

The Inhibition and Reactivation of Enzymatic Processes

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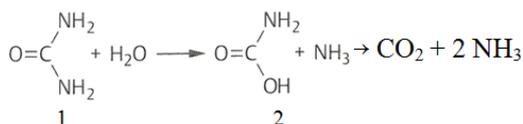
Abstract We present experiments that show the inhibition of the catalytic activity of the enzyme urease on the chemical degradation of urea with copper ions. In addition, we demonstrate the efficiency of the antidotes 2,3-dimercapto-1-propanesulfonic acid (DMPS, 2,3-bis(sulfanyl)propane-1-sulfonic acid) and 2,5-dimercapto-1,3,4-thiadiazole (DMTD) on reactivation of urease by complexation of copper.

Keywords: three-year undergraduate, analytical, electrochemistry, electrochemiluminescence, IR-spectrometry, hands-on learning/manipulatives

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1. Introduction

Urease is one of the most-used enzymatic systems in chemistry teaching. The induced hydrolysis of urea (1) is an example of an enzymatic catalyzed chemical reaction. This hydrolysis leads to the unstable intermediate carbamide acid (2), which gives CO₂ and 2 NH₃. Hydrolyzing CO₂ and NH₃ in water increases both electrical conductivity and pH.



The activity of urease for cleaving urea is exceptionally high: 1 g urease reacts with 60 g urea in one minute at 20°C. Urease has a molecular weight of about 480,000 u and consists of eight catalytic active monomers (60,000 u) with two centered Ni²⁺ ions (Figure 1). Urea catalysis is relatively specific, because molecules with a similar structure to urea (e.g., thiourea or N-substituted urea derivatives) are not catalyzed by urease.

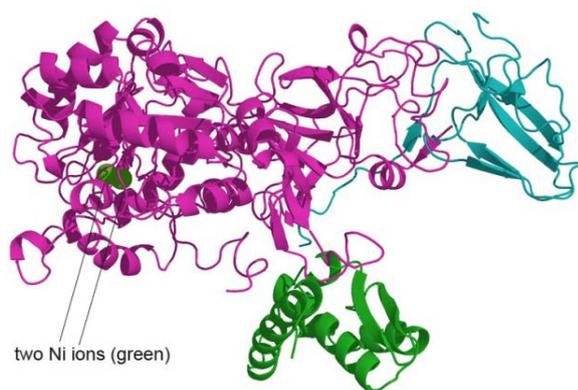


Figure 1. Model of urease (modified after https://de.wikipedia.org/wiki/Urease#/media/File:Urease_2KAU.png)

The reaction of urease with urea can be blocked with heavy metal ions. These ions bind to the thiol moieties of urease and change its configuration. Afterwards, urease can no longer anchor urea. But the heavy metal-urease reaction is reversible. This is interesting in medicine, because heavy metal poisoning can be cured via an infusion of a copper-complexing agent ("antidote") such as 2,3-dimercapto-1-propanesulfonic acid (DMPS) or 2,5-dimercapto-1,3,4-thiadiazole (DMTD) (Figure 2).

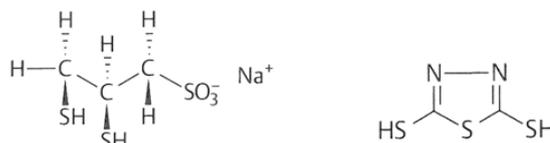


Figure 2. DMPS (left) and DMTD (right)

In 1940, English biochemists found that 2,3-dimercaptopropanole (DMP) detoxifies the arsenic-containing agent Lewisite (2-chloroethenylarsinous dichloride). Therefore, DMP was called "British anti-Lewisite" (BAL). DMPS, which is more water soluble than DMP, is more suited to reducing human metal intoxication. DMPS is a chelating agent that binds the heavy metals through the SH groups, producing 1:1 complexes and 2:1 complexes [1] (Figure 3). Most of these complexes are water soluble.

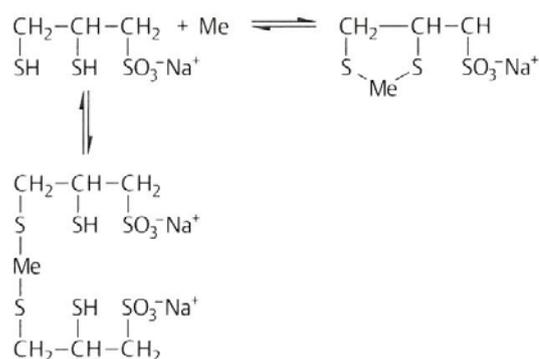


Figure 3. DMPS-metal complexes (1:1 and 2:1). Me: metal

DMPS is commercially available as Dimaval®. Dimaval has been extensively investigated as a scavenger molecule when heavy metals, especially mercury, poison enzymes [2,3,4,5].

DMTD reacts similarly with heavy metals. Unlike Cu-DMPS complexes, Cu-DMTD complexes are only slightly soluble in water. Therefore, they can easily be removed from aqueous solutions.

2. Experiments

Hazards

DMTD causes serious eye irritation. If it comes into contact with the eyes, rinse cautiously with water for several minutes.

Copper(II)sulfate is harmful, irritant and dangerous for

the environment.

Urea causes nausea and vomiting.

2.1. Reaction of Urease, Inhibition with Copper Ions, and Reactivation with DMTD

Chemicals and instruments:

DMTD (1,3,4-Thiadiazole-2,5-dithiol, Sigma Aldrich, D 129006)

Urease from jack beans (Sigma Aldrich, U4002, Type IX, powder, 50,000-100,000 units/g)

Copper sulfate

Urea (Roth, Karlsruhe, Germany, 7638.1)

Conductivity tester with CASSY data acquisition system (Leybold didactic, Germany)

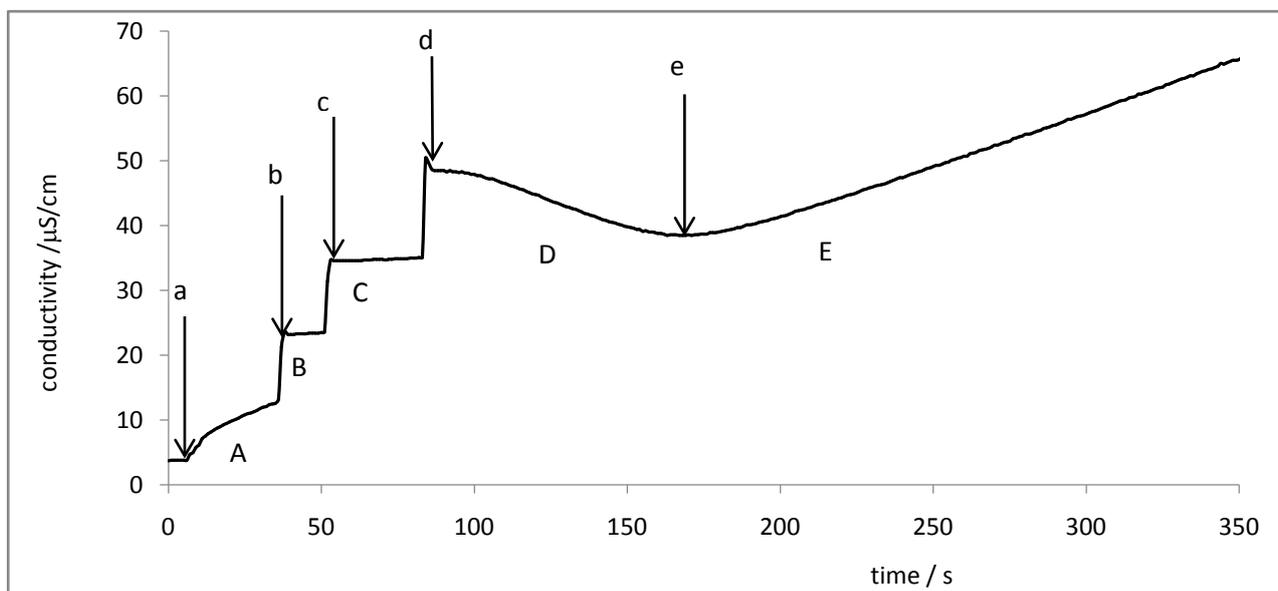


Figure 4. Conductivity as a function of time

When adding urease to an aqueous solution of urea (a, Figure 4), the conductivity increases (A). By adding copper (II) sulfate twice (b, c) the conductivity increases but then remains constant (B, C), because urease activity is hindered and no catalysis of urea results. By adding DMTD (d) the conductivity decreases due to the complexation of the copper ion (D). At 170 s (e), the conductivity increases again (E) due to recovery and reactivation of urease.

The analogous reactions occur if the urease activity is blocked by mercury ions. In this case, too, the blocking can be reverted by adding DMTD.

To investigate the product we compared the IR-spectra of DMTD and Cu-DMTD.

2.2. IR Spectroscopy

Chemicals and instruments:

DMTD (1,3,4-Thiadiazole-2,5-dithiol, Sigma Aldrich, D 129006)

Copper sulfate

Si-SH functionalized silica gel (Silicycle)

FTIR spectrometer (Bruker, Vector 22)

KBr pellets

Hettich centrifuge

A 0.01 molar aqueous solution of copper(II)sulfate was mixed with a 0.01 molar aqueous solution of DMTD. After shaking for about 5 min, the resulting brown precipitation was centrifuged, dried in a drying oven overnight (Figure 5), and an IR spectrum was recorded (Figure 6).



Figure 5. Top left: Copper(II)sulfate solution. Top right: After addition of DMTD and centrifugation. Middle: KBr-Cu-DMTD pellet for IR spectroscopy, bottom: solid Cu-DMTD

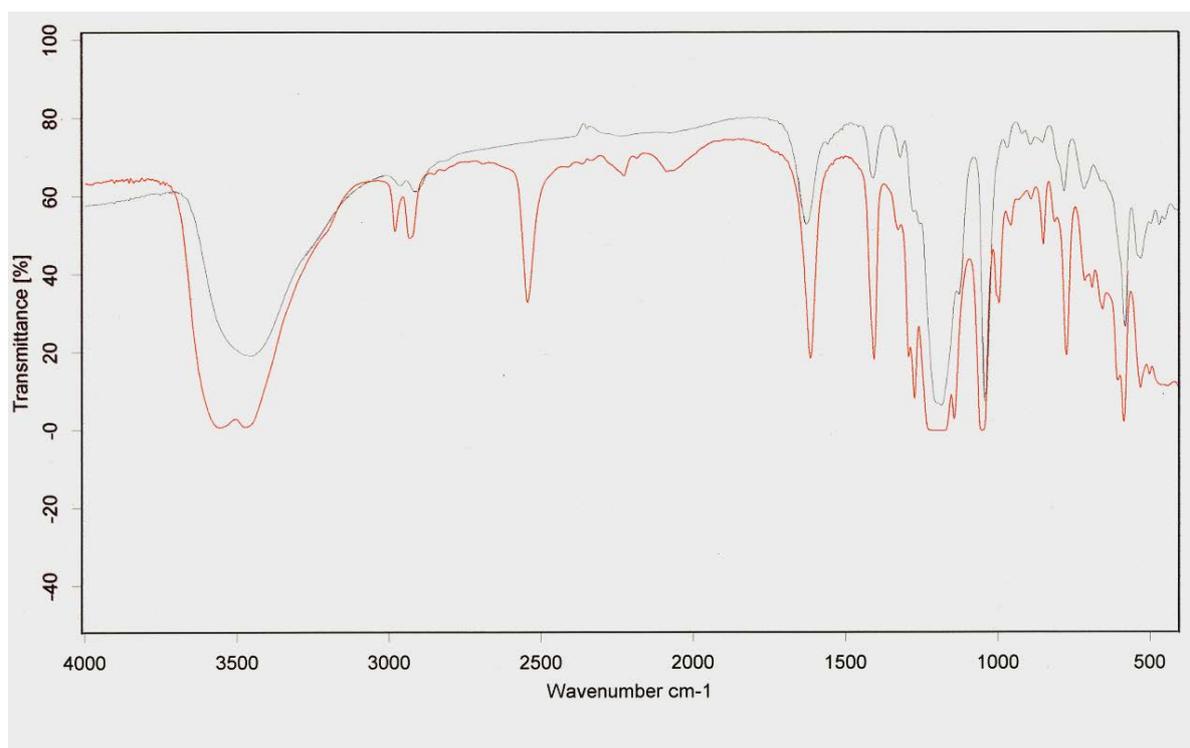


Figure 6. IR spectra of DMTD (red) and Cu-DMTD (dark). The arrow indicates the SH vibration around 2500 cm^{-1}



Figure 7. Left: Solution of copper sulfate (0.01 mol) with the scavenging reagent. Right: Si-thiol silica gel (left) and copper-coated Si-thiol silica gel (right)

Figure 6 shows the IR spectrum of DMTD (red) and Cu-DMTD (dark).

The spectrum indicates that the SH vibration of DMTD at about 2500 cm^{-1} in the Cu-DMTD complex is significantly reduced, because the S moiety is now coordinated to copper. The vibration of the S-Cu bond, however, lies in a region below 400 cm^{-1} . Unfortunately, our FTIR spectrometer only operates between 4000 cm^{-1} and 400 cm^{-1} .

In an analogous way, copper ions can be fixed with a commercial silica gel containing Si-thiol moieties (Figure 7). Intensive stirring results in a green precipitation. After centrifugation and drying, a green solid results, and the aqueous copper solution becomes clear.

However, as Figure 8 shows, this reaction is quite slow and is therefore not suitable for quickly removing heavy metal ions from enzymes. However, the method may be successful in removing heavy metal ions from waste water.

2.3. Electrochemistry

Chemicals and instruments:

DMTD (1,3,4-Thiadiazole-2,5-dithiol, Sigma Aldrich, D 129006)

Copper sulfate

Hettich centrifuge

Potentiostat (μ Stat ECL, DropSens), screen-printed electrodes (DS Au-BT, Au as working electrode, Au as counter electrode, and Ag as reference electrode, DropSens)

Cyclic voltammetry (CV), linear sweep voltammetry (LSV), and electrogenerated chemiluminescence (ECL) are ideal means to investigate the complexation of copper ions with DMTD.

Figure 8 shows the time-dependent precipitation of copper ions with the Si-SH covered silica gel after 6 and 15 minutes, detected with LSV at the SPE.

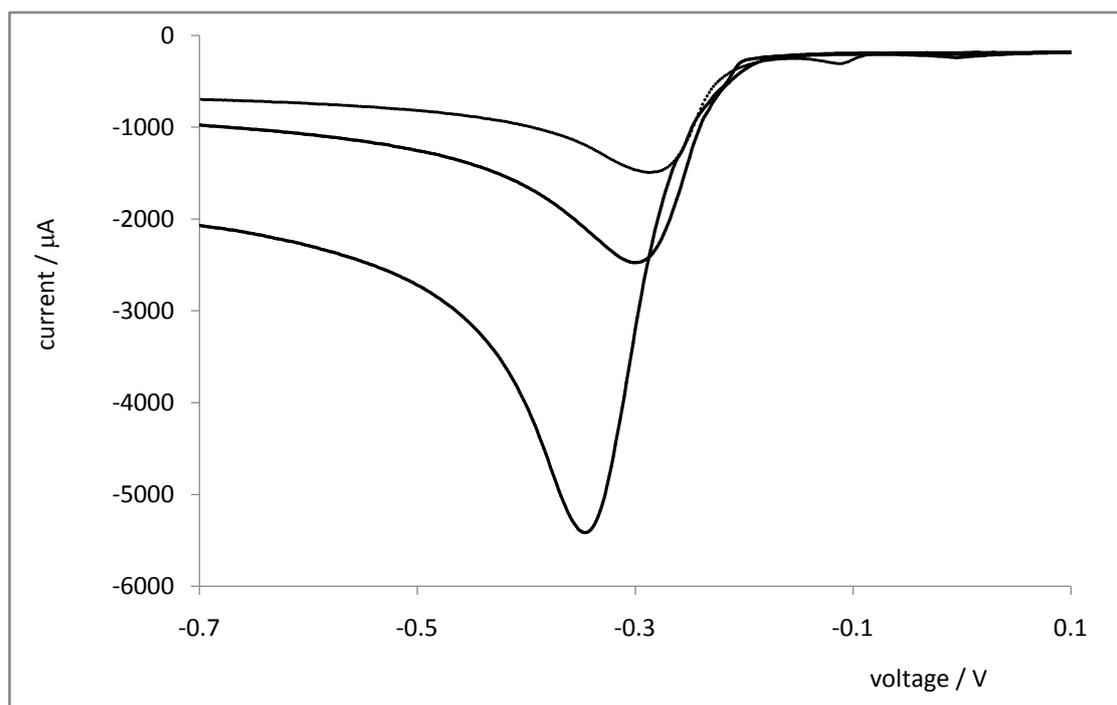


Figure 8. Reduction current peak of copper before (solid line) and after mixing with Si-SH silica gel at different times: dashed after 5 minutes and dotted line after 15 minutes

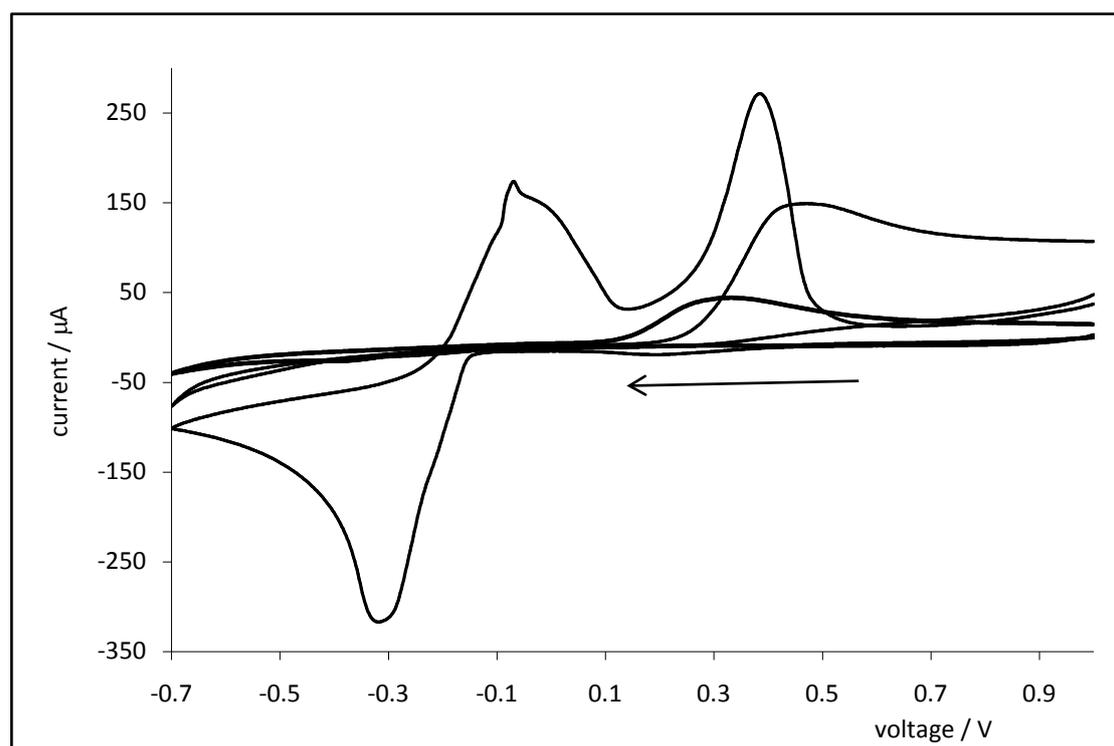


Figure 9. Cyclic voltammogram on a Au-BT SPE. Solid line: copper sulfate (0.01 mol), dotted line: after reaction of copper ions with DMTD, dashed line: only DMTD. Scan rate: 50 mV/s. The arrow indicates the scan direction

Even after 15 minutes, the complexation of copper ions is not completed.

Figure 9 shows the CV of copper sulfate with the SPE. The CV shows two anodic peaks (-0.07 V and 0.38 V) and two cathodic peaks (-0.32 V and 0.20 V). This results in the two redox couples: Cu/Cu^{2+} with $E(1) = [1/2(E_1 + E_2)] = 0.29$ V and $\text{Cu}^+/\text{Cu}^{2+}$ with $E(2) = -0.195$ V.

After adding DMTD in excess, the current peaks of copper disappear immediately, and an oxidation peak of

DMTD arises due to the formation of the Cu-DMTD complex, which is either not electroactive or does not cover the SPE surface.

Figure 10 shows the quenching of the electrogenerated chemiluminescence (ECL) of the system $[\text{Ru}(\text{bpy})_3]^{2+}$ / proline [6] with copper ions. Recovery of the initial ECL by adding DMTD was not totally achieved, because as additional measurements show, DMTD itself quenches the ECL.

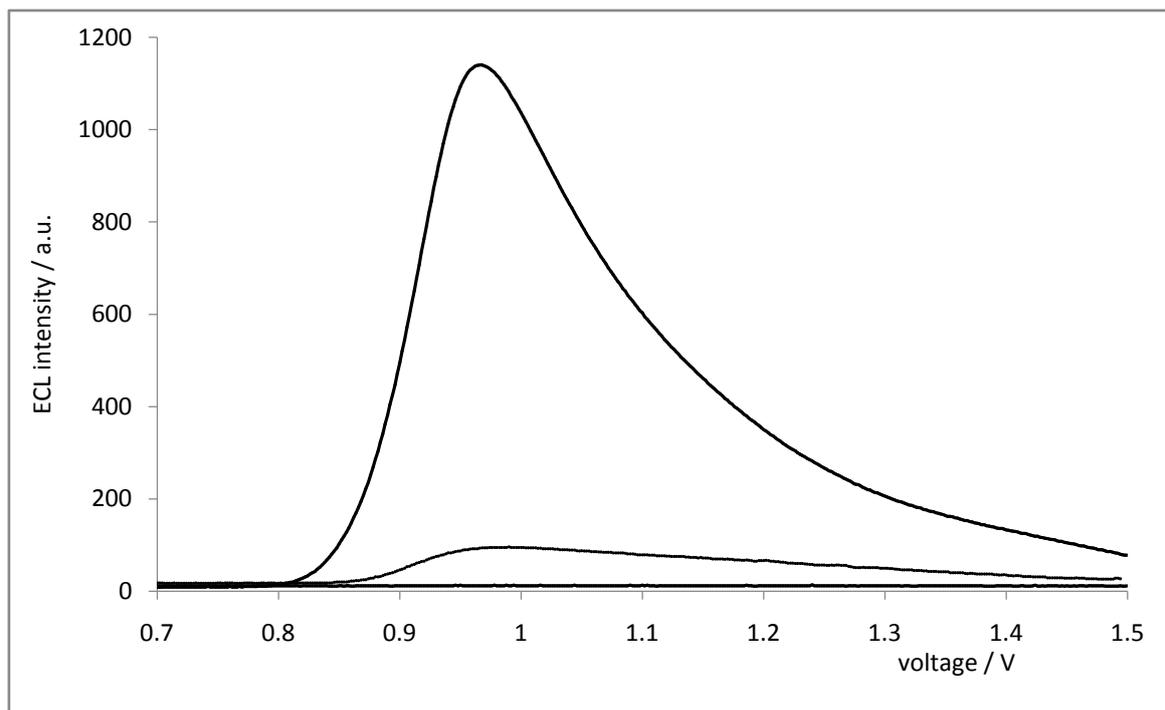


Figure 10. ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ -proline alone (each 1 mmol) (solid line), with added copper ions (dashed line) and with copper ions / DMTD (dotted line)

3. Conclusion

The described experiments demonstrate that adding heavy metal ions to urease inhibits its catalytic activity. This inhibition can be reduced by adding an antidote such as DMTD. IR spectroscopy demonstrated the reaction of DMTD with copper ions by the loss of SH vibration. In summary, urease is a strong example of an enzyme that can be reversibly poisoned by heavy metals and detoxified by DMTD.

We think that the pedagogic benefit of the experimental procedures described is based on the combination of electrochemical and spectroscopic experiments together with simple conductivity measurements. The complexation of copper ions can directly be observed via IR, CV and ECL.

As mentioned in the keywords, the described experiments were conducted during a three-year, undergraduate level university course in chemistry teaching experiments.

Acknowledgements

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