Structural Basis for Differences in Plasma Protein Binding Among Cephalosporins: Insights from Human Serum Albumin Complexes

Cephalosporins are widely used antibiotics that have long been considered safe. However, despite decades of clinical use, fundamental pharmacokinetic details such as plasma protein binding have been limited. This knowledge gap holds clinical significance because protein binding influences drug distribution, duration, and potential interactions with other medications, which is a growing concern amid increasing polypharmacy. In this study, we reported the molecular interactions between two frequently used cephalosporins, ceftriaxone and cefazolin, and human serum albumin. Our structural insights advance understanding of these interactions and contribute to more informed, safer, and effective use of these established therapeutic agents.

Cephalosporins are a subclass of β-lactam antibiotics routinely prescribed for the treatment of bacterial infections. Their structure consists of a conserved cephem core and two side chains, R1 and R2, located at the C7 and C3 positions, respectively (Fig. 1(a)). Modification of these side chains has been the primary strategy to broaden the antimicrobial spectrum of cephalosporins and confer resistance to β-lactamase-mediated hydrolysis. These structural changes also result in varying degrees of plasma protein binding, especially with human serum albumin (HSA). While it has been recognized that cephalosporins differ in their plasma protein binding affinities, the structural mechanisms underlying these differences had remained unclear. Kanis et al. reported that a net negative charge and the presence of a thiomethylene-linked aromatic nitrogen heterocycle at the R2 position are associated with increased plasma protein binding (Fig. 1(b)) [1]. However, the precise binding site for cephalosporins on HSA had not been definitively identified. To address this gap, we determined the crystal structures of HSA in complex with two clinically relevant cephalosporins ceftriaxone and cefazolin [2].

We prepared crystals of the HSA–cephalosporin complexes, transported them to beamlines BL-1A or BL-17A and collected X-ray diffraction datasets. The crystal structure of the HSA–cephalosporin complex revealed that both cephalosporins bind specifically to subdomain IB of HSA (Fig. 2(a)). In both complexes, the cephem core is located at the center of a channel within subdomain IB, while the R1 and R2 side chains extend into distinct cavities at either end of the channel. The binding modes of ceftriaxone and cefazolin are highly similar, suggesting a conserved mechanism of recognition among cephalosporins. A key interaction involves hydrogen bonding between the β-lactam carbonyl and carboxyl groups of the cephem core and Arg117 in HSA (Fig. 2(b, c)). The R1 side chain is accommodated in a hydrophobic chamber formed by Pro118, Met123, and Phe134. In contrast, the R2 side chain is stabilized in a distinct chamber shaped by Tyr138, Ile142, His146, Arg186, Gly189, and Lys190. The structure and composition of this R2 chamber appear to be critical determinants of cephalosporin binding affinity.

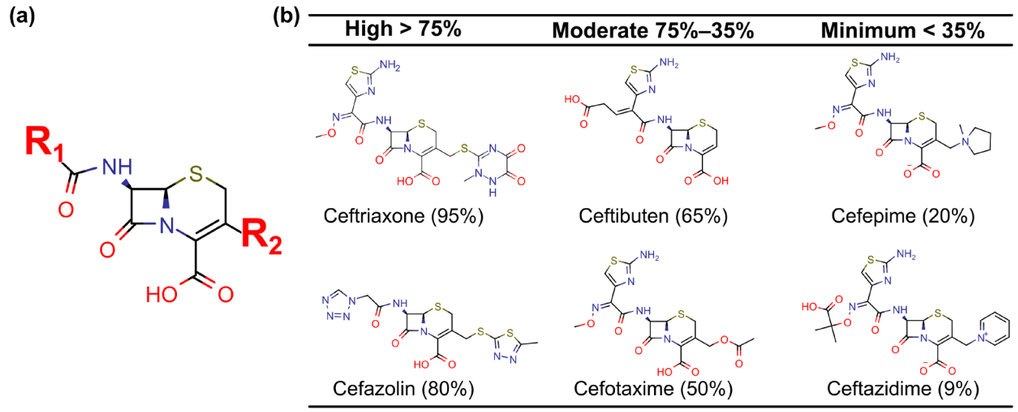

Figure 1: Chemical structure of cephalosporins. (a) Cephem core structure. (b) Representative cephalosporin structures and their plasma protein binding rates.

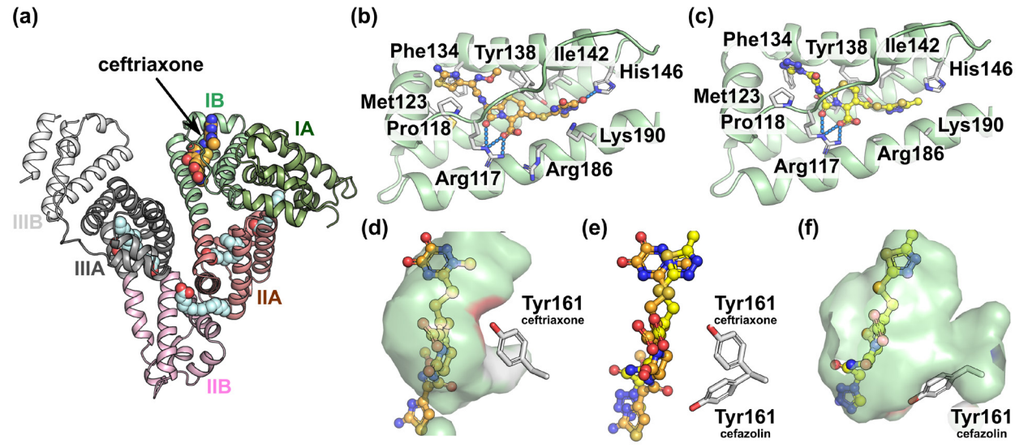

Figure 2: (a) Overall structure of the HSA–ceftriaxone complex. (b–f) Close-up views of the binding sites for ceftriaxone (orange; b, d), cefazolin (yellow; c, f) and superposition of the binding sites (e). Hydrogen bonds are shown as blue dashed lines (b, c). Transparent molecular surfaces are depicted in green (d, f).

One of the most notable findings in our study is the role of Tyr161, whose side chain undergoes a rotameric shift depending on the cephalosporin structure (Fig. 2(d–f)). In the ceftriaxone-bound form, the bulky R1 side chain induces an approximately 80° rotation of Tyr161, reshaping the R2 cavity into a narrower, more compact form. This conformational change creates a space that selectively accommodates the thiomethylene linker present in ceftriaxone. In contrast, in the cefazolin-bound structure, Tyr161 retains a rotamer conformation similar to that observed in the ligand-free state, resulting in a wider R2 cavity that can accommodate slightly bulkier substituents. These observations suggest that Tyr161 acts as a gatekeeper residue, modulating the R2 binding environment in response to the steric characteristics of the bound ligand. Ceftazidime, an agent with low plasma protein binding, also possesses a bulky R1 side chain similar to ceftriaxone (Fig. 1(b)) and would require a similar Tyr161 rotation if it adopted a similar binding pose. However, its R2 side chain is connected via a shorter linker, which is one atom shorter than that of ceftriaxone, to a pyridinium moiety. This structural difference between ceftazidime and ceftriaxone suggests that, although the narrowed space can accommodate the thiomethylene linker of ceftriaxone without significant steric hindrance, it may not be compatible with the shorter linker of ceftazidime. As a result, steric clashes are likely to contribute to ceftazidime's reduced binding to HSA. Furthermore, cephalosporins with moderate plasma protein binding tend to have non-aromatic R2 groups, which may fail to form favorable interactions within the R2 chamber, further diminishing their binding affinity (Fig. 1(b)).

In conclusion, we identify subdomain IB of HSA as the principal binding site for cephalosporins with high plasma protein affinity. Our findings underscore the importance of geometric complementarity within the R2 chamber. The rotameric flexibility of Tyr161 emerges as a key structural feature that allows HSA to accommodate diverse cephalosporin scaffolds. These insights deepen our understanding of the structural basis of cephalosporin–HSA interactions and are expected to inform future antibiotic design and the clinical optimization of dosing strategies.

REFERENCES

[1] E. Kanis, J. Parks and D.L. Austin, *ACS Pharmacol. Transl. Sci.*, **6**, 88 (2023).
[2] A. Kawai, K. Yamasaki, M. Otagiri and Y. Doi, *J. Med. Chem.*, **67**, 14175 (2024).

BEAMLINES

BL-1A and BL-17A

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