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Abstract: Cobalt compounds are intensely explored as efficient catalysts for the oxygen evolution reaction (OER). Since vitamin B12 is a soluble cobalt compound with high enzymatic activity, evaluating its OER activity is of relevance for biomimetic catalyst research. In this work, the temporal evolution of a homogenous vitamin B12 catalyst in the early stages of OER was investigated by an advanced combination of in situ electrochemical liquid transmission electron microscopy (EC-LTEM), in situ UV-vis spectroelectrochemistry, and extended X-ray absorption fine structure (EXAFS) methods. For the first time, we provided direct evidence of diffuse layer dynamics on the working electrode interface. The results suggested that the formation of cobalt oxyphosphate nanoparticles on the working electrode interface and in the presence of phosphate buffer is the initial stage of the catalytic pathway. Computational results confirmed that the ligand oxidation pathway could occur at potentials below the OER thermodynamic barrier (1.23 V vs reversible hydrogen electrode (RHE)), which leads to a Co ion leaching into the electrolyte. This study showed that investigation of the apparent molecular mechanisms of OER with metal complexes requires careful analyses. We illustrate the high precision and sensitivity of EC-LTEM under operational conditions to monitor heterogeneous catalysts generated during OER and to evaluate their actual roles in the catalytic process.

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Understanding the Dynamics of Molecular Water Oxidation Catalysts with Liquid Phase Transmission Electron Microscopy: The Case of Vitamin B₁₂

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Keywords: *in situ* electrochemical liquid TEM, renewable energy, cobalt, vitamin B₁₂, oxygen evolution reaction.

ABSTRACT: Cobalt compounds are intensely explored as efficient catalysts for the oxygen evolution reaction (OER). Since vitamin B₁₂ is a soluble cobalt compound with high enzymatic activity, evaluating its OER activity is of high relevance for bio-mimetic catalyst research. In this work, the temporal evolution of the homogenous vitamin B₁₂ catalyst in the early stages of OER was investigated by an advanced combination of *in situ* electrochemical liquid transmission electron microscopy (EC-LTEM), *in situ* UV-vis spectroelectrochemistry, and extended X-ray absorption fine structure (EXAFS) methods. For the first time, we provided direct evidence of diffuse layer dynamics on the working electrode interface. The results suggested that the formation of cobalt oxyphosphate nanoparticles on the working electrode interface and in the presence of phosphate buffer is the initial stage of the catalytic pathway. Computational results confirmed that the ligand oxidation pathway could occur at potentials below the OER thermodynamic barrier (1.23 V vs. RHE), which leads to Co ion leaching into the electrolyte. This study showed that investigation of the apparent molecular mechanisms of OER with metal complexes requires careful analyses. We illustrate the high precision and sensitivity of EC-LTEM under operational conditions to monitor heterogeneous catalysts generated during OER and to evaluate their actual roles in the catalytic process.

Introduction

Catalysts for the oxygen evolution reaction (OER) are indispensable for water-splitting systems in producing molecular hydrogen as a means to store renewable energy.¹⁻⁴ A wide range of first-row transition metal compounds has been reported as OER catalysts under different conditions.^{2,5-8} On the other hand,

nature makes use of a {Mn₄CaO₅} cluster as an efficient catalyst for OER.⁹⁻¹³

Cobalt salts were first identified as promising catalysts for water-oxidation in the 1980s.¹⁴ Nocera's group reported a cobalt-based OER catalyst with remarkable activity, self-healing properties, and low overpotential under neutral conditions

(pH 7).¹⁵ Other studies further showed that Co-based complexes with different ligands^{16–27} displayed significant catalytic activity in the OER. The first cobalt porphyrinoid OER catalyst reported by the Nocera group, a cobalt “hangman” β -octafluoro corrole complex, exhibited a high activity at 1.6 V vs. RHE at pH 7, with a turnover frequency (TOF) of 0.81 s⁻¹.²⁵ Sakai et al. investigated several Co(III) porphyrins for OER catalysis via photoinitiation in the presence of Ru^{II}(bpy)₃²⁺/Na₂S₂O₈, with a high TOF (0.16–0.35 s⁻¹) at pH 11.6.²⁰ A bimolecular oxyl-oxyl coupling pathway for the OER was proposed in such systems. The decomposition of the catalyst was reported to have been due to the production of the highly oxidizing (> 1.8 vs. RHE) sulfate radical anions, which also resulted in the generation of singlet dioxygen (¹O₂).²⁰ The fluorination of the Co(III) porphyrin catalyst was reported by the same group to confer higher stability and efficiency (TOF = 1.1 s⁻¹) for the OER.²¹ In contrast to the previous mechanism adopted by the Co porphyrin catalyst,²⁰ a nucleophilic attack OER pathway was proposed for the fluorinated Co(III) porphyrin.²¹ Groves and Wang reported a series of cationic cobalt porphyrins as efficient OER catalysts,¹⁸ which yielded an OER rate of 170 nmol cm⁻² min⁻¹ (*k*_{obs} = 1.4 × 10³ s⁻¹) with a near 90% Faradaic efficiency at 1.5 V vs. RHE at pH 7. A Co^{IV}-O porphyrin cation radical species was proposed as an intermediate during the OER.¹⁸ Despite the results of the studies reviewed above, there is still some controversy regarding the true catalyst under water oxidation conditions for such molecular systems, since decomposition of the complexes to cobalt oxides (CoO_x) is a pathway that cannot be excluded *per se*.^{17,26}

The formation of cobalt oxide nanoparticles from precursors of cobalt complexes during water oxidation was reported for the first time by the Fukuzumi group.²³ Cobalt SALEN (SALEN: N,N'-bis (salicylidene) ethylenediamino) was reported as a precatalyst of cobalt oxide under different water oxidation conditions.^{24,27} Generally, cobalt oxide particles can be very efficient in water oxidation, which calls for new and powerful methods for identifying them as possible *in situ* formed competitors of molecular catalysts.^{14,16,19,22} A detailed understanding of the catalytic process of OER requires the identification of the true catalysts under operational conditions.

Vitamin B₁₂ belongs to the cobalamin family of cobalt tetraazamacrocyclic complexes; it is water-soluble, exhibits versatile bio-activity, and excels through environmentally friendly properties.^{28–30} The central cobalt ion can adopt the oxidation states Co(III) (CN 6), Co(II) (CN 5), or Co(I) (CN4), giving rise to derivatives acting as electrophiles, radicals, or nucleophiles, respectively. Depending on the axial ligands, different forms of a given oxidation state can coexist.^{28–30}

A linear dependence of the standard potential of vitamin B₁₂ was observed over different pH ranges, which indicated a change in the molecular structure.^{28–30} In acidic conditions (pH < 2.9), the nucleotide side-chain opens, and a water molecule enters to occupy a ligand position of the Co(III) center.^{14,15,25} Above pH 7.8, the water molecule in the axial position is deprotonated into OH⁻.^{28–30} Co(III) porphyrin-like catalysts with high stability and efficiency for OER keep attracting a lot of interest.^{14,18,21,25}

During OER, especially when performing cyclic voltammetry (CV) of vitamin B₁₂ (**1**), cobalt oxide³¹ phases could be deposited and dissolved.²¹ In this regard, it is not possible to detect

true catalyst on the electrode interface with conventional surface characterization methods.

Although the cobalt-oxide formation from pre-cursors of cobalt complexes during OER is known, the details of this reaction is not clear. Advances in the fabrication of micro-electrochemical cells for operando microscopy have opened up new horizons to perform real-time investigations of electrochemical dynamics on the electrode-electrolyte interface.^{32–40} Liquid phase transmission electron microscopy could be a promising method to directly observe the structural changes during electrochemical reactions.^{41–44} Peña et al. reported *in situ* electrochemical transmission electron microscopy to investigate electrocatalysts of during OER in the presence of Co₃O₄ nanoparticles. Using this method, the observation of the chemical, morphological, and structural changes was possible under OER conditions. The surface amorphization of Co₃O₄ and then cobalt (oxyhydr)oxide-like phase formation occurs during OER.⁴⁴ Lee et al investigated Li–O₂ battery during the discharge reaction by liquid-phase transmission electron microscopy and observed the growth of toroidal Li₂O₂ discharge product in the electrolyte.⁴¹

In this regard, we present new investigations of vitamin B₁₂ (**1**) as an OER precatalyst. We combined *in situ* electrochemical liquid transmission electron microscopy (EC-LTEM) with complementary cutting-edge computational methods to understand the dynamic behavior of **1** in OER on the electrode interface in the nanoscale regime. The results shed new light on the advanced identification and understanding of true active species emerging from molecular OER catalysts.

Experimental section

Characterizations

SEM and EDX were carried out with VEGA/TESCAN-XMU. Electrochemical experiments (see Figures S1 and 2 for setup) were performed using an EmStat³⁺ from PalmSens (Netherlands). Voltammetry studies were carried out with a conventional three-electrode setup, in which FTO, Ag|AgCl (KCl: 3.0 M), and a platinum foil served as working, reference, and auxiliary electrodes, respectively. All potentials in this project were reported as values vs. Ag|AgCl (all potentials reported versus normal hydrogen electrode, reversible hydrogen electrode (RHE)). The distance between two opposite sides of the FTO electrode was measured by a digital caliper MarCal 16ER model (Mahr, Germany). The temperature was measured with a Laserliner 082 (Germany).

Oxygen evolution measurement

The O₂ evolution measurement in solution was carried out at 25.0 °C using an optical-probe oxygen meter (HQ40d from Hach, Düsseldorf, Germany) (Scheme S1).

XAS measurement

XAS data were collected at the SuperXAS beamline at the Paul Scherrer Institute (PSI). XAS at the Co *K*-edge for **1** and the references (LiCoO₂, CoO, and Co metallic foil) in transmission mode and the post-catalytic films on FTO electrode in fluorescence mode. The measured *k*³-weighted extended X-ray absorption fine structure (EXAFS) spectra *k*³ $\chi(k)$ were extracted by standard data reduction, absorption edge energy calibration, and background subtraction as implemented in ATHENA.⁴⁵

The spectra were reduced into the range $\Delta k \approx 3\text{--}12 \text{ \AA}^{-1}$ and Fourier transformed to $\text{FT}[k^3\chi(k)]$ into the real-space interval $\Delta R \approx 0\text{--}6 \text{ \AA}$. To calculate main values for interatomic distances, coordination numbers, and Debye-Waller factors σ^2 nonlinear least-squares fitting of the $\text{FT}[k^3\chi(k)]$ spectra was carried out with ARTEMIS⁴⁶ using an atomic cluster of modified Co-Cyanocobalamin (model PDB code: 1CCW), generated by ATOMS⁴⁶ as implemented in IFEFFIT⁴⁵. Amplitudes and phases shift for single-multiple scattering paths were calculated using FEFF6.⁴⁶

In situ electrochemical TEM experiments

All the *in situ* TEM experiments were performed on a 300 kV super-high-resolution GRAND ARM (JEM-ARM300F), Cs corrected electron microscope. All images were obtained after calibration of the dose to avoid beam damage effects. All the electrochemical measurements were performed with a commercially available Poseiden liquid cell (ECT-45PT-10, Protochips Inc., Raleigh, NC, USA) connected to Gammy potentiostat (model Reference 600) that allowed us to perform the electrochemical experiments in liquid TEM cell. The electrochemical cell was mounted in the tip of the TEM holder. The top part (ECT-45PT-10) contains a Si chip with a Pt ring electrode as a counter, one printed Pt rod as a reference electrode, and one Pt as a working electrode (WE). The WE was mounted on the 550×40 microns Si_3N_4 window, which is transparent to the e-beam. The bottom part of the Si chip was a 300×90 micron window with 500 nm spacer to have a proper fluid layer thickness and stable electrolyte flow on the working electrode (ECB-39GF-10, Protochips Inc., Raleigh, NC, USA). The bottom and top parts are sealed within the tip by one gasket (Figure S3). All components and the preparation steps are described in supporting information.

Computational Methodology

Geometry optimizations were performed with the Gaussian 16⁴⁷ package using the M06L hybrid functional.⁴⁸ The double-zeta Pople basis set 6-31g(d)^{49–52} is first employed to optimize the full vitamin B₁₂ structure.⁵³ Afterwards, the energies are refined with single-point energy calculations employing the def2-TZVP basis set,⁵⁴ including solvent corrections according to the SMD-model.⁵⁵ All calculations were performed assuming the lowest possible total spin state of the total system. All computational methodology and steps are described in supporting information.

Results and Discussion

CV of **1** showed high OER activity compared to a bare fluorine-doped tin oxide coated glass slide (FTO) (Figure 1a). Three notable features were evident from linear sweep voltammetry (LSV) (Figure 1b). First, the potentials for the onset of OER were calculated as 1.88 V and 2.03 V for **1** and a bare FTO, respectively, indicating that the overpotential of FTO in the presence of **1** was 150 mV below a bare FTO. Second, the water oxidation activity was 1.8 times higher with the FTO electrode at 2.35 V in the presence of **1**. Finally, no apparent faradaic redox peaks were observed for **1** in either cyclic voltammograms (CV) or linear sweep voltammograms (LSV) at pH 11.0 (Figures 1a and 1b). Square wave voltammetry (SWV) is a promising method for detecting weak peaks. In contrast to CV and LSV results, the SWV of **1** at pH 11.0 showed a peak at 2.25 V, corresponding to an oxidation reaction (Figure 1c). Consecutive

CVs were performed to investigate the stability of **1**, the results indicated that the current density for **1** at 2.35 V decreased to 2250 ($\mu\text{A}/\text{cm}^2$) for the first CVs (Figure 1d and 1e). Then, an increase in current density to 2800 ($\mu\text{A}/\text{cm}^2$) was observed.

Finally, a slight decrease to 2280 ($\mu\text{A}/\text{cm}^2$) was recorded after 250 cycles. The oxygenmetries for a bare FTO electrode in the presence (1.85 mM) and the absence of **1** at 2.35 V were recorded, which showed the important role of **1** for OER (Figure 1f). In the presence of **1**, OER for the FTO electrode showed (Figure 1f) high amounts of O₂ evolution with a Faradaic efficiency of > 80%.

Although a wide range of metal complexes have been reported as OER catalysts so far, whether they play the role of true catalysts or precatalysts is still a matter of controversy.^{22–24} In this regard, the formation of uncomplexed metal ions, metal oxides, or other heterogeneous species on the surface of the electrode during OER in the presence of **1** will have critical effects on the OER Mechanism. In the case of **1** and other relatively stable metal complexes, a simple comparison to Co salt as a reference material is not informative (Figures S4 and S5). This study aims to directly analyze the formed nanoparticles in the presence of vitamin B₁₂ during OER.

Figure 1. (a) CVs and (b) LSVs of **1** (pink; 184.5 μM) and a bare FTO electrode (blue). (c) SWV (amplitude: 100 mV; frequency: 10 Hz) of **1** (184.5 μM), and for FTO at pH 11.0. (d) Consecutive CVs of **1** (184.5 μM). (e) Current at 2.35 V in each CV of the consecutive CVs. (f) Oxygenmetry for a bare FTO electrode in the presence (1.85 mM) (pink) and absence (blue) of **1** at 2.35 V. Conditions: phosphate buffer (0.25 M, pH 11.0) at a scan rate of 100 mV/s.

In order to screen the Co ion leaching to the electrolyte and its redeposition on the surface of the FTO, after 250 consecutive

CVs in the presence of **1**, the electrode was rinsed and transferred to a fresh buffer solution. Interestingly, the operated electrode (hereafter FTO-1) showed high activity for OER in the absence of **1** (Figure 2a). After EDTA (0.01 M) treatment of FTO-1, the OER activity significantly decreased (Figure 3a), suggesting that the metal-containing product(s) deposited on the surface of the electrode were at least one of the true catalysts for OER. EDTA obviously removed these true catalysts on the surface of the electrode, thereby diminishing the water-oxidation substantially.

Figure 2. CVs of FTO (a) in the presence (pink) or absence (black) of **1**, CV after the consecutive CVs in the presence of **1** (FTO-1) in a fresh buffer (blue) and CV of FTO-1 after treatment by EDTA (green). Chronoamperometry at 2.35 V for a bare FTO, **1** and FTO-1 (b). UV/vis spectra of **1** (1.85 mM) during chronoamperometry at 2.35 V for 1 h (c). Conditions: phosphate buffer (0.25 M, pH 11.0) at a scan rate of 100 mV/s.

The chronoamperometry data for **1**, FTO-1, and a bare FTO electrode at 2.35 V are presented in Figure 2b. The FTO electrode in the presence of **1** showed an increase in OER during the chronoamperometry (Figure 2b). This increase in activity might have been caused by the formation of a true catalyst on the FTO surface during the chronoamperometry. As shown in Figure 2b, FTO-1 displayed higher activity than a bare FTO. A decrease in the current density of FTO-1 (Figure 2b) could correspond to the separation of nano-sized particles from the surface of FTO-1. In the next step, the stability of **1** at 2.35 V was studied by in situ UV-vis spectroelectrochemistry under the bulk electrolysis conditions. As shown in Figure 2c, the band intensities of the UV-vis absorption spectra corresponding to **1** decreased over time, indicating the decomposition of **1** on the surface of the electrode during the chronoamperometry.

It has been reported that even trace amounts of cobalt salt showed high activity in OER under similar conditions.¹³ In the presence of **1**, the detection of the cobalt compound on the surface of the electrode is not easy, especially for the first CVs or during short-time chronoamperometry. X-ray photoelectron spectroscopy (XPS) analyses could not detect such species during the short-time chronoamperometry (< 10 min) (Figure S6). Energy-dispersive X-ray spectroscopy coupled with scanning

electron microscopy (SEM-EDX) after one-hour chronoamperometry (2.35 V, pH = 11, phosphate buffer) showed no nano-sized cobalt compound on the surface of the electrode (Figures S7-S10), but Co ions were detected on the surface of SnO₂ on the surface of FTO.

The surface of FTO investigated by analytical scanning electron microscopy (SEM) after the OER reaction was monitored during long-time chronoamperometry at pH 11.0 for 120 hours. As shown in Figure S9, after applying a potential of 2.35 V for 120 h, particles with a spherical morphology (ca. 300-600 nm diameters) were observed on the surface of the electrode. SEM images indicated that each spherical particle consisted of small nanoparticles (15-22 nm) (Figure S9). EDX (10 kV) spectroscopy was used to characterize the surface, and although a significant portion of the signal came from the FTO support, the analysis showed the presence of Co (4.1%), P (5.62%), Cl (0.9%), and K (6.35 %) on the surface of the electrode (Figure S10).

PXRD did not give rise to diffraction patterns corresponding to cobalt compounds, which indicated that either the amount of compound was small or it was amorphous (Figure S10).

To analyze the electrodeposited film on the FTO electrode after 120 h of chronoamperometry of **1**, its structural features were studied with XAS. As shown in Figure 3a, the XANES spectra of **1**, the post-catalytic sample, and the Co-based compounds (LiCoO₂, CoO, and Co(OH)₂) (as references) were investigated. The average oxidation state (AOS) of the Co center in **1** and its post-catalytic states was estimated from the linear dependence of the Co K-edge position at the energy corresponding to a $\mu(E)$ -value of 0.5 of the normalized XANES spectra (Figure 3b).⁵⁶ The formal Co oxidation state varies between +2 and +3. The calibration curve showed an AOS of 3.07 and 2.07 for **1** and post-catalytic samples, respectively (Figure 3b). Previous XAS studies on Co-cyanocobalamin showed that the AOS of the Co center in the Co-cyanocobalamin structure was slightly higher than +3, which is in line with our results.⁵⁷ The post-catalytic sample showed that a reduced species was deposited on the FTO electrode, and the Co K-edge absorption edge dramatically changed to a lower energy level by ~4.5 eV.

The shape of the spectra in the pre-edge, white-line, and the XANES regions differed clearly between **1** and the post-catalytic samples. In the case of **1**, the pre-edge absorption in the K-edge (around 7710 eV) reflected resonant 1s electron excitation into unoccupied valence levels with the partial Co(3d) character,⁵⁷ which totally disappeared in the post-catalytic sample (Figure 3a). Also, the **1** edge fine structure showed two defined peaks in 7726.82 and 7734.44 eV, corresponding to 1s-4p_z and 1s-4p_{x,y} transitions, respectively.⁵⁸ In contrast to XANES data of **1**, the Co K-edge of the post-catalytic sample did not show any sub-transition, which indicated a significant difference between the film deposited on the FTO and the structure of **1**.

Simulated Fourier-transform EXAFS spectra of **1** (Figure 3c) showed a main peak arising from the first coordination shell of the corrin ring, and the axial CN ligand and the smaller features at higher distances were mostly related to the second coordination shells between Co center and the carbon atoms in the corrin ring. The fitting parameters calculated for interatomic distances, atomic coordination numbers (N), and Debye-Waller factors (σ^2) are listed in Table S1.

Comparing Fourier-transform EXAFS spectra of **1** and the post-catalytic sample (Figure 3d) revealed a new coordination shell at around 1.96 Å. Furthermore, the intensity of the coordination shell around 2.5 Å was increased, which can also be attributed to the increasing electron density in this path. These changes in the FT-EXAFS confirmed the formation of a different, non-soluble Co-based species on the FTO electrode during 120 h of chronoamperometry (2.35 V) of a solution of **1** (pH = 11, KOH/H₃PO₄ on FTO electrode).

To further investigate the nature of the post catalytic sample, Wavelet transform (WT) analysis of the Co K-edge EXAFS oscillations was performed. The conventional FT-EXAFS simulation is not able to discriminate an unknown or complex system with overlapped scattering pathways in R space. In such cases, WT of EXAFS spectra can resolve the contribution of each pathway in R and k space simultaneously.⁵⁹ WT facilitates the discrimination of atoms by their elemental nature, including the local contribution of each scattering pathway and its ability to distinguish between light atoms such as N, C, and O with close distance in R space.^{60,61} The WT maxima can be affected by different factors such as path length (R), Debye-Waller factors (σ^2), energy shift (ΔE), and atomic number (Z).^{62,63} Typically, heavier elements showed a maximum in the higher k range.⁶²

In Figure 3e, the WT contour plot of **1** in the first coordination shell showed one major intensity maximum at $k \approx 6 \text{ Å}^{-1}$, which mainly represented the Co-N corrin ring. The asymmetrical shape of this maximum could be explained by the presence of an axial CN ligand with overlapped backscattering pathways in R space. Other than **1**, the WT-EXAFS spectrum of the post catalytic sample (Figure 3f) showed a major intensity maximum at $k \approx 8.7$, which represented a major structural change in the initial coordination of **1**. These structural changes can be explained by replacing the first nitrogen and carbon shells with heavier elements such as oxygen or phosphorous, as would be expected in the case of Co oxide/phosphate species formation during the OER.

In situ electrochemical liquid TEM approach

In this study, the in situ EC-LTEM approach has been applied to directly visualize the early stages of solid growth on the surface of the working electrode (WE) in the nanoscale regime (more details on the instrumental setup in SI and Figures S3 and S12). As evident from XAS spectroscopy, the solid formed on the surface of the electrode was a cobalt-containing degradation product of **1**. Due to the phosphate sources from the electrolyte, these most likely decomposition-based compounds on the WE surface can be oxide or phosphate-based species. This hypothesis was checked by applying high-resolution in situ EC-LTEM to identify the catalyst on the WE under operational conditions.

Figure 3. (a) Co K-edge XANES spectra of **1** and reference oxides used for energy calibration. (b) Linear regression of K-edge energies of **1**, post-catalytic sample on FTO, and references at 0.5 normalized intensity of XANES spectra in Figure 4a used as a calibration curve for AOS calculation. (c) Fitting of the Fourier transforms EXAFS spectra $FT|k^3\chi(k)|$ of **1**. The dashed green line represents the fitting range on the Å scale. (d) Comparison of the Fourier-transform EXAFS spectra of **1** and the post-catalytic sample. Morlet wavelet transformation of Co K-edge EXAFS spectra for (e) **1** and (f) post catalytic samples with $\eta = 5$ and $\sigma = 1$.

Surprisingly, TEM image sequences showed that, after only one CV cycle, structural changes and nanoscale degradation process could be observed on the WE (Figures 4a and b). The in situ electrochemical STEM-EDX analysis of the working electrode interface supported the idea that the particles generated on the electrode surfaces were agglomerated cobalt oxide, and phosphate species (Figures 4c-f). The phosphorus EDX mapping also showed the presence of P, most likely as phosphate, on the surface of the WE (Figure 4f). Interestingly, the cobalt/phosphate film deposited on the WE surface showed a semi-reversible behavior during CV cycling (Figure 5 and SI, movie S1).

Figure 4. TEM image sequences of WE interface (a) before and (b) after CV cycling. (c) STEM image (d) and BF-STEM of electrode interface and electrochemically generated particles on the WE surface. (e) BF-STEM EDX mapping of (e) cobalt and (f) phosphorous from the selected area in (d).

Both EC-LTEM and STEM-EDX showed that in the first CV cycle, during the anodic sweep at 1401 mV vs. RHE (Figure 5 and SI, in movie S1), a phosphate film was formed on the WE. In contrast, in the cathodic sweep, this layer was dissolving. On the other hand, at 1841 mV vs. RHE (Figure 5 and SI, in movie S1), cobalt oxide/phosphate species formed on or near the phosphate layer. Similar to the phosphate layer, in the cathodic sweep, cobalt oxide/phosphate species were dissolving. This phenomenon showed that post-performance analyses, even at the highest detection limits, may leave a heterogeneous catalyst on the surface of the electrode undetected.

This reversible behavior shows that during the operational conditions, a meta-stable Co phosphate/oxide-rich species were formed on or near the surface of the working electrode, which was acting as an active compound under operating conditions. The thickness of the phosphate-rich layer increased as the potential escalated (Figure 5). This observation is of exceptional importance for molecular catalysis because it shows in real-time that the active compound can undergo dissolution/deposition during the OER reaction. We speculate that a high concentration of phosphate ions on the surface of the working electrode could induce the degradation of the molecular structure of vitamin B₁₂ and other molecular structures of metal complexes. It is known¹⁵ that Co phosphates are usually very insoluble in water; thus, their precipitation can be the driving force to remove the central atom from the otherwise stable coordination within the multidentate ligand.

As one of the critical challenges in liquid phase TEM is to avoid any electron beam damage and radiolysis effects,⁶⁴ we performed all experiments in the low dose TEM in order to minimize the electron beam-induced artifacts during our EC-LTEM

studies. Moreover, all our in situ TEM studies were carried out under the same, carefully adjusted illumination conditions (emission, spot size, condenser aperture, condenser lens value, magnification, and proper electrolyte flow rate) to stay below the threshold for any detectable nanostructure changes in a field of view. In this study, we further used phosphate buffer, which has a very high buffer capacity and solubility, rendering it a suitable electrolyte to maintain the pH in the desired range and to minimize the pH changes due to any possible local radiolysis during the EC-LTEM experiments.

The above experiments showed in their entirety that **1** was an unstable compound not only during long-time operation but also under short-time operation. Furthermore, in situ electrochemical TEM showed that **1** was not even stable after the first cyclic voltammogram.

Computational Results

The electrochemically induced leaching of Co from **1** was verified computationally with a complete **1** model, employing the M06L hybrid functional.⁴⁸ To the best of our knowledge, we present the first detailed modeling of the decomposition pathway for a molecular OER catalyst. The structures were optimized at M06L/6-31g(d), followed by single point energy refinement employing the M06L/def2-TZVP with implicit water solvation via the SMD approach.⁵⁵ A more detailed description of the computational methodology is provided in the Supporting Information. The OER pathway is the single site (or mononuclear) mechanism typically approximated by a 4-step electrochemical reaction between neutral intermediates **M***, **M-OH**, **M=O** and **M-OOH**,⁶⁵⁻⁶⁷ placed in equilibrium commonly using H₂O, H₂ and O₂.

This procedure provides insights into the OER thermodynamics, which we compare here with one of the plausible pathways leading to the release of Co-species into the solution. To make this comparison, we have assumed that the Co-CN group in **1** is quickly replaced by a Co-OH group, representing the state of **M-OH** in Figure 6. Besides, the figure represented a comparison between the single-site oxygen evolution pathway (full lines) and the ligand oxidation pathway (dotted lines).

The highest thermodynamic step in the oxygen evolution process is hydrogen abstraction from **M-OH**, resulting in the **M=O** species, which requires 1.90 eV or a bias anode potential of 1.90 V versus the pH-independent reversible hydrogen electrode (RHE). Therefore, the calculated oxygen evolution overpotential would be 0.67 V. Alternatively, if, from the state of **M-OH**, the ligand undergoes sequential hydrogen abstractions breaking the bonds within it and eventually releasing Co³⁺, all four required electrochemical steps would remain below 1.23 eV as the threshold for oxygen evolution. In the first ligand oxidation step taken into account (**M-OH** → **M-Lox1**), the hydrogen of the ligand (dotted arrow) is abstracted at 1.09 V vs. RHE. From **M-Lox1**, second hydrogen abstraction and corresponding C-C bond breakage result in **M-Lox2**, a spontaneous step at 0.24 V vs. RHE. To study the next steps, we added four explicit water molecules to **M-Lox2**, which resulted in a **M-Lox2*** state that was only -0.23 eV lower in energy. This is quite a low energy difference and a consequence of the already present implicit solvation in the computational methodology. From **M-Lox2** to **M-Lox3**, several reactions take place within an overall exer-

gonic electrochemical step (-0.20 eV): another hydrogen abstraction, cleavage of a C-N bond, and reaction with a water molecule.

Figure 5. Degradation of **1** during an anodic and cathodic scan at indicated applied potential during cyclic voltammetry in phosphate buffer solution (pH = 11, KOH/H₃PO₄, applied potential vs. RHE). The thickness of phosphate layers is 200-300 nm or 300-400 nm (pink) at 1401 and 1841 mV (red), respectively.

At the state **M-Lox3**, the ligand is already degraded; however, four Co-N coordination bonds remain and need to be broken or hydrolyzed before the Co³⁺ species can leach into the

solution. Another electrochemical step involving hydrogen abstraction from **M-Lox4** allows the breaking of a first C-N coordination bond; however, the required energy is 1.08 eV (corresponding to a bias voltage of 1.08 V vs. RHE). If we allow the complex to rearrange, breaking another Co—N coordination bond, this chemical step would amount to another 0.48 eV, resulting in **M-Lox4'** (Figure 7). Finally, adding two water molecules to **M-Lox4'** allows for generating Co(OH)₃(H₂O)₃ and a four-fold oxidized ligand, and this step would also be endothermic by about 0.68 eV. However, if chemical reactions go hand in hand with other ligand oxidation steps and become an electrochemical reaction step, these last two steps could most probably also be catalyzed electrochemically. In summary, we have shown that the release of Co-species from **1** is certainly feasible under electrochemical conditions, which explains the monitored Co-oxide film formation during OER (see also Figure S13, Tables S2, and S3).

Figure 7. Electrochemical reaction pathways vs RHE (at zero-potential: (eU = 0 V), pH= 0, and T = 298 K) from a derivative of **1** (Co-CN replaced by Co-OH in M-OH) are indicated in red and regular chemical pathways in black. The OER pathway (full lines) is compared with the ligand oxidation pathway (dotted lines). Via 4 consecutive ligand oxidation steps from M-OH (red dotted lines, abstracting a proton/electron pair) combined with regular chemical steps (black dotted lines), the Co³⁺ to ligand coordination can be broken at potentials below 1.23 V vs. RHE, while the oxygen evolution process requires potentials of 1.90 V vs RHE. (a) Schematic diagram showing Co-ligand coordination; (b) free energy level diagram.

Conclusions

In summary, vitamin B₁₂ (cyanocobalamin) (**1**) is a representative and easily accessible water-soluble cobalt compound with an attractive corrole structure. Therefore, its properties as a homogeneous pre catalyst for OER were newly examined here at pH 11.0. However, EDTA tests, EXAFS, XANES and in situ electrochemical TEM results for **1** during OER demonstrated that it underwent decomposition to nano-sized cobalt oxide/phosphate particles on the surface of the FTO. Most likely, these decomposition products represent the true OER catalysts, at least to a significant extent. Furthermore, in situ electrochem-

ical TEM investigations showed that even after the first CV cycle, a solid was formed on the surface of the electrode. These results are fully in line with DFT calculations. The present study demonstrated that careful operando analyses are necessary to detect such nanoparticles, whose formation may be reversible during electrochemical processes, rendering them easy to overlook even with high-resolution ex-situ analyses. In situ monitoring is thus indispensable for identifying the type of true OER catalysts in electrochemical water oxidizing systems.

We demonstrated that in situ electrochemical TEM analyses are among the most sensitive and powerful tools to directly visualize the formation of such true solid catalysts on electrodes, even in the initial stages of electrochemical processes. Our

study indicates that the continuous development of dual monitoring strategies, such as in situ TEM in combination with DFT calculations, will shed completely new light on the integrity and the transformation pathways of molecular catalysts under operational conditions. This insight is vital for the future design and application of tailored and tunable molecular components in water splitting setups.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Details of the in situ electrochemical liquid TEM setup; in situ visible spectroelectrochemistry setup; XRD; SEM-EDX; XPS spectra; complementary EIS data; complementary voltammetry data; complementary computational data; and XAS fitting data (PDF)

In situ EC-LTEM image sequences (AVI)

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Notes

The authors declare no conflict of interest.

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