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Introduction

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Introduction

Since the observation in 1959 by Van Heyningen (Nature, **184**, 194–195) that diabetes complications such as cataracts can be linked to the formation of polyols such as sorbitol, the polyol pathway has been the object of numerous studies. Aldose reductase (ALR2), the first enzyme in the polyol pathway, reduces glucose into sorbitol. The mechanism includes a hydride donation by NADPH, which becomes $NADP^+$, and a proton donation by the enzyme. ALR2 in particular has been studied, including structure determination by X-ray crystallography, and a large number of ALR2-inhibitor complexes have been developed and analyzed. However, only one of these inhibitors (epalrestat) is currently in the market, so that developing new inhibitors is the goal of a continuous effort. The purpose of the multi-author review series presented here is to provide up-to-date information about ALR2, its inhibitors and efforts to develop new ones.

In the first article, Mark J. Petrash describes the structural and functional features of ALR2 from the biological perspective. He focuses on the conformational changes involved in $NADP^+$ release, and on the residues involved in proton donation. He also describes the general kinetic features of the enzyme, which has a rather poor efficiency for glucose, implying that ALR2 is maximally active only when intracellular hexose concentrations rise to abnormally high levels. He then reviews the roles of ALR2 in metabolism and signalling, including osmotic regulation, detoxification and protein kinase C activation. He also reviews the genetic complexity of AR and related enzymes, such as FR-1, MVDP and tumor-associated aldo-keto reductases. This complexity suggests that AR inhibitors might have different pathways, including unexpected targets.

In the second article, El-Kabbani et al. review the development of ALR2 inhibitors (ARIs). He starts with the identification of the active site residues involved in the enzymatic mechanism, which provide the anchoring point for inhibitors. This chapter includes a detailed review of the proposals for the catalytic mechanism. He then classifies the existing inhibitors, which contain either a cyclic imide group, such as a spirohydantoin group or a spirosuccinimide group, or an acetic acid moiety. These head groups bind in the active site, notably to His 110, Tyr 48 and Trp 111. They occupy the 'anionic pocket', defined by His 110, Tyr 48 and $NADP^+$. Another common feature among the various inhibitors is the presence of one or more aromatic groups, which bind in the hydrophobic 'specificity' pocket of ALR2, bordered by the Trp111, Phe122 and Leu300 residues, either through hydrogen bonding or hydrophobic contact. El-Kabbani et al. then review inhibitor design based on active site residues, leading to an overview of structure-based and de novo design of ARIs and to crystallographic and modelling studies of ALR2-inhibitor complexes. These modelling studies include a three-dimensional database for ligand screening and molecular docking, and more advanced techniques such as free energy perturbation (FEP) methods. Finally, El-Kabbani et al. cite site-directed mutagenesis studies.

The recent determination of several complexes at subatomic resolution has revealed new details of protein structure and of inhibitor binding. These results are reviewed in the third article, by Podjarny et al. The structures reviewed are the complexes of ALR2 with IDD 594 (resolution: 0.66 Å, IC50 (concentration of inhibitor that produced half-maximal effect): 30 nM, space group: P21), IDD 393 (resolution: 0.90 Å, IC50: 6 nM, space group: P1), Fidarestat (resolution: 0.90 Å, IC50: 9 nM, space group: P21) and Minalrestat (resolution: 1.10 Å, IC50: 73 nM, space group: P1). The article compares the structures, which are highly reproductible within the same space group (RMS deviations: 0.15 ~ 0.3 Å). It then analyses the mode of binding of the carboxylate inhibitors IDD 594 and IDD 393. Binding of the carboxylate head can be accurately determined by the subatomic resolution structures, since both the protonation states

and the positions of the atoms are very precisely known. The differences appear in the binding in the specificity pocket. The high-resolution structures explain the differences in IC50, which are confirmed both experimentally by mass spectrometry measures of VC50 and theoretically by FEP calculations. The article then reviews binding of the cyclic imide inhibitors fidarestat and minalrestat, focusing on the observation of a Cl^- ion which binds simultaneously with fidarestat. The presence of this anion, binding also to the active site residue His 110, leads to a mechanism in which the inhibitor can bind in a neutral state and then become charged inside the active site pocket. This mechanism can explain the excellent in vivo properties of cyclic imide inhibitors. In summary, the complete and detailed information supplied by the subatomic resolution structures can explain the differences in binding energy of the different inhibitors.

In the fourth article, Lecomte et al. go further in the interpretation of the subatomic resolution diffraction data by interpreting it with a multipolar model, in which the electron density around each atom is expanded in a spherical harmonics series. The multipolar model, as implemented in the program MOPRO, allows a more precise refinement of the deviations of the electron density from that corresponding to a spherical model (deformation density). In his review, Lecomte et al. explain the methodology underlying the multipolar model approach, and its implementation in the program MOPRO, which uses an existing database of multipoles as a starting point of refinement. They then describe how the multipolar model allows a direct calculation of the electrostatic potential, and application to three cases: toxin II of the Androctonus Australis Hector scorpion (0.96 Å resolution), the protein crambin (0.54 Å resolution) and the ALR2-IDD594 complex (0.66 Å resolution).

In the fifth and last article of the series, Klebe et al. describe their efforts to model in silico the interactions of ALR2 with its inhibitors. ALR2 is known to operate on a broad palette of structurally diverse substrates ranging from small aliphatic and aromatic aldehydes up to steroid-type ligands. The enzyme achieves its substrate promiscuity via pronounced induced-fit adaptations; a comparison of existing crystallographic structures shows four major conformations suitable for binding. To treat this particular case, Klebe et al. followed two distinct methods. In the first one, both a conformation and ligands which bound favorably to this conformation were selected. In the second, a superposition of molecules bound to the four different conformations was used as a scaffold to which possible new ligands were aligned. Both strategies resulted in ligands binding in the micromolar range.

The full series of articles gives an overall accurate review of the state-of-the-art techniques used in AR research, ranging from biochemical and genetic observations and experimentally determined complex structures to electrostatic energy potentials obtained from multipolar models and algorithms to computationally obtain new possible ligands from existing structures. The unique characteristic of the AR studies is that the subatomic resolution structures have enable bridging the gap between biology and physics, e. g. by showing the detailed electronic basis for ligand binding. This information can then be successfully used for the design of new inhibitors.

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