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Intentional hydrolysis to overcome the hydrolysis problem: detection of Ce(IV) by producing oxidase-like nanozymes with F⁻†

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Polyvalent metal ions are susceptible to hydrolysis, making their reproducible detection by sensors and biosensors difficult. We herein turned this disadvantage into an advantage to develop a high selectivity colorimetric method for detecting Ce(IV) by intentionally hydrolyzing it through heating, where subsequent addition of F⁻ recovered the activity, allowing a detection limit of 3.8 μM Ce(IV).

Analysis of polyvalent metal ions is often complicated by metal hydrolysis, leading to time-dependent results and poor reproducibility. A typical method to deal with this problem is using strong acids to fully dissolve the hydrolyzed products. While this method is ideal for certain analytical instruments such as inductively coupled plasma (ICP) spectroscopy, strong acids are less desirable for sensors and biosensors. We reason that another possibility is to achieve full hydrolysis, which may also help avoid time-dependent changes in sample properties.

Cerium is an important rare earth metal with rich redox and catalytic activities.[1–3] Cerium dioxide (CeO_2) is a very useful material for catalysis[4–7] and sensing applications.[8–10] It has two stable oxidation states: Ce(III) being similar to other trivalent lanthanides as a hard Lewis acid, whereas Ce(IV) is a strong oxidant that is easily hydrolyzed in water. A lot of efforts have been made for detecting cerium.[11] For example, determination of Ce(IV) was achieved through oxidation of organic compounds, such as arsenazo III,[12] H_2-receptor antagonists,[13] tribromoarsenazo,[14] sulphanilic acid,[15] phenothiazine derivative,[16] and ascorbate.[17] However, these reactions are unlikely to be specific and other oxidation reagents might also achieve similar results. Recently, Ce(III) was detected by promoting the catalytic activity of gold nanoparticles.[18] Biological probes for cerium, such as DNAzymes, have also been reported.[19,20]

Ce(IV) in water exists mainly as $Ce(OH)^{3+}$ and $Ce(OH)_2^{2+}$ along with a dimer $(Ce–O–Ce)^{6+}$, leading to time-dependent and unstable aqueous chemistry even in strong acids.[21,22] Such hydrolysis makes reliable and reproducible measurements difficult. We recently reported that F^- can drastically improve the oxidase-like activity of CeO_2 nanoparticles.[23] This acceleration is unique to CeO_2 among all the tested metal oxides.[24] Since the hydrolysis of Ce(IV) may lead to products similar to CeO_2, it might be possible to use this reaction to detect Ce(IV). We herein intended to turn the disadvantage of Ce(IV) hydrolysis into an advantage in efforts to distinguish it from other metal ions.

We first reacted various metal ions (50 μM each) with a chromogenic substrate 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, 0.2 mM). The freshly prepared Ce(IV) produced a green color, indicating oxidation of ABTS,[25] while the Fe(III) sample also appeared slightly green (inset of Fig. 1A). However, the other metals, including Ce(III) and Cu(II), did not show much color change. This is reasonable since Ce(IV) is a strong oxidant, and Fe(III) has a lower oxidation potential. We then performed the same reactions with 400 μM F^- added. The color of the Ce(IV) sample became even more intense, while the Fe(III) sample appeared less green. To quantitatively measure them, we used UV-vis spectrometry and plotted the absorption peak at 415 nm (Fig. 1). Indeed, F^- promoted the activity of Ce(IV) but inhibited Fe(III), while none of the other metals had any activity, regardless of F^-. We then tested more metal ions, including Sc(III), Y(III), La(III), Pr(III), Nd(III), Sm(III), Eu(III), Gd(III), Tb(III), Dy(III), Ho(III), Er(III), Tm(III), Yb(III), Al(III), Ca(II) and Mg(II). They also failed to oxidize ABTS even with F^- (Fig. S1, ESI†). In this regard, Ce(IV) is unique for its high activity for oxidation of ABTS, and its selectivity was further increased by F^-.

Ce(IV) has a strong tendency to hydrolyze, making the reproducible measurement difficult. For example, after storing freshly prepared Ce(IV) in water for 2 weeks, its activity towards oxidation of ABTS was significantly decreased (comparing the first and second sets of tubes in Fig. 1B). Interestingly, F^- still boosted the oxidation activity of this aged Ce(IV) to a similar level to the fresh Ce(IV). We further challenged the system by heating freshly prepared Ce(IV) in water at 90 °C for 1 h and 6 h to further promote hydrolysis (the last two sets of tubes in Fig. 1B).

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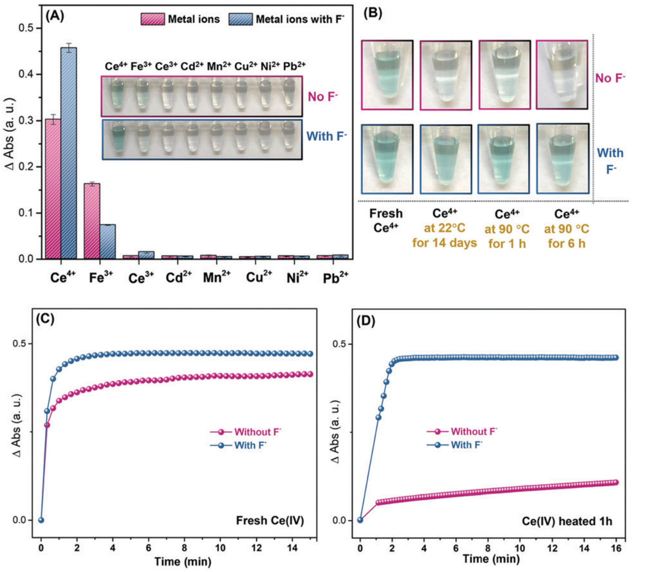

Fig. 1 (A) Oxidation of 0.2 mM ABTS (monitored at 415 nm) by various metal ions (50 μM each) without and with F⁻ (400 μM). Inset: The corresponding photographs. (B) Photographs of reacting Ce(IV) (50 μM) after various thermal treatments with 0.2 mM ABTS without and with F⁻ (400 μM). Effect of 400 μM F⁻ on the kinetics of 0.2 mM ABTS oxidation (monitored at 415 nm) in the presence of 50 μM (C) freshly prepared Ce(IV), and (D) Ce(IV) heated for 1 h at 90 °C. The buffer was 20 mM acetate, pH 4.

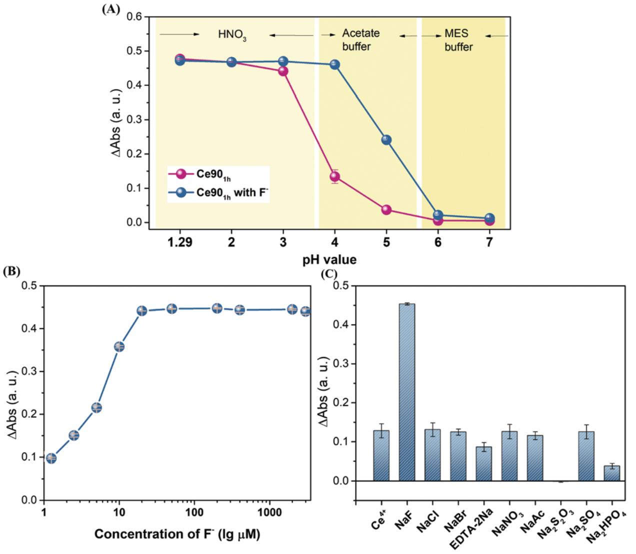

Fig. 2 (A) Effect of pH on ABTS (0.2 mM) oxidation monitored at 415 nm in the presence of 50 μM Ce90$_{1h}$ without and with 400 μM F⁻. pH 1.29 to 3 was adjusted by HNO_3, pH 4 and 5 were with acetate buffer (20 mM), and pH 6 and 7 were with MES buffer (20 mM). (B) Effect of F⁻ concentration on ABTS (0.2 mM) oxidation in the presence of Ce90$_{1h}$ (50 μM) in pH 4 acetate buffer. (C) Effect of various anions (400 μM each) on the oxidation of ABTS by Ce(IV). The anions were incubated with Ce90$_{1h}$ for 15 min before measurement.

After heating, the activity of ABTS oxidation was also reduced significantly. Yet, F⁻ still brought the activity back. The color of the products was quantitatively measured (Fig. S2, ESI†). In all the cases, the samples with F⁻ showed a similar level of activity. Therefore, F⁻ can normalize the activity of hydrolyzed Ce(IV) with a different extent of hydrolysis.

We further measured the reaction kinetics. For the freshly prepared Ce(IV), the reaction was very fast and most of the reaction took place immediately after mixing (Fig. 1C, red trace). After that, although the reaction continued, the rate decreased significantly. With F⁻, the reaction finished in ∼1 min, where no color change was observed after that. Meanwhile, for the heat treated samples, the initial oxidation became much lower (Fig. 1D). After adding F⁻, the extent of oxidation became similar to that of the freshly prepared Ce(IV), although the initial kinetics were slightly slower. With F⁻, it took ∼2 min for the heated sample to fully oxidize ABTS. The kinetics were overall quite robust with different heating temperatures (40 °C to 90 °C) and times (0.1 to 6 h) (Fig. S3, ESI†). Although we cannot ensure that all Ce(IV) in solution were hydrolyzed by the heat treatment, the insensitivity to the heating time and temperature is important for reproducible analytical assays.

Since both hydrolysis of Ce(IV) and oxidation of ABTS are strongly pH-dependent,[26–28] we then studied the effect of pH. We heated fresh Ce(IV) at 90 °C for 1 h and denoted this sample Ce90$_{1h}$. Ce90$_{1h}$ (50 μM) was then dispersed in buffers of different pH. At very low pH (*e.g.* below 3), the activity was high regardless of F⁻ (Fig. 2A), suggesting that the acid dissolved the hydrolyzed products and recovered the activity.[2] Thus, the effect of F⁻ was not obvious under very low pH conditions. At pH 4, the effect of F⁻ was the greatest. When the pH was higher than 6, the reaction was inefficient even in the presence of F⁻, since ABTS oxidation generally requires an acidic pH. Thus pH 4 was used for most reactions.[25] Therefore, the best condition to show the effect of F⁻ was pH 4.

We then studied the effect of F⁻ concentration. For 50 μM Ce90$_{1h}$, we added up to 3 mM F⁻ (Fig. 2B). Initially the activity increased linearly with the logarithm of F⁻ concentration and saturated activity was observed at around 20 μM F⁻. After that, even 3 mM F⁻ did not further increase or inhibit activity. Although the concentration of Ce(IV) was only 50 μM, after hydrolysis, the surface exposed cerium might be significantly less. Therefore, to activate hydrolyzed Ce(IV), a sub-stoichiometric amount of F⁻ was sufficient.

To test if this reaction is specific for F⁻, we also added 9 types of other sodium salts to Ce90$_{1h}$ (Fig. 2C). Only F⁻ promoted the activity. $Na_2S_2O_3$ is a strong reducing agent and could react with Ce(IV), resulting in a suppressed color change. Phosphate also showed a significant inhibition effect, likely due to its strong affinity with Ce(IV), forming cerium phosphate to inhibit the oxidation activity. EDTA also showed a weak inhibition effect. Therefore, this system has excellent selectivity for F⁻. We previously showed that we can detect F⁻ with CeO_2.[24] The mechanism of action could be similar here, especially for the heat treated samples. Since directly using CeO_2 might be more convenient than using heat treated Ce(IV) for detecting F⁻, the analytical work here focused on the detection of Ce(IV).

The change in absorbance after a 15 min reaction was plotted as a function of the concentration of Ce90$_{1h}$ (Fig. 3A). With 200 μM ABTS, a linear relationship was obtained in the

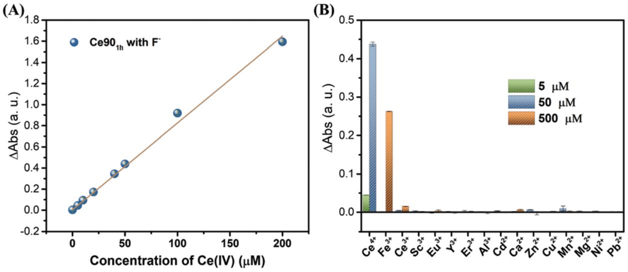

Fig. 3 (A) Effect of Ce90$_{1h}$ (prepared by heating Ce(IV) at 90 °C for 1 h) concentration on ABTS (0.2 mM) oxidation monitored at 415 nm in the presence of 400 μM F$^-$. (B) Selectivity test for the detection of Ce(IV). All the metal ions were heated for 1 h. Concentration of ABTS: 0.2 mM, F$^-$: 400 μM and the ABTS oxidation was performed for 15 min in 20 mM acetate buffer, pH 4.

0–200 μM Ce(IV) range. The regression equation is ΔAbs = 0.00339 + 0.00823 [Ce(IV)] (μM), r^2 = 0.996. The limit of detection (LOD) was calculated to be 3.8 μM according to 3σ/slope (σ = standard deviation of the blank signal). The relative standard deviation (RSD) was 1.75% for the detection of 50 μM Ce(IV) (n = 9).

For the selectivity test, we measured 16 heated metal ions (Fig. 3B). Only a high concentration of Fe^{3+} (500 μM) gave an obvious signal, but the signal was still weaker than that of 50 μM Ce(IV), indicating high specificity. We also compared 50 μM Ce(IV) and its mixtures with various metal ions (Fig. S4, ESI†). The coexistence of other metal ions (50 μM) had no obvious influence on the activity of heated Ce(IV), although 500 μM Ca(II) showed some inhibition. Ce(III) showed no activity and thus this method is specific for Ce(IV). One may still use it for Ce(III) by first oxidizing it to Ce(IV) followed by hydrolysis. To test its application in real samples, a water sample collected from a contaminated site in Quebec was spiked with Ce(IV) (Fig. S5, ESI†), and a detection limit of 2.1 μM Ce(IV) was obtained.

After showing the concept of intentional hydrolysis using Ce(IV) as an example, we then wanted to understand the role of F$^-$. Two possibilities exist: (1) breaking the hydrolyzed products to produce Ce(IV) ions and (2) acting on the hydrolyzed products just like how F$^-$ acts on CeO$_2$.[29] To understand the mechanism, we designed the following experiments. First, Ce90$_{1h}$ was respectively incubated in pH 4 acetate buffer for 0, 1.5, 3 and 12 h before F$^-$ and ABTS were added (Fig. 4A). The kinetics of the reactions were all very fast and showed no obvious difference between these samples. Without F$^-$, however, even storing Ce90$_{1h}$ at pH 4 for 12 h still resulted in low activity (Fig. S6, ESI†). We then incubated Ce90$_{1h}$ at pH 4 with F$^-$ for various periods of time before adding ABTS (Fig. 4B). Interestingly, the reaction rate decreased with longer pre-incubation of F$^-$.

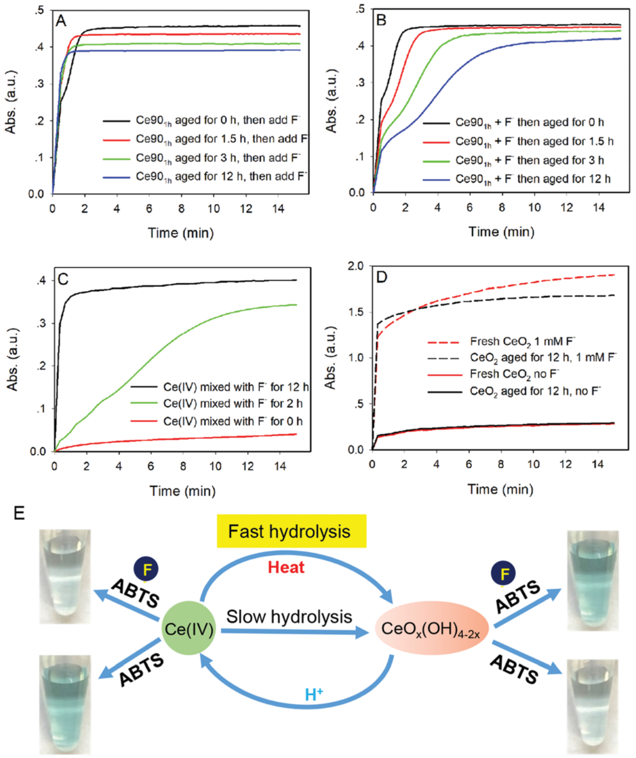

Fig. 4 Kinetics of 0.2 mM ABTS oxidation (monitored at 415 nm) of 50 μM CeH1 incubated in 20 mM pH 4 acetate buffer (A) in the absence of F$^-$ for a different time, then adding 400 μM F$^-$ and ABTS to initiate the reaction, and (B) in the presence of 400 μM F$^-$ for a different time and then adding ABTS to initiate the reaction. (C) Reaction of freshly prepared 50 μM Ce(IV) and fresh Ce(IV) incubated with 400 μM F$^-$ for 2 and 12 h before reacting with ABTS. (D) Effect of F$^-$ incubation time with 200 μg mL^{-1} CeO$_2$ for oxidation of ABTS (0.5 mM). (E) A scheme showing the principle of heat promoted hydrolysis and boosted activity by F$^-$, while F$^-$ inhibited the activity of free Ce(IV).

After adding F$^-$ to freshly prepared Ce(IV) and immediately testing it, its activity slightly increased (Fig. 1C). However, when fresh Ce(IV) and F$^-$ were incubated for 2 h, its activity was significantly decreased, and was nearly fully inhibited after overnight incubation with F$^-$ (Fig. 4C). F$^-$ can form stable complexes with Ce(IV),[30–32] and such complexes are inactive. The kinetics of the complex formation appeared to be slow, yet adding F$^-$ had a transient promoting effect, likely by breaking some unstable partially hydrolyzed species. At last, we can conclude that forming CeF$_4$ was not the reason for the promoted activity of hydrolyzed Ce(IV). We then studied the extent of hydrolysis by centrifugation and measuring the UV-vis spectrum of the supernatant (Fig. S7, ESI†). It appeared that most of the Ce(IV) remained in the supernatant and thus the hydrolyzed products were not large particles.

Finally, we did the same experiment with CeO$_2$ nanoparticles. F$^-$ promoted the activity of CeO$_2$ to a similar extent regardless of the pre-incubation time with F$^-$ (Fig. 4D). The kinetic trend of the heated Ce90$_{1h}$ sample (Fig. 4B) behaved between those of Ce(IV) and CeO$_2$. Therefore, we reason that the hydrolyzed Ce(IV) did not form rigid CeO$_2$, and thus can still be slowly disrupted by F$^-$. The mechanism of promotion should be similar for the hydrolyzed Ce(IV) and CeO$_2$. The hydrolyzed Ce(IV) had a more stable response than freshly prepared Ce(IV) upon incubation with F$^-$ (comparing the 12 h incubation data in Fig. 4B and C). Therefore, intentional hydrolysis can produce more robust sensors. The effect of hydrolysis and F$^-$ is summarized in Fig. 4E.

In conclusion, we proposed the concept of intentional hydrolysis of polyvalent metal ions to achieve a more stable sensor response. Using this concept, we have developed a highly selective colorimetric method to detect Ce(IV) based on the effect of F^- to boost the oxidation ability of hydrolyzed Ce(IV). Compared to other sample treatment approaches using strong acids, this detection system can avoid strong acidic conditions. The products of hydrolysis for other metal ions might also have their own unique properties to be explored for their detection. In particular, the concept of nanozymes can be explored for these metal containing species.[27,28,33–37]

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Conflicts of interest

There are no conflicts to declare.

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