Anion–π Interactions

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Relevant Anion–π Interactions in Biological Systems: The Case of Urate Oxidase**

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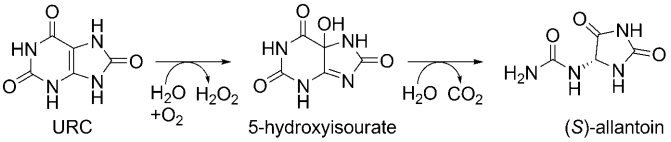
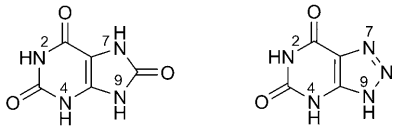
Supramolecular chemistry involves the intelligent utilization of noncovalent interactions between molecules. All biological systems are based on these impressively efficient interactions.[1] Interactions between predesigned binding centers can lead to complex functions in highly organized molecular systems, which is one fundamental aspect of supramolecular chemistry.[2] The importance of anion–π interactions has been widely recognized[3] and has led to many theoretical and experimental investigations.[4] Several pioneering theoretical studies revealed that these interactions are energetically favorable.[5] Anion–π interactions are gaining significant interest; for instance, Matile and co-workers have reported remarkable synthetic ion channels based on anion–π interactions.[6] However, clear evidence of anion–π interactions that likely play a key role in enzymes is lacking in the literature. The ultimate step to prove the importance of this noncovalent interaction is to demonstrate its crucial role in a biological system.

Herein we report several selected examples retrieved from the Protein Data Bank (PDB).[7] In these examples, relevant anion–π interactions are present in the active site of the urate oxidase enzyme, and lead either to interactions with the substrate (uric acid) that inhibits the enzymatic activity or to interactions with the inhibitor (8-azaxanthine). In addition, taking advantage of quantum mechanical calculations, we demonstrate that the interactions observed in the solid state are relevant and energetically favorable.

Urate oxidase (UOX, EC 1.7.3.3) is a homotetrameric cofactorless enzyme, which in the presence of molecular oxygen catalyzes the hydroxylation of uric acid (URC) to (*S*)-allantoin through a specific enzymatic cascade that involves the formation of 5-hydroxyisourate (see Scheme 1). Curiously, this enzyme is absent in humans, which is considered to be an evolutionary step since URC is a powerful antioxidant. Recently, this enzyme has been the object of several investigations and the X-ray structures of several complexes of UOX with the substrate, the inhibitor, and both have been resolved.[8]



Scheme 1. Uric acid degradation pathway.



It is known that UOX is inhibited in solution by cyanide ions, which results in a loss of enzyme activity of 90%, and to a lesser extent by azide ions (up to 40% loss of enzyme activity).[9] The location of the cyanide ion, which probably replaces either the molecular dioxygen involved in the hydroperoxy intermediate formation or the water molecule involved in the hydroxylation of the dehydrourate intermediate, suggests that the cyanide ion hinders any access to the peroxo site in the course of the reaction. The inhibition of UOX by cyanide ions is noncompetitive with urate and is perfectly reversible.[10] In Figure 1 we show the X-ray structure (asymmetric unit) of the UOX/URC/CN^- complex retrieved from the PDB.

Unfortunately, all attempts to crystallize the enzyme with the substrate and molecular oxygen have been unsuccessful. However the complex of one inhibitor of this enzyme, that is, 8-azaxanthine (AZA), with molecular oxygen and UOX has been resolved by oxygen-pressurized X-ray crystallography.[8b] The ternary UOX/AZA/O_2 complex is shown in Figure 2 (left). The molecular oxygen binds within the active

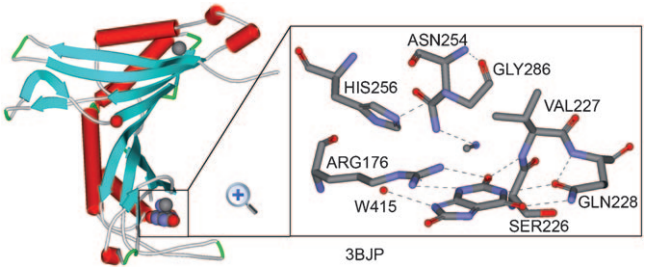


Figure 1. Representation of one subunit of UOX (PDB code 3BJP) and an enlargement of the active site with indication of the amino acids.

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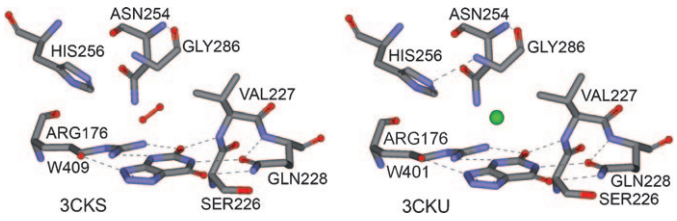

Figure 2. Representation of the active site of the UOX complexed to the inhibitor 8-azaxantine (AZA) and dioxygen (left, 3CKS) or a chloride ion (right, 3CKU).

Table 1: Interaction energies without and with the BSSE correction (E and E_{BSSE}).[a]

Complex	aug-cc-pVDZ		aug-cc-pVTZ		CBS
	E	E_{BSSE}	E	E_{BSSE}	
URC···CN^-	−18.9	−16.4	−18.1	−16.9	−17.7
AZA···Cl^-	−16.4	−14.8	−16.3	−15.4	−16.2
URC···OH_2	−4.9	−3.2	−4.2	−3.4	−4.0
AZA···OH_2	−4.0	−3.0	−3.7	−3.1	−3.5

[a] BSSE = basis set superposition error. Values are given in kcal mol^{-1}, and were obtained by using the RI-MP2 method and aug-cc-pVXZ (X = D and T) basis sets and CBS.

site at a location where the cyanide ion inhibitor is observed in 3BJP. Interestingly, the crystallization of UOX in the presence of sodium chloride shows that one chloride ion occupies the same location as the oxygen atom or the cyanide ion (Figure 2, right).

Both URC and AZA can be considered strong π-acidic rings. As a matter of fact, the anion–π binding ability of related rings such as cyanuric acid and hypoxanthine has been demonstrated both experimentally and theoretically.[11] A close examination of the UOX/URC/CN^- and UOX/AZA/Cl^- complexes revealed that the anions are located above the URC or AZA molecular planes, thus leading to anion–π interactions. These ternary complexes are shown in Figure 3.

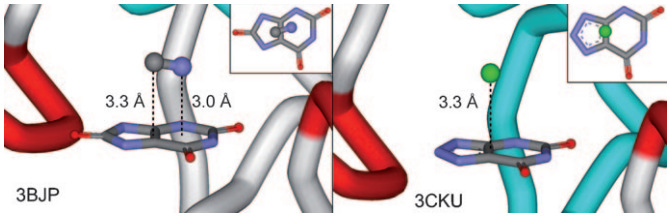

Figure 3. Representation of the anion–π interactions observed in the active site of UOX complexed to URC/CN^- (3BJP, resolution = 1.8 Å) and AZA/Cl^- (3CKU, resolution = 1.7 Å).

The distances between the carbon and the nitrogen atom of the CN^- ion and the mean plane of the URC molecule in 3BJP are 3.2 and 3.0 Å, respectively. The distance between the Cl^- ion and the mean plane of the AZA molecule is 3.3 Å. These observed distances are consistent with the reported theoretical values, which range from 3.1 to 3.3 Å.[11]

We have performed a high level ab initio study in order to characterize the anion–π complexes (see the Supporting Information for details). In Table 1 we summarize the interaction energies of the complexes of URC with cyanide ions and AZA with chloride ions at three levels of theory, that is, RI-MP2/aug-cc-pVXZ (X = D,T) and RI-MP2/CBS (CBS = complete basis set). The interaction energies are negative, thus indicating that the binding of anions to these π-acidic rings is favored. The difference between the interaction energies of both complexes is small, and is slightly more favorable in the URC···CN^- complex. Experimental evidence[8] indicates that a water molecule can be located at the peroxo site in the same position as the nitrogen atom of the cyanide ion, therefore we carried out calculations of the complexes of uric acid and 8-azaxanthine with water (lone pair (lp)–π interactions). The interaction energies are shown in Table 1, and they suggest that the binding of water is also favored. As expected, the interaction energies of the lp–π complexes are modest compared to the anion–π interactions. In Figure 4, the molecular electrostatic potential (MEP) of URC is shown (sphere and cylinder slices). The blue contours indicate favorable regions for anion interactions, which are mainly located in the molecular plane, although they also reach the region above the molecular plane. In the active center, the molecular plane is occupied by amino acids that leave the region above the molecular plane available for interactions with negative charges.

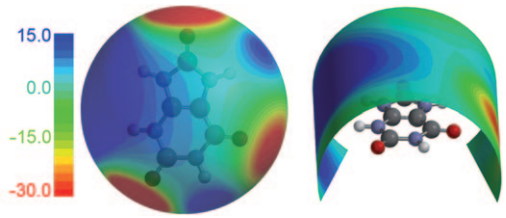

Figure 4. Molecular electrostatic potential [kcal mol^{-1}] of URC computed in spherical and cylindrical slices.

With the help of the theoretical results described above, we have demonstrated that neutral URC and AZA are able to interact favorably with cyanide and chloride ions, respectively and both neutral URC and AZA react with water. However, a close examination of the active center reveals that the uric acid is present in its anionic form. It is clear that the X-ray structure of the ternary UOX/URC/CN^- complex represents a static view of an intermediate state of the reaction. The exact charge state of the bound ligand (URC) observed in the active site is still under discussion.[8] However, in the case of AZA it is clear that the monoanionic state is present since the compound lacks a proton at N7, which is the position where the enzymatic base generates the urate dianion. Furthermore, since the pK_a values of the URC are 5.3 and 10.3, the form that is monodeprotonated at N3 of the substrate is likely to be the species that first binds at biological pH. We computed how the pK_a values of URC and AZA change in the enzyme because of the presence of the arginine (ARG) side chain (see the Supporting Information for computational details). We estimated the pK_a values by using the methodology of Namazian et al.[12] As a result, the

pK_a value of URC changes from 5.3 to 3.1 in the presence of Arg and a similar reduction in pK_a value is observed for AZA (from 4.9 to 2.7).

We have extended our theoretical study to the analysis of the anion-binding ability of uric acid in its anionic form (urate) that interacts with the amino acids of the active center, in order to know if the anion–π interaction is also favorable. We have used models of the amino acids in order to make the calculation possible. For the same reason we used the RI-MP2/aug-cc-pVDZ level of theory, since this level gives comparable results to the RI-MP2/CBS level of theory, as can be seen from the energetic data in Table 1. In Figure 5, the

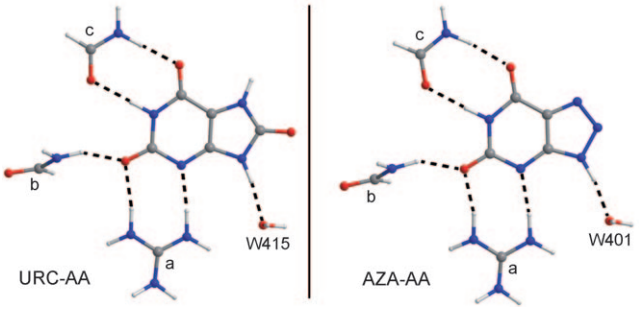

Figure 5. Urate and deprotonated 8-azaxanthine complexed to the amino acid models and a water molecule. a: ARG176 model, b: VAL227 model, and c: GLN228 model.

optimized structures of the URC and AZA anions interacting with the neighboring amino acid models and a water molecule (denoted as URC-AA and AZA-AA respectively) are shown and the energies of their anion–π complexes are summarized in Table 2. As mentioned before, one protonated arginine

Table 2: Interaction energies.[a]

Complex	E [kcal mol^{-1}]
URC-AA···CN$^-$	−7.1
AZA-AA···Cl$^-$	−3.2
PHE-URC-AA···CN$^-$	−7.4

[a] Values obtained by using the RI-MP2/aug-cc-pVDZ level of theory.

(ARG176, see Figure 2) is present in the active site of the enzyme, thus forming a salt bridge with the URC or AZA units. Therefore, the global charge of the URC-AA and AZA-AA moieties is neutral. The binding energies are smaller than those calculated for the non-anionic forms of URC and AZA (see Table 1). However, they are still favorable, hence indicating that the anion–π interaction is stabilizing even in the anionic form of either the substrate or the inhibitor that interacts with the protonated ARG. For more details on the electrostatic potential surfaces (EPS) and quadrupole moments (Q_{zz}) computed for uric acid, the urate monoanion, and urate complexed to the guanidinium counterion see the Supporting Information and Figure S1 in the Supporting Information.

Finally, we wish to emphasize the presence of a phenylalanine residue in the active site of the enzyme, which forms a π–π stacking interaction with the urate and it is located at the opposite face of the cyanide ion (see Figure 6). Several theoretical works have studied the influence of the π–π

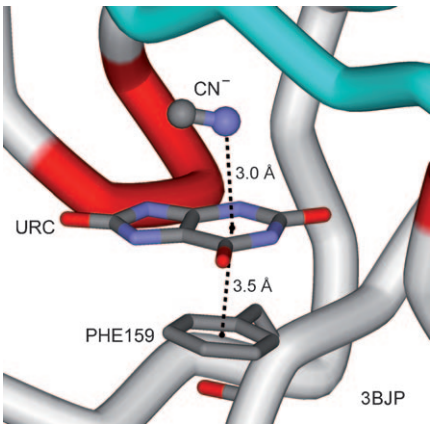

Figure 6. Representation of the anion–π and π–π interactions observed in the active site of UOX complexed to URC and the cyanide ion.

stacking interaction on the anion–π interaction in ternary complexes where both interactions coexist.[13] It has been demonstrated that, depending on the nature of the aromatic rings, favorable cooperativity effects are present in these anion–π–π complexes. We have computed the binding energy of a cyanide ion that interacts with the urate bonded to the amino acid models and stacked with benzene as a model of phenylalanine (denoted as PHE-URC-AA, see Figure 7). The interaction energy is −7.4 kcal mol^{-1} (see Table 2), which is slightly more negative than the interaction energy of the URC-AA···CN$^-$ complex, thus indicating that the presence of the PHE159 enhances the interaction energy of the anion with the urate π system. We have also performed a Bader's atoms-in-molecules (AIM)[14] analysis of the PHE-URC-AA···CN$^-$ complex (see the Supporting Information).

In conclusion, we have demonstrated that anion–π interactions are present in the active site of the UOX enzyme and that they are energetically favorable. To the

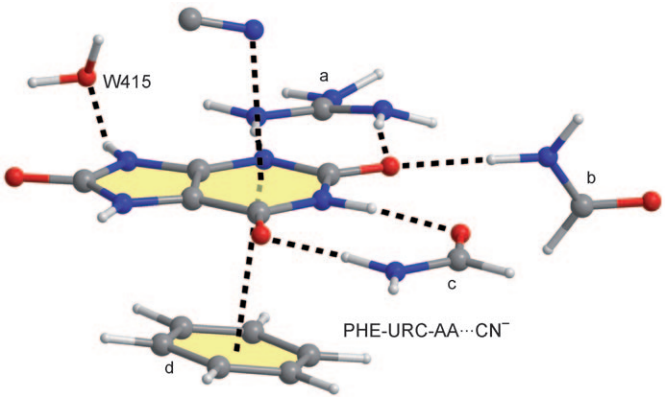

Figure 7. The theoretical model URC-AA interacting with benzene and the cyanide ion simultaneously. a: ARG176 model, b: VAL227 model, c: GLN228 model, and d: PHE159 model.

best of our knowledge, this is the first example where the presence of an anion–π interaction between an inhibitor and an enzymatic substrate is proposed to be crucial in the inhibition of an enzyme. This investigation extends the relevance of anion–π interactions to an important field such as enzyme chemistry.

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