Inhibitors of the catalytic domain of mitochondrial ATP synthase

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Abstract
An understanding of the mechanism of ATP synthase requires an explanation of how inhibitors act. The catalytic F1-ATPase domain of the enzyme has been studied extensively by X-ray crystallography in a variety of inhibited states. Four independent inhibitory sites have been identified by high-resolution structural studies. They are the catalytic site, and the binding sites for the antibiotics aurovertin and efrapeptin and for the natural inhibitor protein, IF1.

Introduction
The ATP synthases (F1Fo-ATPases) are multisubunit assemblies found in the membranes of bacteria, the thylakoid membranes of chloroplasts and the inner membranes of mitochondria. They couple the transmembrane PMF (protonmotive force) with the synthesis of cellular ATP from ADP and P↓ν. They consist of two main structural domains, a globular catalytic domain known as F1 and a proton-translocating membrane-embedded domain, F↓o, linked by central and peripheral stalks [1]. The architecture of the mitochondrial enzyme is shown in Figure 1. The F↓1 has the subunit stoichiometry α↓3β↓3γ↓δε. The γ-, δ- and ε-subunits form the central stalk in which an α-helical coiled coil in the γ-subunit penetrates into the α↓3β↓3 subassembly and provides a central axis around which the three β-subunits are arranged and interspersed by the three α-subunits [2]. The lower region of the central stalk is attached to a ring of hydrophobic c-subunits in the F↓o domain. The synthesis of ATP is coupled with the PMF by a mechanical rotary mechanism. The passage of protons from the intermembrane space into the matrix via channels at the interface between the c-ring and another hydrophobic protein, subunit a (or ATPase-6), drives a rotation of the c-ring and attached central stalk [3]. The three catalytic sites are in the β-subunits at interfaces with the non-catalytic α-subunits. Their nucleotide binding properties and catalytic activities are modulated in a cyclical manner by the clockwise rotation (as viewed from the membrane) [4] of the central α-helical structure of the γ-subunit to promote ATP synthesis. During hydrolysis, energy released from the hydrolytic reaction drives the counterclockwise rotation of the central stalk and c-ring, and protons are ejected from the mitochondria. The peripheral stalk [subunits b, d, F↓6 and OSCP (oligomycin-sensitivity conferral protein)] links subunit a to the external surface of the F↓1 domain, and it is thought to act as a stator to counter the tendency of the α↓3β↓3 subcomplex to follow the rotation of the central stalk [5].

In the first crystal structure of the F↓1-ATPase, referred to as the reference state, one subunit (β↓DP) binds ADP, the second (β↓TP) binds ATP and the third subunit (β↓E) is in a conformation that has very low affinity for nucleotide [2]. These conformations correspond to the ‘tight’, ‘loose’ and ‘empty’ or ‘open’ states of the binding change mechanism respectively.

Figure 1 | Schematic diagram of the mitochondrial ATP synthase
The β-subunit closest to the viewer is not shown for clarity. The components of the rotor of the enzyme are outlined in black. Minor subunits (e, f, g and A6L) in the F↓o domain are not shown. They have no known functions in the enzyme’s mechanism. The inhibitor protein IF↓1 is also not shown.

Key words: ATPase synthase, aurovertin, catalytic domain, crystal structure, inhibitory site, mitochondrion.

Abbreviations used: p[′NH]↓3pA, adenosine 5-[(α,γ-imidohydroxypentyl)phosphophate; PMF, protonmotive force.

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**Figure 2** Inhibitory sites in F$_1$-ATPase from X-ray crystallography

(A) View of the F$_1$-ATPase inhibited by efrapeptin [12] along the central axis (left panel) from the membrane towards the catalytic sites. Side view (right panel) showing the interacting β-subunit and the γ-subunit. Panels (B) and (C) show same views as above of the F$_1$-ATPase inhibited by aurovertin B [13] and IF$_1$ [14] respectively. Each of the inhibitors binds to a distinct site separate from the catalytic site. The α-, β- and γ-subunits are shown in red, yellow and blue respectively. The inhibitors are shown in green and nucleotides are coloured black. Images were produced with the program PyMOL (DeLano Scientific, San Carlos, CA, U.S.A.).
The rotary mechanism of ATP synthesis is consistent with the cyclic modulation of nucleotide affinity in the catalytic β-subunits required by the binding change mechanism [6].

Inhibitor studies

A variety of covalent and non-covalent inhibitors of the mitochondrial F₁-ATPase have been identified. For a recent review, see [7]. Covalent inhibitors include Nbf-Cl (4-chloro-7-nitrobenzofurazan), DCCD (dicyclohexylcarbodi-imide) and several reactive derivatives of ATP and ADP. The noncovalent inhibitors include non-hydrolysable substrate analogues, azide, the natural inhibitor protein IF₁, the efrapeptins, the aurovertins, dietary phyto-polyphenols, non-pentidyl lipophilic cations and amphiphilic peptides.

Over the past 10 years, bovine F₁-ATPase has been studied extensively by X-ray crystallography. Several structures have been identified intermediates in the catalytic cycle of the enzyme, while others have allowed the determination of the binding site for inhibitors. Four sites of inhibition in the bovine F₁-ATPase have been defined by structural analysis. They are the catalytic site and three allosteric inhibitory sites. The non-hydrolysable nucleotide triphosphate analogues p[NH]ppA (adenosine 5′-β,γ-imido) triphosphate), and several metallofluoride complexes (ADP-AlF₃, ADP-AlF₄ and ADP-BeF₃⁻) bind to the catalytic nucleotide-binding sites found predominantly in the β-subunits (with essential contributions, notably Arg₃⁷₅) from the adjacent α-subunits. p[NH]ppA and ADP-BeF₃⁻ represent good analogues of ATP [2,8]. The coplanar arrangement of the fluorine atoms in the aluminium fluoride complexes mimic the pentacoordinate transition state in the hydrolysis reaction involving a trigonal bipyramidal phosphoryl group [9,10]. Azide has recently been shown to bind to the βD₃ catalytic site where it stabilizes the state with bound ADP [11]. The series of structures inhibited by these analogues has helped to define the catalytic pathway of ATP hydrolysis by F₁-ATPase. The covalent inhibitors of ATP hydrolysis act by modification of residues at or near the catalytic sites. For a review, see [7].

The allosteric inhibitory sites include the binding sites for the aurovertins, the efrapeptins and for the natural inhibitor protein IF₁ (see Figure 2). The efrapeptins are a family of peptide antibiotics produced by the fungus Tolypocladium niveum, and are potent inhibitors of the ATP synthase in mitochondria and some bacterial species. The structure of bovine F₁-ATPase in complex with efrapeptin shows that the antibiotic binds to a unique site across the central cavity of the enzyme. It interacts with the γ- and βE-subunits, and with the two adjacent α-subunits via hydrophobic contacts (Figure 2A). It prevents the βE-subunit from closing and gaining the capacity to bind nucleotides, as required by the binding change mechanism [12].

The aurovertin family of antibiotics also inhibit the ATP synthase in mitochondria and many bacterial species. In the structure of bovine F₁-ATPase in complex with aurovertin B, the inhibitor is bound to two equivalent sites in subunits βD₃ and βE in a cleft between the nucleotide-binding and C-terminal domains (Figure 2B). The two sites vary in their affinities for aurovertin and the higher affinity site is interpreted to be in the βD₃ subunit. In the βD₄ subunit, the binding site is inaccessible to the inhibitor. Aurovertin appears to inhibit F₁-ATPase by preventing closure of the catalytic interfaces necessary for the cyclic interconversion of catalytic sites [13].

In mitochondria, the hydrolytic action of ATP synthase is prevented under conditions when the PMF has collapsed, for example during ischaemia, by a natural inhibitor protein, IF₁. The active bovine IF₁ is an α-helical dimer with monomers associated via an antiparallel coiled coil between their C-terminal regions. In the structure of the dimeric F₁–IF₁ complex, the N-terminal regions of the dimeric inhibitor are bound to two F₁-ATPase complexes. IF₁ is bound to the catalytic interface between the C-terminal domains of the αD₃- and βD₄-subunits, and makes contacts with the γ-subunit (Figure 2C). Inhibition is achieved primarily through the prevention of conformational changes necessary for rotary catalysis [14]. Other amphiphilic α-helical peptides may bind at or near the same inhibitory site as IF₁ [15].

Concluding remarks

Inhibited forms of the bovine mitochondrial F₁-ATPase have proved amenable to high-resolution structural analysis. These studies have defined the catalytic pathway of ATP hydrolysis and elucidated the binding sites and mechanisms of action of several inhibitors. A detailed understanding of the inhibitors of ATP synthase may also be of significant medical interest. The structures of the F₁-ATPase in complex with the medically relevant dietary phyto-polyphenols resveratrol, piceatannol and quercetin have now been determined and have enabled elucidation of their binding sites and modes of action (J.R. Gledhill, A.G.W. Leslie and J.E. Walker, unpublished work). In addition, a high-resolution structure of the inhibited F₁–IF₁ complex formed in the presence of ATP has been determined, providing additional information regarding the mechanism of action of the inhibitor protein (J.R. Gledhill, M.G. Montgomery, A.G.W. Leslie and J.E. Walker, unpublished work).

References


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