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Non-covalent binding of the heavy atom compound \([Au(CN)_2]^\text{−}\) at the halide binding site of haloalkane dehalogenase from \textit{Xanthobacter autotrophicus} GJ10


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The Na[Au(CN)_2] heavy atom derivative contributed considerably to the successful elucidation of the crystal structure of haloalkane dehalogenase isolated from \textit{Xanthobacter autotrophicus} GJ10. The gold cyanide was located in an internal cavity of the enzyme, which also contains the catalytic residues. Refinement of the dehalogenase–gold cyanide complex at 0.25 nm to an R-factor of 16.7% demonstrates that the heavy atom molecule binds non-covalently between two tryptophan residues pointing into the active site cavity. At this same site also chloride ions can be bound. Therefore, inhibition of dehalogenase activity by the Au(CN)_2; presumably occurs by competition for the same binding site as substrates.

1. INTRODUCTION

Haloalkane dehalogenase from \textit{Xanthobacter autotrophicus} GJ10 converts 1-haloalkanes to their corresponding alcohols and halide ions in a two-step reaction mechanism, via a postulated covalently bound intermediate ester [1–3]. In this reaction no oxygen or cofactors are used, only water is needed as a co-substrate for the hydrolysis of the intermediate ester [3,4] (Fig. 1). Since a broad range of terminally halogenated alkanes are substrates for the enzyme [1], it has a high potential to be applied in the biological degradation of these environmentally toxic compounds [5].

The X-ray structure of haloalkane dehalogenase has been determined by Franken et al. [3] by means of multiple isomorphous replacement (MIR) techniques to solve the phase problem. Na[Au(CN)_2] turned out to provide an excellent single-site heavy atom derivative, which contributed substantially to the successful elucidation of the enzyme’s crystal structure [3]. The gold cyanide site appeared to be located in a buried cavity in the interior of the enzyme, which constitutes the enzyme’s active site with residues Asp^{24}, His^{39} and Asp^{66} in a catalytic triad arrangement [3].

As gold cyanide inhibits the dehalogenation reaction in solution, we decided to analyse in detail the binding mode of this compound. Therefore we refined the structure of the dehalogenase–gold cyanide complex at 0.25 nm, and determined the exact interactions of the heavy atom compound with the enzyme. One cyanide moiety is positioned between two tryptophan residues – Trp^{125} and Trp^{175} – in the active site cavity. This same site was also found to bind chloride ions as we could demonstrate from a crystal structure of the dehalogenase complexed with chloride. The other cyanide part might be stabilized by electrostatic interactions with the aromatic ring of Phe^{128}.

2. MATERIALS AND METHODS

2.1. Crystal growth and soaking experiments

Haloalkane dehalogenase purified from \textit{Xanthobacter autotrophicus} GJ10 [1] was crystallized according to Rozeboom et al. [6]. Nicely shaped crystals, suitable for data collection and soaking experiments with heavy atom derivatives, were grown within three weeks by vapor diffusion from a 62% (w/v) saturated (at 0°C) ammonium sulphate solution in a 100 mM bis-Tris-HSO₄ buffer of pH 6.2 [6]. The space group of the crystals is P2₁2₁2 with unit cell dimensions of a = 9.48 nm, b = 7.28 nm and c = 4.14 nm, respectively [3,6]. One crystal was soaked at room temperature for one day in a mother liquor solution (62% (w/v) saturated (at 0°C) (NH₄)_2SO₄ in 100 mM bis-Tris-HSO₄, pH 6.2) containing 3 mM Na[Au(CN)_2] [3]. To analyse chloride binding, a crystal was soaked for one day at room temperature in mother liquor with 10 mM NaCl. Both crystals were isomorphous with the native dehalogenase crystals. The unit cell dimensions of the crystals did not undergo significant changes upon soaking with the different compounds.
2.2 Data collection and processing
X-ray intensities of the crystallized dehalogenase complexed with
the heavy atom derivative were collected at room temperature to a
maximum resolution of 0.25 nm on a FAST area detector (Enraf
Nonius, Delft, The Netherlands) with graphite monochromatized Cu-
Kα radiation from an Elliot GX21 rotating anode [3]. The MADNES
package [7] was used for data collection and processing, profile fitting
and local scaling of the data set was done according to Kabsch [8], and
software from the Groningen BIOMOL crystallographic protein
structure determination package was used for merging of the data. A
similar data collection set-up was used for the dehalogenase crystal
soaked in 10 mM NaCl. More details of the data statistics are summa-
rized in Table I.

2.3. Refinement
Independent refinement procedures of the dehalogenase-gold cya-
nide complex and the dehalogenase-chloride complex were performed
with the least-squares reciprocal-space refinement package TNT [9],
which was adapted in order to implement the scattering factors of the
gold ion [10]. The starting model for both refinements was the native
dehalogenase at pH 6.2 refined to 0.19 nm [11] from which all solvent
molecules had been deleted. Initial stereochemical geometry for the
gold cyanide was generated using standard bond lengths and angles
as listed in the ‘Tables of Interatomic Distances and Configuration in
Molecules and Ions’ [12].

Cycles of coordinate and temperature factor refinement were altern-
ated with manual rebuilding on a PS390 Evans and Sutherlands
graphics system using the programs PRODO [13] and O [14].

2.4. RESULTS AND DISCUSSION
The crystal structure of haloalkane dehalogenase
complexed with the heavy atom derivative [Au(CN)2]−

Table I
Statistics on the data collection and quality of the refined models

<table>
<thead>
<tr>
<th></th>
<th>Dehalogenase-gold cyanide</th>
<th>Dehalogenase-chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 6.2</td>
<td>pH 6.2</td>
</tr>
<tr>
<td>Number of crystals</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Space group</td>
<td>P2₁2₁2</td>
<td></td>
</tr>
<tr>
<td>Unit cell dimensions (nm)</td>
<td>a = 9.48 nm, b = 7.28 nm, c = 4.14 nm</td>
<td></td>
</tr>
<tr>
<td>Resolution range</td>
<td>1.50–0.25 nm</td>
<td>1.50–0.23 nm</td>
</tr>
<tr>
<td>Total number of observations</td>
<td>34,819</td>
<td>47,049</td>
</tr>
<tr>
<td>Number of unique reflections</td>
<td>6,467</td>
<td>12,008</td>
</tr>
<tr>
<td>Rmerge (%)*</td>
<td>4.88% (on I)</td>
<td>6.09% (on I)</td>
</tr>
<tr>
<td>Completeness of the data</td>
<td>88.6% (1.5–0.25 nm)</td>
<td>94.8% (1.5–0.23 nm)</td>
</tr>
<tr>
<td>Completeness of the last shell</td>
<td>71.5% (0.258–0.250 nm)</td>
<td>49.8% (0.236–0.234 nm)</td>
</tr>
<tr>
<td>Number of residues</td>
<td>1,310</td>
<td>1,310</td>
</tr>
<tr>
<td>Number of water molecules</td>
<td>115</td>
<td>180</td>
</tr>
<tr>
<td>Number of ligand molecules</td>
<td>1 ([Au(CN)2]−)</td>
<td>1 (Cl−)</td>
</tr>
<tr>
<td>Overall R-factor (nm²)</td>
<td>0.084 nm²</td>
<td>0.110 nm²</td>
</tr>
<tr>
<td>Final R-factor (%)**</td>
<td>16.7%</td>
<td>16.4%</td>
</tr>
<tr>
<td>Estimated coordinate error (determined from a σA plot [15])</td>
<td>0.015 nm</td>
<td>0.018 nm</td>
</tr>
<tr>
<td>r.m.s. difference for all non-solvent atoms with native dehydrogenase</td>
<td>0.035 nm</td>
<td>0.024 nm</td>
</tr>
<tr>
<td>r.m.s. deviations from ideality for:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>covalent bond lengths</td>
<td>0.0013 nm</td>
<td>0.0010 nm</td>
</tr>
<tr>
<td>bond angles</td>
<td>3.4°</td>
<td>3.0°</td>
</tr>
</tbody>
</table>

*Rmerge (%) = \frac{\sum_{hkli} |F_{hkli}| - \langle |F_{hkli}| \rangle}{\sum_{hkli} |F_{hkli}|} \times 100

**R-factor (%) = \frac{\sum |F_{obs}| - |F_{calc}|}{\sum |F_{obs}|} \times 100
was refined to a final $R$-factor of 16.7% for 9,467 unique reflections between 1.50 and 0.25 nm. The final model contains 115 solvent molecules together with one gold cyanide ion and shows good stereochemical quality as can be seen from Table I. The r.m.s. difference for all non-solvent atoms is 0.035 nm compared to the refined 0.19 nm native dehalogenase structure [11]. The present structure of the dehalogenase–gold cyanide complex clearly demonstrates the binding position of the heavy atom compound in the active site cavity of the dehalogenase. Since gold is a strong atomic scatterer, series termination effects tend to decrease the electron density for the covalent bonds of the gold cyanide compound (Fig. 2). The heavy atom derivative is tightly bound in the internal active site cavity, as is clear from the low average $R$-factors of the atoms of the reagent (Table II). One of the partially negatively charged cyanide moieties of the compound lies on the intersection of the planes of the side-chains of two tryptophan residues pointing into the cavity – Trp$^{125}$ and Trp$^{173}$ – and is stabilized by their slightly positively charged ring nitrogens. We could identify this same site as a halide binding site in the dehalogenase enzyme by soaking experiments with chloride (Table I and II, Fig. 3). The other cyanide part of the reagent is lying in the plane of the aromatic ring of Phe$^{128}$, interacting with the somewhat positively charged ring hydrogens. Negatively charged groups have been shown before to interact preferentially with the hydrogens in the plane of aromatic rings [16,17], while positively charged groups are attracted more by the $\pi$-electron cloud below and above the aromatic rings [18–20]. More details about the geometry of the compound environment and distances are summarized in Table II.

The binding of the large complex $[\text{Au(CN)}_2]^-$ anion in the crystal structure of the dehalogenase has caused...
A. Dehalogenase-gold cyanide complex

B-factor (nm$^2$)  

<table>
<thead>
<tr>
<th>Atom</th>
<th>Distance (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trp$^{32}$N$_1$</td>
<td>0.31</td>
</tr>
<tr>
<td>Trp$^{32}$N$_1$</td>
<td>0.36</td>
</tr>
<tr>
<td>Phe$^{22}$O</td>
<td>0.35</td>
</tr>
<tr>
<td>Phe$^{22}$C</td>
<td>0.34</td>
</tr>
<tr>
<td>Glu$^{66}$C$_{6}$</td>
<td>0.34</td>
</tr>
<tr>
<td>Trp$^{32}$N$_1$</td>
<td>0.32</td>
</tr>
<tr>
<td>Glu$^{66}$C$_{6}$</td>
<td>0.37</td>
</tr>
</tbody>
</table>

B. Dehalogenase-chloride complex

B-factor (nm$^2$)  

<table>
<thead>
<tr>
<th>Atom</th>
<th>Distance (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trp$^{32}$N$_1$</td>
<td>0.34</td>
</tr>
<tr>
<td>Trp$^{32}$N$_1$</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Acknowledgements

It is a pleasure to thank Dr D. B. Janssen and co-workers for their generous supplies of protein material and useful comments, and all members of the Groningen Protein Crystallography Group for their many stimulating discussions.

REFERENCES

[11] Of the covalently bound intermediate ester [2,3,11] (Fig. 1). The volume of the cavity containing this water molecule is reduced, allowing space for just one solvent molecule instead of two as has been found in the native dehalogenase structure [11] (Fig. 3), although however, we cannot exclude the possibility of missing electron density for this solvent molecule, due to the limited 0.25 nm resolution reflection data set.

From $\mathrm{^{35}Cl}$ and $\mathrm{^{81}Br}$ nuclear magnetic resonance studies [21] it is known that cyanide compounds can act as competitors towards halogens for high-affinity binding sites in a number of proteins. From the crystal structures of the dehalogenase-gold cyanide and the dehalogenase-chloride complexes, we have shown here that indeed the gold cyanide binds non-covalently with its cyanide part at the halide binding site between the two tryptophan residues - Trp$^{25}$ and Trp$^{75}$ - pointing into the cavity of the dehalogenase. Since modeling studies of 1,2-dichloroethane in the active site cavity indicate that the substrate binds with its chloride ion between the two tryptophan side-chains, gold cyanide most likely acts as an inhibitor of the dehalogenation reaction in solution by binding on the substrate binding site in the active site cavity of the haloalkane dehalogenase.

The structure of the dehalogenase-gold cyanide complex presented here, is a structural confirmation of the findings of Norne et al. [21] that kinetically inert complex anions like $[\text{Au(CN)}_2]^{-}$ and $[\text{Pt(CN)}_2]^2$ may bind non-covalently on high-affinity anion-binding sites of proteins. Since such compounds are chemically inert, they also cause less structural modifications in the proteins [21]. Therefore they may provide very useful heavy atom derivatives for the elucidation of crystal structures of proteins [22-24].