Effect of the sialic acid residues upon the binding of beta blocker propranolol to human serum alpha-1 acid glycoprotein

Robert Kerep*, Tino Šeba, Mario Gabričević
University of Zagreb, Faculty of Pharmacy and Biochemistry, A. Kovačić 1, Zagreb, Croatia
e-mail: rkerep@pharma.hr

Background

Plasma protein binding is a focus of great importance in the pharmaceutical science. Alpha-acid glycoprotein (AGP) is largely selective for basic and neutral drugs like propranolol (PRO) [1]. In healthy patients, the basal plasma concentration of AGP is cca 20 mM, whereas in some disease states it can increase up to 7-fold [2].

Since carbohydrate content of AGP is 45 %, which contain 14 sialic acid residues per molecule [3], it is believed that those residues might cause different binding affinity of drugs. As such, the free fraction of drug could change in plasma, which then affects the pharmacokinetics of drug itself.

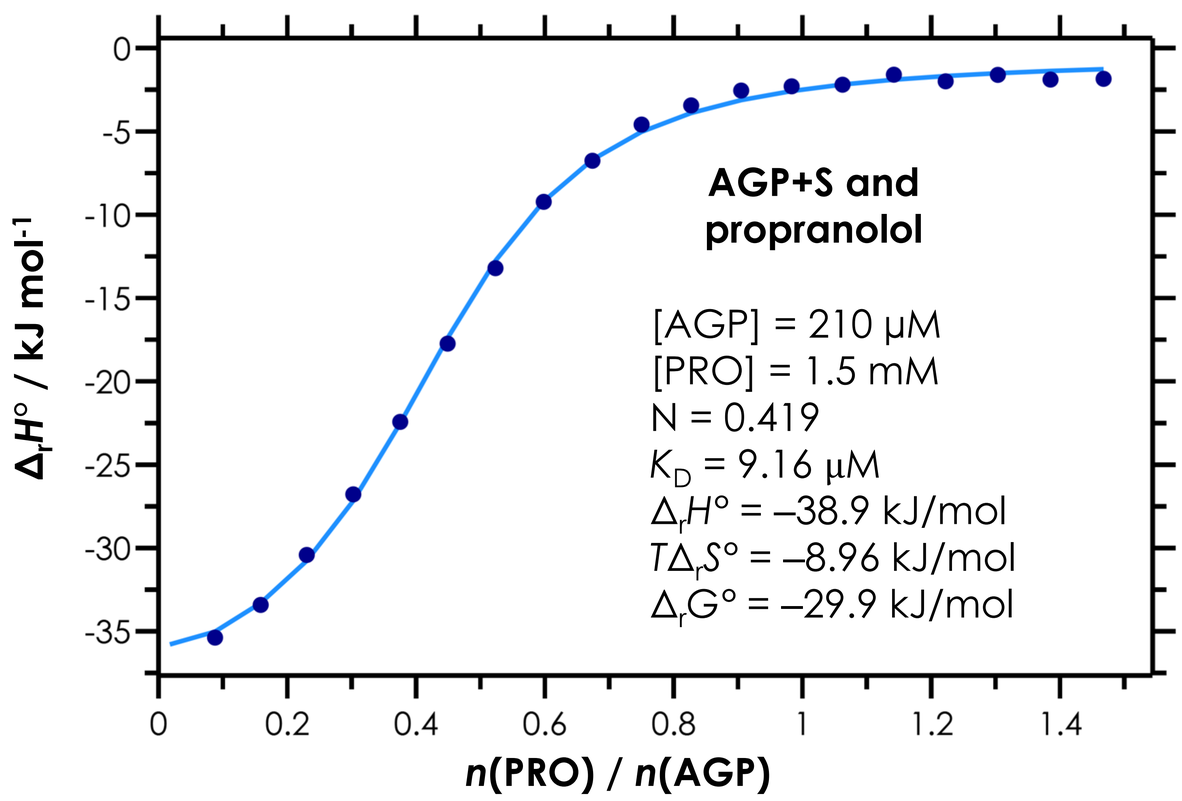
Determination of thermodynamic parameters, such as dissociation constant (K_D) could be valuable in preclinical studies where it is important to know the exact dosage of drug. Therefore, isothermal titration calorimetry (ITC) is very useful in the evaluation of thermodynamic parameters nedeed in drug development and further in dose optimization as well.

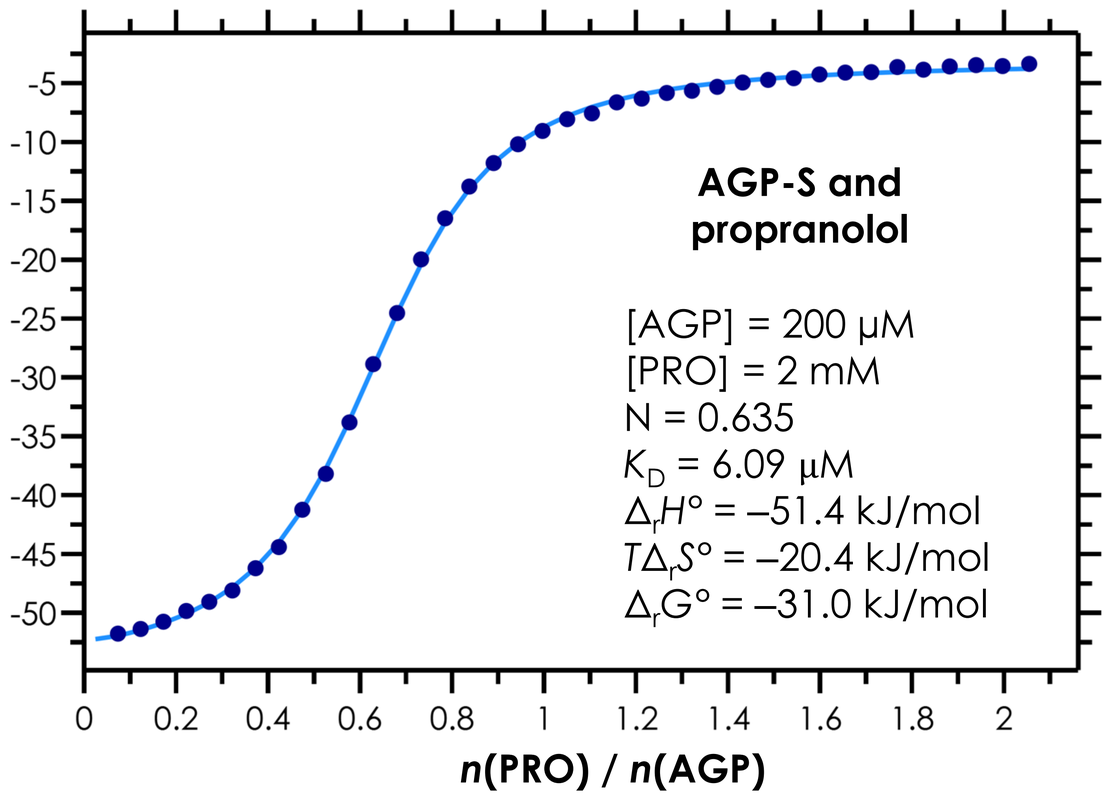
Experimental

Human AGP and PRO were obtained from Sigma-Aldrich. All other reagents were of analytical grade or better. Desialylated AGP was prepared by incubation of immobilized sialidase beads (SialEXO®) in the native human serum AGP buffered solution (2.5 mg/mL AGP, 25 mM HEPES, pH 7.4) at room temperature.

A MicroCal PEAQ-ITC calorimeter (Malvern, UK) was used for thermodynamic binding experiments. AGP samples in 25 mM HEPES buffer pH 7.4 were filled into the sample cell and titrated with a propranolol solution in protein buffer dialysate at 250 s intervals. The cell contents were stirred constantly at 700 rpm.

Results





Binding isotherms for the interaction of PRO with AGP forms monitored at 37 °C. Both charts show the binding enthalpy (kJ/mol) as a function of the PRO-AGP molar ratio. The insets show the thermodynamic parameters for interaction derived from a one site model. Values were negative for both $\Delta_r H°$ and $\Delta_r G°$ indicating an exothermic and spontaneous interaction and leads to favorable enthalpy meaning that interaction is dominant by hydrogen bonding and electrostatic forces while values for $\Delta_r S°$ are negative with unfavorable conformational change.

Conclusion

This study reported that ITC method could provide some valuable information to understand protein-drug interactions. ITC suggest that the interaction of PRO and AGP froms is an exothermic process and is driven mainly by enthalpy. The ITC measurements indicated that there is no significant difference in binding of PRO to AGP+S or AGP-S, although AGP+S did show weaker binding upon removal of sialic acid for 50 % compared to AGP-S.

References

[1] Israili ZH., Dayton PG., Drug Metab. Rev. 2001, 33, 161–235.
[2] Fernandez CL., Ligabue-Braun R., Verli H. Glycobiology 2015, 25, 1125-33.
[3] Schmid, K., et al., Biochim. Biophys. Acta 1977, 492, 291–302.

Acknowledgments

This work was supported by the Croatian Science Foundation under the project number IP-2016-06-3672 and EU European Regional Development Fund under the projects KK.01.1.09.0031 and KK.01.1.1.07.0055. The contents of this poster are the sole responsibility of the Faculty.