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Characterization of binding mode of imatinib to human α_1-acid glycoprotein

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ABSTRACT

Imatinib (IMT) is a selective tyrosine kinase inhibitor, used in the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors. Its strong plasma protein binding was found to belong to the F1*S genetic variant of α_1-acid glycoprotein (AGP). In this work, comparative AGP binding studies were performed with IMT fragment molecules to reveal which parts of the molecule are important in the high-affinity interaction provoking specific spectral changes. Molecular modeling calculations indicated that IMT docked into the X-ray structure of AGP/F1 adopts a bent, compact conformation. This binding mode is similar to those found in its complexes with some low-affinity kinases and a quinone reductase, being strikingly different from the extended conformation of IMT in its high-affinity kinase targets.

1. Introduction

Binding of drugs to blood serum proteins influences their pharmacokinetic and pharmacodynamic action [1]. Among serum proteins, the most abundant albumin component is of highest importance [2]. Human α_1-acid glycoprotein (AGP) is an acute-phase serum component, with diverse biological activity [3]. Though its concentration in healthy subjects is about 30 times lower as compared to that of albumin, it plays decisive role in the plasma binding of many drug molecules [4]. The AGP molecule consists of a single polypeptide chain of 183 amino acids and of five asparaginyl-linked glycans. Native AGP isolated from plasma is not homogeneous, in addition to the heterogeneity of glycans, the protein part shows genetic polymorphism. AGP of most individuals is a mixture of two or three major genetic variants (F1 and/or S and A) which are encoded by two different genes [5]. Variants F1 and S (i.e. ORM1) are encoded by the alleles of the *AGP-A* gene, while variant A (i.e. ORM2) is the product of the *AGP-B/B'* gene [6]. F1 and A differ by 22/183 amino acid units, while F1 and S have only a single Gln/Arg substitution at position 20 [7]. Commercial pooled AGP can be separated by chromatography resulting in about 70% F1 and S mixture (F1*S) and 30% variant A [8]. The genetic variant composition of individuals, however, shows large variability [9]. The F1*S and A genetic variants were found to have different ligand binding characteristics [10–14]. The exact tertiary structure of AGP has been reported only recently, determining the X-ray structures of the recombinant unglycosylated F1 [15] and A [16] variants. It was also proven that the oligosaccharide moieties have negligible effects on the structural and ligand binding properties of AGP [17]. The lipocalin-type protein fold comprises an eight-stranded β-barrel, with a central cleft-like ligand-binding cavity. The binding pocket of the F1 variant is wide and consists of three lobes (I–III), while in the A variant lobe III is missing.

Imatinib (Glivec®) is a rationally designed specific tyrosine kinase inhibitor, effectively binds to a limited number of kinases [18,19]. It is very successful in the treatment of chronic myeloid leukemia, gastrointestinal stromal tumors and may have further therapeutic applications [19,20]. Some other molecular targets have also been revealed by chemical proteomic approach [21]. Imatinib mesylate (IMT, Fig. 1) has strong plasma protein binding, which can be referred to the AGP component [22,23]. Since AGP is a positive acute phase protein, the large interindividual variability in its plasma concentration is a factor to be considered in IMT therapy [24]. The role of enhanced AGP level was proven in preventing the effect of IMT in some patients [25] and in mice with pulmonary fibrosis [26]. In those cases, the activity of IMT could be restored in the presence of drugs, e.g. clindamycin (CLI) and erythromycin, which are able to displace IMT bound to AGP.

Abbreviations: AGP, α_1-acid glycoprotein; IMT, imatinib; TNS, 2-(p-toluidino)naphthalene-6-sulfonic acid; ANS, (8-anilinonaphthalene)-1-sulfonic acid; QR, quinaldine red; CLI, clindamycin; ICD, induced circular dichroism; CE, Cotton effect.

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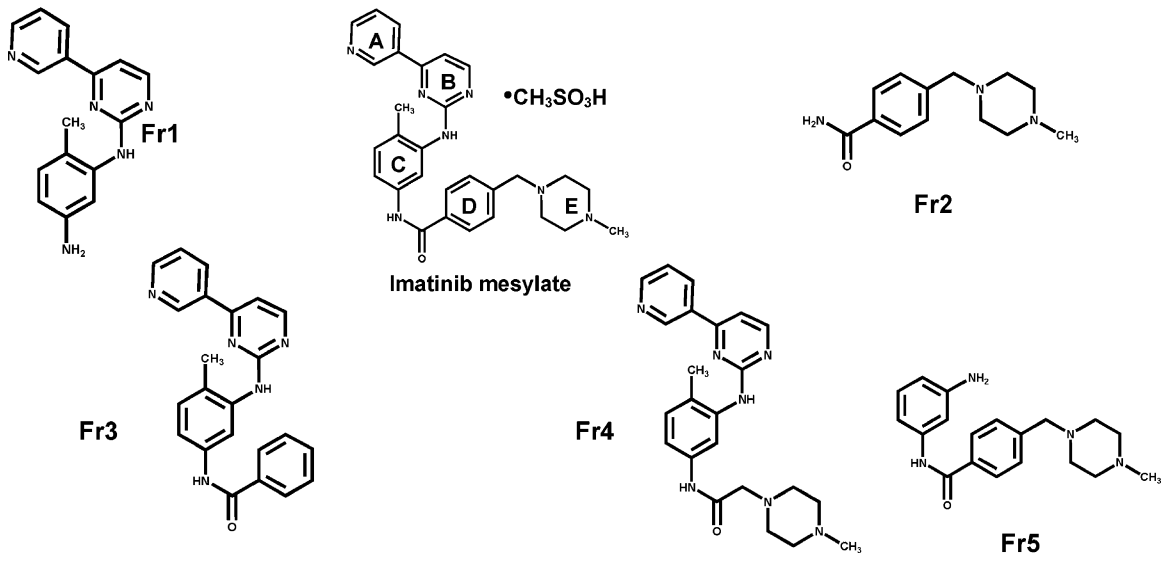

Fig. 1. Chemical structures of ligands.

In an earlier study [27] we proved that the high-affinity and specific AGP binding of IMT belongs to the F1*S genetic variant. The association binding constant (K_a) value is $1.7(\pm 0.2) \times 10^6\ M^{-1}$ and the found number of binding site (0.94) indicates no difference between the F1 and S variants. This interaction is manifested in induced circular dichroism (ICD) and difference UV spectra, as well as in quenching of the intrinsic fluorescence of the protein and displacement of specific label molecules. IMT binding to the A variant is weaker and lacks specific spectral changes. The binding of two IMT fragment molecules, Fr1 and Fr2 (Fig. 1) was also studied, proving impaired binding without ICD for Fr1 and lack of binding for Fr2. In this work, further studies were carried out to characterize the molecular details of IMT binding to AGP. In order to identify which parts of the five-ring molecule are important in the specific interaction, comparative binding studies were performed with fragment molecules Fr3, Fr4 and Fr5 (Fig. 1), applying CD–UV and fluorescence spectroscopic methods. Molecular modeling calculations were also carried out to characterize the variant selective accommodation of IMT within AGP, using the crystal structures of the recombinant F1 and A variants. The conformation of IMT bound

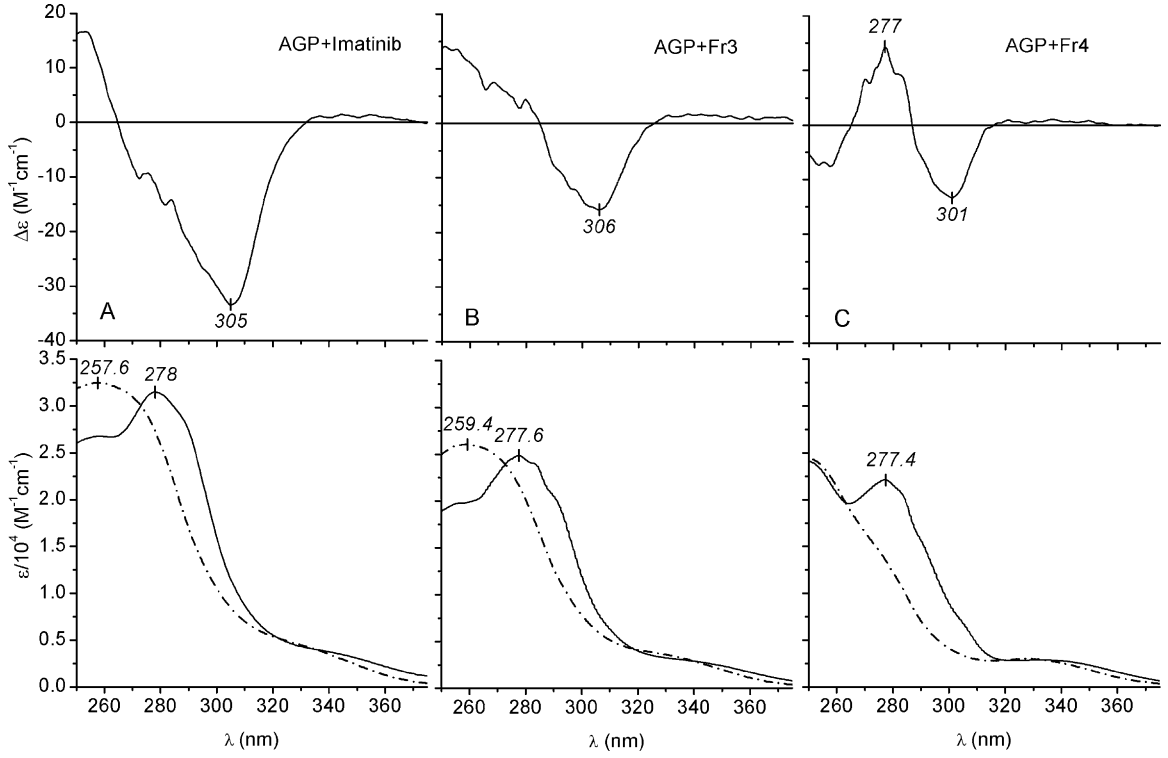

Fig. 2. Induced CD and difference UV spectra of IMT, Fr3 and Fr4 bound to AGP/F1*S variant, as well as UV spectra of free ligands (dotted lines). Molar parameters were calculated from spectra taken in solutions containing (A): 10 μM IMT and 34 μM AGP, (B): 13 μM Fr3 and 42 μM AGP, (C): 13 μM Fr4 and 42 μM AGP, using total ligand concentrations.