Fluorescence polarization binding characterization of dipyridamole and imatinib on alpha-1-acid glycoprotein

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Introduction

Alpha-1-acid glycoprotein (AGP) and albumin are two main plasma proteins involved in the binding and transport of many endogenous and exogenous ligands (Bteich, 2019). Apart from the ability to bind basic and neutral lipophilic drugs, AGP has some properties that differ from those of albumin such as relatively low concentration and one binding site which makes drug-binding to AGP saturable and displaceable. Altered AGP plasma concentration is found in various pathophysiological states which can potentially change the free drug fraction in plasma and possibly affect their pharmacokinetic and pharmacodynamic properties (Huang and Ung, 2013), thus, knowing the equilibrium binding constants is of great importance.

In this study, we present fluorescence polarization spectroscopy (FP), one of the techniques for protein-ligand binding measurements. It is based on the observation of emitted light with a certain degree of polarization as a result of ligand excitation by polarized light. The degree of polarization is inversely proportional to the molecular rotation rate. When a ligand (usually smaller than 1500 Da) is excited by polarized light, it emits depolarized light. If the ligand is bound to protein (usually above 10 kDa), emitted light will be polarized due to the significant reduction in the rotation of the ligand (Moerke, 2009). Fluorescence polarization assays are widely utilized in high-throughput screening and drug discovery due to the homogenous format, robust performance and relative insensitivity to some types of interferences, such as inner filter effects and low intrinsic fluorescence change of a protein upon ligand binding in tryptophan fluorescence quenching assays.

Materials and methods

The native human alpha-1-acid glycoprotein and dipyridamole (Dip) were obtained from Sigma-Aldrich (USA), and metasulfonic salt of imatinib (Ima) from LC Laboratories (USA). All other reagents were of analytical grade or higher. Fluorescence polarization ($\lambda_{excitation}$ = 414 nm; $\lambda_{emmision}$ = 494 nm) of samples was obtained on Tecan (Switzerland) Spark microplate reader using a black, 96-well, flat-bottom microplate.

Direct binding measurement of Dip to AGP

The titration experiment was carried out in a 25 mM sodium phosphate buffer with pH adjusted to 7.4. It consisted of 15 data points where the concentration of Dip was kept constant (0.5 µM) and AGP concentration ranged from 0 to 7.5 µM.

Competitive binding measurement of Ima to AGP in the presence of Dip as a fluorescent probe

A competitive binding assay was also carried out in a 25 mM sodium phosphate buffer with pH adjusted to 7.4. It consisted of 15 data points where the concentration of AGP and Dip was kept constant (5 µM and 0.5 µM respectively) and Ima mesylate concentration ranged from 0 to 250 µM.

Results and discussion

Fluorescence polarization data were analyzed in Microsoft Excel using Solver add-in. Association constant uncertainties were estimated using the "jackknife" procedure described by Harris (Harris, 1998).

AGP-Dip direct binding data analysis

The proposed binding model is given by the equation (1):

$$AGP + Dip \rightleftarrows AGP\text{-}Dip \quad (1)$$

Using the quadratic equation described by Roehrl et al. in 2004 and Pollard in 2010 for fitting the binding data, we obtained the equilibrium binding constant of **$2.80 \pm 0.03 \cdot 10^{-7}$ M**.

AGP-Ima competitive binding data analysis in the presence of Dip as a fluorescent probe

In competitive binding assay Dip and Ima compete for AGP's binding site. Binding is mutually exclusive and the overall binding model is given by the equations (1) and (2).

$$AGP + Ima \rightleftarrows AGP\text{-}Ima \quad (2)$$

A more complex cubic equation described by Roehrl et al. in 2004 is used for fitting competitive binding data. We obtained the AGP-Ima equilibrium binding constant of **$1.48 \pm 0.27 \cdot 10^{-7}$** M, while the AGP-Dip binding constant must be known beforehand.

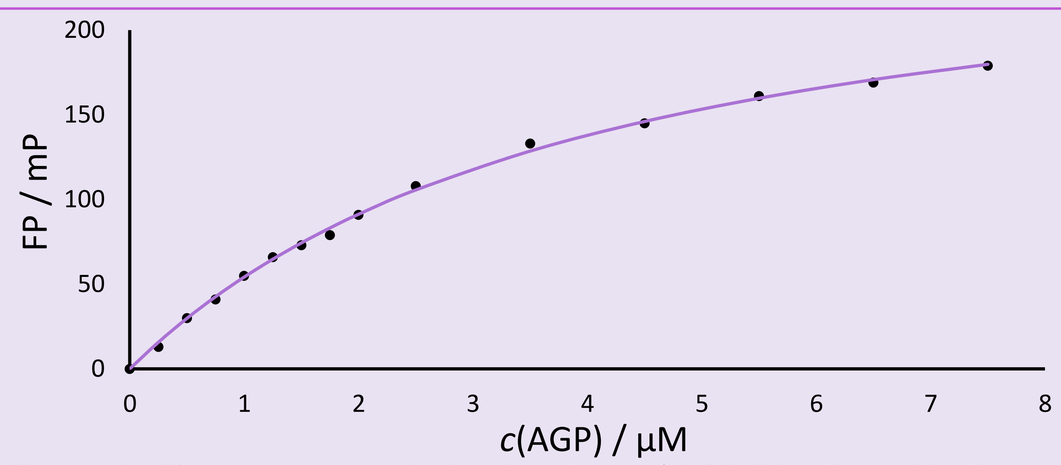

Figure 1. Fluorescence polarization data of AGP-Dip direct binding.

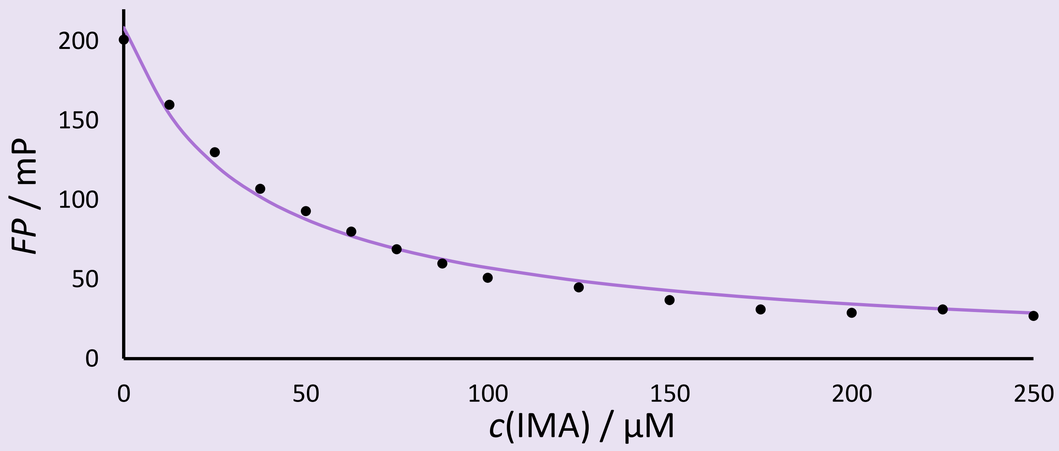

Figure 2. Fluorescence polarization data of AGP-Ima competitive binding in the presence of Dip as a fluorescent probe.

Conclusion

As we demonstrated, FP is a simple technique for AGP-drug binding characterization and can be used for other protein-ligand interactions. It is a simple mix-and-read protocol and does not require the separation of bound and free species. The choice of a fluorescent probe in FP assay is of key importance. Excitation and emission spectra must differ from the wavelengths of other molecules present in the solution to minimize autofluorescence and they must have sufficient Stokes shift to reduce the negative influence of light scattering.

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