Supporting information

Unexpected In Crystallo Reactivity of the Potential Drug Bis(maltolato)oxidovanadium(IV) with Lysozyme

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Figure S1 Anomalous difference electron density map (at 3.0 $\sigma$ level) of the (bis)V-containing fragment. Atoms from a symmetry related molecule are highlighted with the asterisk (*) and coloured in grey.
Figure S2 Interaction of the (bis)V-containing fragment bound to the side chain of Lys1. Atoms from a symmetry related molecule are highlighted with the asterisk (*) and coloured in grey. The side chain of Ser86* adopts two difference conformations.
Fig. S3 Proposed reactions of pyrylium ions.\textsuperscript{1}
Figure S4 Maltol cleavage by diketone cleaving enzyme via molecular oxygen and subsequent hydrolytic decomposition of the products.\textsuperscript{2}
Figure S5 Oxidation products of maltol: A) lactic acid and B) 3-hydroxyacrylic acid.\(^3\)
**Figure S6** Structure of the cross-linked HEWL dimer formed in the crystals of the protein exposed to BMOV. The symmetry related molecule is coloured in grey. Asp87 from the symmetry-related molecule is highlighted with the asterisk (*).
Figure S7 MALDI MS spectra of the peptide mixture obtained from *in situ* hydrolysis of non-metalated HEWL (band 1.1 in Figure 4 of the main text): magnification of 1110-1150 (A) and 2600-2800 (B) m/z ranges.
Figure S8 MALDI MS spectra of the peptide mixture obtained from *in situ* hydrolysis of cross-linked HEWL dimer (band 2.1 in Figure 4 of the main text): magnification of 1110-1150 m/z range. The signals present in the reference (not metalated HEWL, band 1.1 in Figure 4 of the main text) are marked with asterisks (*).
Figure S9 MALDI MS spectra of the peptide mixture obtained from *in situ* hydrolysis of metalated monomeric HEWL (band 2.2 in Figure 4 of the main text) (A) and cross-linked HEWL dimer (band 2.1 in Figure 4 of the main text) (B): magnification of 3000-3100 m/z range.
<table>
<thead>
<tr>
<th><strong>Data collection</strong></th>
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<tr>
<td><strong>PDB code</strong></td>
<td>9FMY</td>
</tr>
<tr>
<td><strong>Crystallization condition</strong></td>
<td>1.1 M NaCl 0.1M sodium acetate at pH 4.0</td>
</tr>
<tr>
<td><strong>Soaking time</strong></td>
<td>21 days</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>P4\textsubscript{3} 2\textsubscript{1} 2</td>
</tr>
<tr>
<td>a (\text{Å})</td>
<td>78.11</td>
</tr>
<tr>
<td>b (\text{Å})</td>
<td>78.11</td>
</tr>
<tr>
<td>c (\text{Å})</td>
<td>37.21</td>
</tr>
<tr>
<td>α/β/γ (°)</td>
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<tr>
<td><strong>Molecules for asymmetric unit</strong></td>
<td>1</td>
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<td><strong>Resolution range (Å)</strong></td>
<td>55.23-1.09 (1.11-1.09)</td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td>1112770 (44263)</td>
</tr>
<tr>
<td><strong>Unique reflections</strong></td>
<td>48619 (2359)</td>
</tr>
<tr>
<td><strong>Completeness (%)</strong></td>
<td>99.9 (99.1)</td>
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<tr>
<td><strong>Redundancy</strong></td>
<td>22.9 (18.8)</td>
</tr>
<tr>
<td><strong>Rmerge (%)</strong></td>
<td>0.056 (1.455)</td>
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<tr>
<td><strong>Average I/σ(I)</strong></td>
<td>29.0 (2.2)</td>
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<tr>
<td><strong>CC\textsubscript{1/2}</strong></td>
<td>0.999 (0.822)</td>
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<tr>
<td><strong>Anom. completeness (%)</strong></td>
<td>100.0 (99.5)</td>
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<td><strong>Anom. Multiplicity</strong></td>
<td>12.1 (9.7)</td>
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<table>
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<th><strong>Refinement</strong></th>
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<td><strong>Resolution (Å)</strong></td>
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<tr>
<td><strong>N\textsuperscript{*} reflections</strong></td>
<td>45192</td>
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<tr>
<td><strong>N\textsuperscript{*} reflections in working set</strong></td>
<td>2841</td>
</tr>
<tr>
<td><strong>Rfactor/Rfree</strong></td>
<td>0.129/0.152</td>
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<td><strong>N\textsuperscript{*} non-H atoms in the refinement</strong></td>
<td>1272</td>
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<td><strong>B-factor overall (Å\textsuperscript{2})</strong></td>
<td>19.11</td>
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<tr>
<td><strong>Estimated occupancy of V (1)</strong></td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Estimated occupancy of V (2)</strong></td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Estimated occupancy of [VO(H\textsubscript{2}O)\textsubscript{5}]\textsuperscript{2+}</strong></td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Estimated occupancy of (bis)V-containing fragment</strong></td>
<td>0.60</td>
</tr>
<tr>
<td><strong>B-factor of V (1) (Å\textsuperscript{2})</strong></td>
<td>27.47</td>
</tr>
<tr>
<td><strong>B-factor of V (2) (Å\textsuperscript{2})</strong></td>
<td>33.43</td>
</tr>
<tr>
<td><strong>B-factor of V [VO(H\textsubscript{2}O)\textsubscript{5}]\textsuperscript{2+} (Å\textsuperscript{2})</strong></td>
<td>22.86</td>
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<tr>
<td><strong>B-factor of (bis)V-containing fragment (Å\textsuperscript{2})</strong></td>
<td>15.4±2.7</td>
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<td><strong>Ramachandran values (%)</strong></td>
<td></td>
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<tr>
<td><strong>Most favoured/Additional allowed</strong></td>
<td>96.52/3.48</td>
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<tr>
<td><strong>Outliers</strong></td>
<td>0.00</td>
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<tr>
<td><strong>R.m.s.d. from ideality</strong></td>
<td></td>
</tr>
<tr>
<td><strong>R.m.s.d. bonds (Å)</strong></td>
<td>1111</td>
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<tr>
<td><strong>R.m.s.d. angles (°)</strong></td>
<td>1515</td>
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Table S2: Anomalous difference electron density peaks interpreted as V centers.

<table>
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<tr>
<th>Assignment</th>
<th>Occupancy of the V center in the model</th>
<th>Anomalous difference e.d. map peaks σ level</th>
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<tbody>
<tr>
<td>V (1) of bis(V)-containing fragment</td>
<td>0.60</td>
<td>21.96</td>
</tr>
<tr>
<td>V (2) of bis(V)-containing fragment</td>
<td>0.60</td>
<td>16.90</td>
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<tr>
<td>V (1)</td>
<td>0.30</td>
<td>8.31</td>
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<tr>
<td>V (2)</td>
<td>0.60</td>
<td>4.61</td>
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<tr>
<td>V of [VO(H₂O)₅]²⁺</td>
<td>0.50</td>
<td>11.23</td>
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Table S3 Specific m/z of signals recorded in MALDI-MS and ESI-LC-MSMS analysis of metalated monomeric HEWL (band 2.2 of SDS-PAGE reported in Figure 4 of the main text) and of cross-linked HEWL dimer (band 2.1 of SDS-PAGE reported in Figure 4 of the main text). Signals are due to peptide mixtures deriving from hydrolysis with Asp-N protease followed by trypsin. Expected Molecular Weight (MW, Da) values are also reported.

<table>
<thead>
<tr>
<th>Experimental m/z (MALDI-MS) - Metalated monomeric HEWL</th>
<th>Experimental m/z (ESI-LCMSMS) - Metalated monomeric HEWL</th>
<th>Experimental m/z (MALDI-MS) - Cross-linked HEWL dimer</th>
<th>Experimental m/z (ESI-LCMSMS) - Cross-linked HEWL dimer</th>
<th>Expected MW (Da)</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>1111.61</td>
<td>555.76</td>
<td>1111.66</td>
<td>-</td>
<td>1110.39</td>
<td>[62-68] + V + V=O</td>
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<tr>
<td>1127.60</td>
<td>-</td>
<td>1127.66</td>
<td>-</td>
<td>1126.39</td>
<td>[62-68] + 2V=O</td>
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<tr>
<td>2699.31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2699.28</td>
<td>[74-97] K_{96} Ac, (Na')</td>
</tr>
<tr>
<td>2713.43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2713.28</td>
<td>[74-97] K_{96} Ac (C-propionamide), (Na')</td>
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<td>2742.37</td>
<td>1371.70, 915.10</td>
<td>-</td>
<td>-</td>
<td>2744.28</td>
<td>[74-97] K_{96} Ac + V=O</td>
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<td>2756.36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2575.28</td>
<td>[74-97] K_{96} Ac (C-propionamide) + V=O</td>
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<td>2776.35</td>
<td>1389.14, 926.43</td>
<td>-</td>
<td>-</td>
<td>2776.35</td>
<td>[22-45] K_{33} Ac</td>
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<tr>
<td>-</td>
<td>-</td>
<td>4974.19</td>
<td>-</td>
<td>4974.22</td>
<td>[1-17] + [74-96] + (bis)V-containing fragment + VO_{3}</td>
</tr>
</tbody>
</table>
Notes and references

