MS analysis** reveals the presence of $[Pt(NH_3)Cl^+]$, $[Pt(NH_3)_2OH_2^+]$ and $[Pt(NH_3)_2^{2+}]$ fragments bond to the protein;
- The number of **CDDP** fragments embedded by protein increases with time;
- **Monodentate** and **bidentate** mode of Pt binding to the protein are possible, the former preceding the latter in coordination of the protein;
- Up to 3 **CDDP** molecules can bind the protein.

The interactions β-lactoglobulin with other metallodrugs is under investigation. Further efforts will be spent toward a rational design of new biomaterials based on metallodrug/β-lactoglobulin adduct nanoparticles.

Acknowledgments

I would like to thank the head of my research group, Prof Antonello Merlino and all the people who are involved in this progect, in particular:
Dr Giarita Ferraro;
Dr Nicole Balasco;
Prof Maria Monti;
PhD student Ilaria Iacobucci;