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Transition metal cations catalyze $^{16}O/^{18}O$ exchange of catechol motifs with $H_2{}^{18}O$†

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Catechol motifs are ubiquitous in nature, as part of plant, animal and microbial metabolites, and are known to form complexes with various metal cations. Here, we report for the first time that complexation with transition metal cations, especially Fe(III), results in rapid $^{16}O/^{18}O$ exchange of the catecholic hydroxyl groups with $H_2{}^{18}O$. We discuss the implications of this finding for mechanistic studies using $H_2{}^{18}O$ and potential relevance for production of ^{18}O-labeled catechol derivatives.

Isotopic labeling studies are widely used in many fields of chemistry, typically in combination with mass spectrometric detection. For example, $^{18}O_2$ and $H_2{}^{18}O$ are often used to investigate chemical, enzymatic and microbial oxidation and degradation reactions.[1–4] In addition, when compounds of interest are available in a stable isotopically labeled form, they can be used for isotope dilution mass spectrometry,[5–7] determination of kinetic isotope effects,[8–10] mass spectrometric monitoring of reaction kinetics,[11,12] and even to follow the metabolic fate of biologically active compounds in human or animal studies.[13,14] In recent (to be published) work, we employed $H_2{}^{18}O$ to investigate degradation reactions of several catechol derivatives in the presence of metal cations, and observed unexpected rapid $^{16}O/^{18}O$ exchange between the $H_2{}^{18}O$ and the aromatic hydroxyl groups under mild conditions (37 °C, neutral or slightly acidic pH). $^{16}O/^{18}O$ exchange of catecholic hydroxyl groups with $H_2{}^{18}O$ has been previously reported, yet only under harsh catalyst-free conditions (3 M HCl, 150 °C, 20 days).[15] To the best of our knowledge, rapid exchange under mild conditions has not been previously described. We investigated this metal-induced $^{16}O/^{18}O$ exchange in further detail, as it may open up a new and simple approach to produce stable ^{18}O-labeled catechol derivatives. Additionally, this communication serves as a warning for researchers who intend to study the reactions of catechol derivatives in the presence of metal cations using $H_2{}^{18}O$.

The starting point of our work was the observation that 3,4-dihydroxybenzoic acid (3,4-DHBA, Fig. 1) showed rapid $^{16}O/^{18}O$ exchange of two oxygen atoms in the presence of various metal cations. These ^{18}O labels were exclusively inserted on the aromatic hydroxyl groups, as evidenced by the neutral loss of 44 Da (*i.e.*, unlabeled CO_2) in both unlabeled and ^{18}O labeled catechols (Fig. 2).

Many metal cations are known to form catecholato complexes in the presence of catechol and its derivatives.[16] Seemingly, this complexation somehow catalyzes the $^{16}O/^{18}O$ exchange with $H_2{}^{18}O$. To investigate this phenomenon in greater detail, we first screened the ability of four metal cations to catalyze this $^{16}O/^{18}O$ exchange. Hereto, catechol and 3,4-DHBA were incubated with the chloride salts of Fe(III), Al(III), Cu(II), and Zn(II) in $H_2{}^{18}O$. We selected these metal cations because they are all able to form catecholato complexes,[17,18] but are fundamentally different in terms of redox activity.

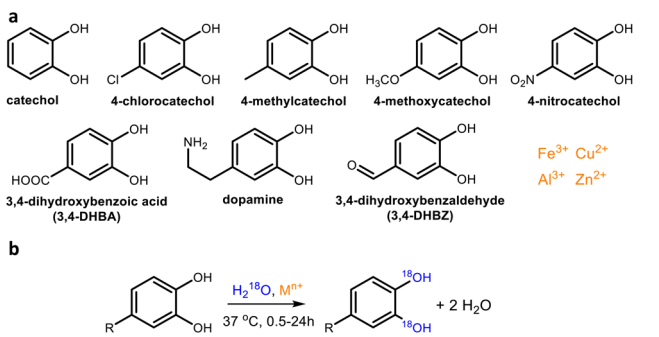

Fig. 1 Catechol derivatives and metal cations used for the ^{18}O labeling study (a) and schematic representation of the observed ^{18}O labeling (b). Unless stated otherwise, cations and catechols were used in equimolar concentrations. The R-group in figure (b) represents any of the substituents shown in figure (a).

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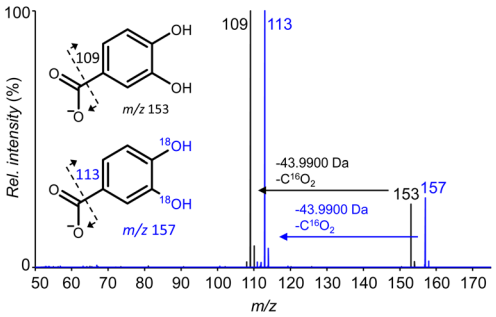

Fig. 2 Overlayed Higher-energy Collisional Dissociation (HCD) fragmentation patterns of unlabeled (black) and doubly ^{18}O-labeled (blue) 3,4-DHBA obtained using high resolution ESI-MS in negative ionization mode. The spectrum of doubly labeled 3,4-DHBA was obtained after incubation with $FeCl_3$ (equimolar, 1 h, 37 °C). Fragmentation patterns of other (un)labeled catechol derivatives can be found in Fig. S1.†

Whereas Fe(III) and Cu(II) have been described to oxidize catechol derivatives,[19] Al(III) and Zn(II) are redox inactive.[20] Samples of the incubation mixture were taken at 2 and 24 h, and analyzed using high resolution RP-UHPLC-PDA-ESI-MS to determine the percentage of single and double ^{18}O labeling. As complexation of catechol motifs to metal cations has been reported to be pH-dependent,[21] these screening experiments were performed at both pH 7 and pH 3.

For both catechol and 3,4-DHBA, incubation with $FeCl_3$ resulted in extensive labeling at pH 3 and 7, already after 2 hours (Fig. 3). $ZnCl_2$ at pH 7 and $CuCl_2$ at both pH 3 and 7 were found to catalyze ^{16}O/^{18}O exchange in the case of catechol, but no effect was observed in the case of 3,4-DBHA. The higher labeling yield at pH 7 as compared to pH 3 is in line with the expected increased complexation of catechol at elevated pH (Fig. S2†).[22] Despite its ability to form catecholato complexes,[23] Al(III) did not catalyze ^{18}O labeling (Fig. 3 and Fig. S2†). The observed ^{16}O/^{18}O exchange in the presence of Fe(III) and Cu(II) could be the result of valence tautomerism within the catecholato complexes. Such tautomerism has, indeed, been described for several complexes of transition metal cations with partly filled 3d orbitals.[24–26] A $H_2{}^{18}O$ molecule coordinated to the metal or present in the bulk could attack the activated keto-tautomer of the catechol and thereby replace the original ^{16}O hydroxyl group (Fig. 4). Such a mechanism seems, furthermore, fully in line with the lack of ^{16}O/^{18}O exchange in the presence of the redox-inactive Al(III). The proposed mechanism, however, cannot explain the observation of ^{16}O/^{18}O exchange in the presence of Zn(II). Since Zn(II) has a completely filled 3d orbital, valence tautomerism, forming Zn(I), is expected to be highly unfavorable. In addition, when catechol incubations were performed with Mn(II), rapid ^{16}O/^{18}O exchange was observed at pH 7 (Fig. S3†). As we expect valence tautomerism between Mn(II) and Mn(I) to be highly unlikely, this finding also challenges valence tautomerism as the only mechanism. To explore other potential mechanisms, we investigated whether molecular oxygen could, somehow, promote ^{16}O/^{18}O exchange, by repeating the incubations of

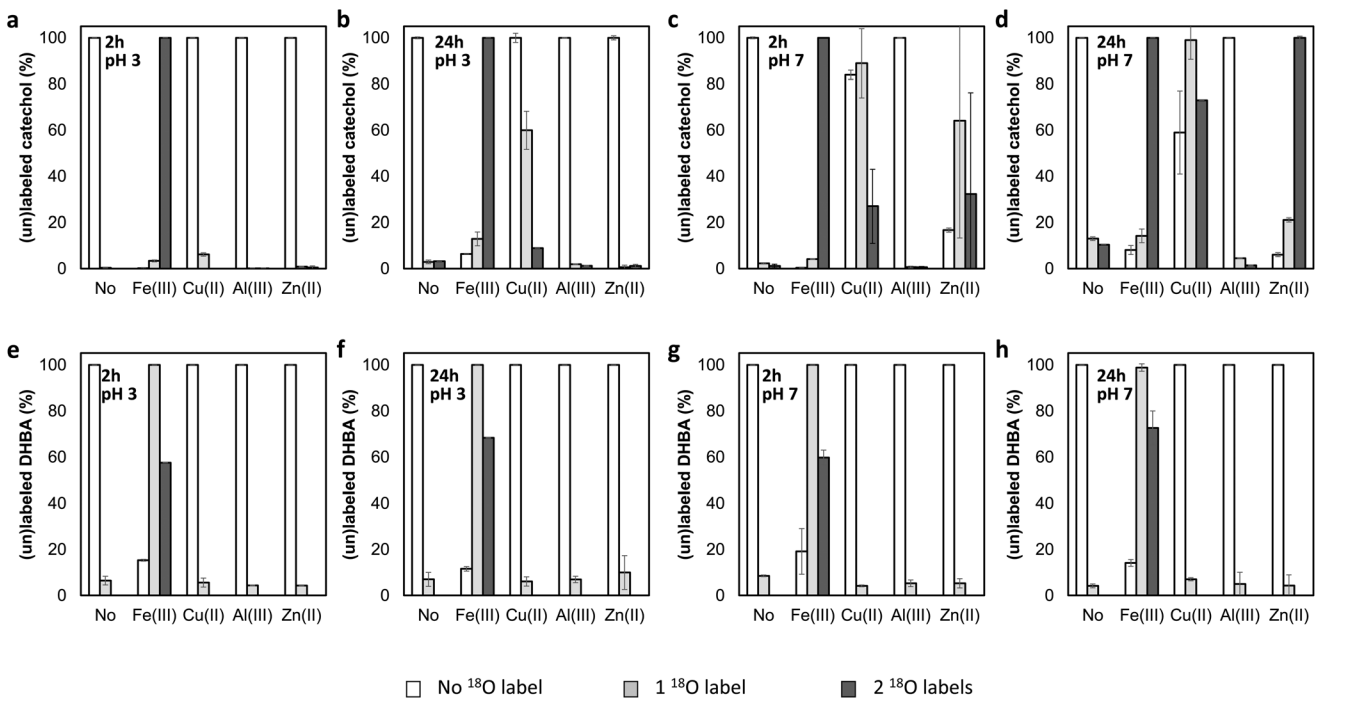

Fig. 3 Percentages of unlabeled, singly labeled, and doubly labeled catechol (a–d) and 3,4-DHBA (e–h) after equimolar incubations (1 mM) with $FeCl_3$, $CuCl_2$, $AlCl_3$, or $ZnCl_2$ at 37 °C for 2 or 24 h, as determined by RP-UHPLC-PDA-ESI-MS. Data are presented as average and standard deviation of two separate incubations.

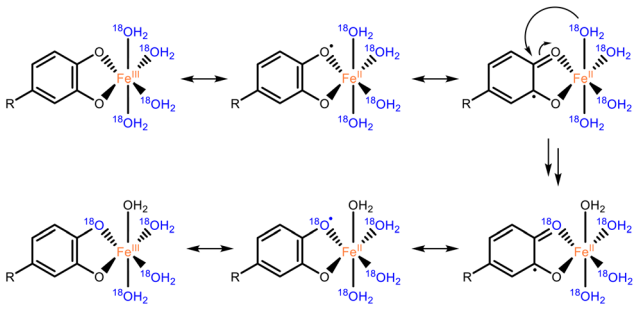

Fig. 4 Proposed mechanism for the Fe(III) or Cu(II) catalyzed $^{16}O/^{18}O$ exchange of catechol motifs, here depicted with Fe(III) as an example. The $H_2{}^{18}O$ attacking the catecholato ligand may also originate from the bulk. Examples of valence tautomerism as a mechanism underlying catalysis in Fe and Cu–catecholato complexes have been reported in earlier research.[25,26]

catechol with $FeCl_3$, $CuCl_2$, and $ZnCl_2$ after N_2 purging of the $H_2{}^{18}O$ solvent and headspace. In none of the incubations, a decrease in ^{18}O labeling was observed after N_2 purging (Fig. S4†), excluding a potential role of O_2 in the $^{16}O/^{18}O$ exchange. We therefore propose that the exchange in the presence of Fe(III) and Cu(II) occurs *via* the mechanism depicted in Fig. 4, whereas the exact mechanism of the Zn(II)-catalyzed exchange remains to be elucidated.

As Fe(III) showed the highest extent of ^{18}O labeling in all cases, we continued our research using only $FeCl_3$. No notable differences were observed between the labeling percentages at pH 3 and pH 7 in $FeCl_3$ incubations. Because of the relatively higher solubility of Fe(III) at acidic conditions,[27] further experiments were performed at pH 3.

Since the initial screening showed limited differences between the timepoints tested, and the $^{16}O/^{18}O$ exchange of catechol seemed already complete within 2 h, we proceeded to follow the exchange kinetics by using time-resolved ESI-MS. Hereto, catechol, 3,4-DHBA, 4-chlorocatechol, 4-methylcatechol, and dopamine solutions in $H_2{}^{18}O$ (pH 3) were mixed with equimolar amounts of $FeCl_3$ in $H_2{}^{18}O$ (pH 3), and infused for ~60 min into an ion trap MS. The kinetics were found to be strongly dependent on the substitution of the aromatic ring. Catechol, 4-chlorocatechol, and 3,4-DHBA were found to undergo near-to-complete ^{18}O labeling within 30 min (Fig. 5a, b and d), whereas 4-methylcatechol and dopamine were labeled considerably slower and reached around 60% labelling within the duration of the experiment (Fig. 5c and e).

No labeling was observed in $H_2{}^{18}O$ in absence of $FeCl_3$, confirming that $^{16}O/^{18}O$ exchange with $H_2{}^{18}O$ only occurs in the presence of a suitable catalyst (Fig. 5f). The latter also indicates that the isotopic labeling will be stable after removal of $FeCl_3$ from the product. When phenol was used instead of the catechols, no labeling was observed after 24 h, which underscores the importance of the catechol motif for the isotope exchange (data not shown).

Although the above described $^{16}O/^{18}O$ exchange may provide an interesting new approach for production of stable ^{18}O-labeled catechols, Fe(III) has been reported to induce oxidative degradation of various catechols.[28] Therefore, we simultaneously determined the labeling yield and recovery of all eight catechol derivatives, shown in Fig. 1, at various time points, using high resolution RP-UHPLC-PDA-ESI-MS. As can be observed from Table 1, complete (*i.e.*, 100% doubly labeled compound) or near-to-complete labeling (*i.e.*, >90% doubly labeled compound) was obtained for catechol, 4-chlorocatechol, 4-methylcatechol, 3,4-DHBA and dopamine with high (*i.e.*, >69%) to very high (*i.e.*, >95%) recoveries. In the case of catechol and 4-chlorocatechol, complete labeling and very high recoveries were obtained for incubations at 0.5 and 1 h. Prolonged incubations (24 h) decreased the recoveries, presumably due to oxidative degradation. For 4-methylcatechol,

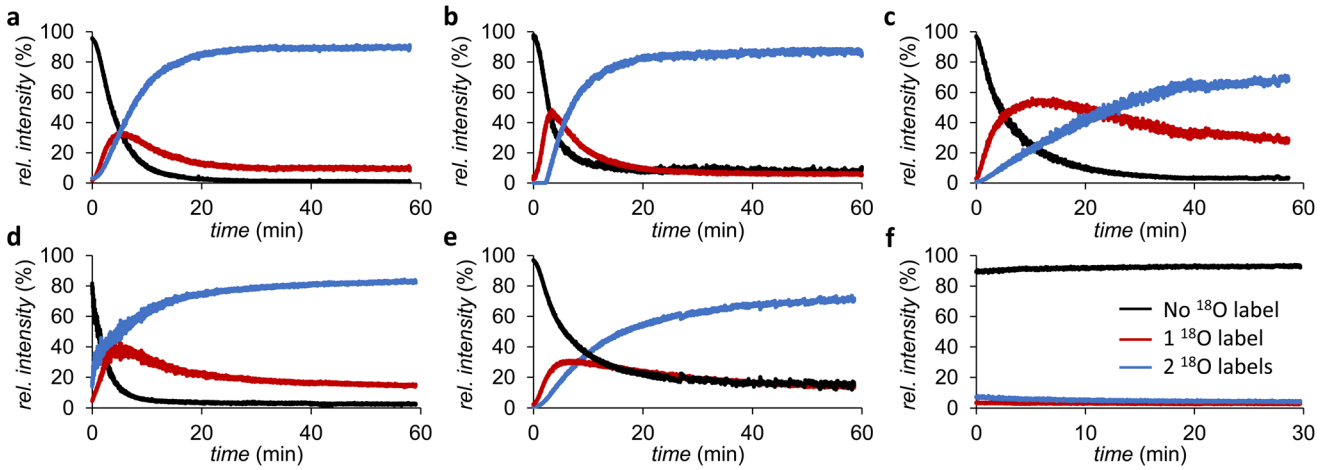

Fig. 5 Kinetics of $FeCl_3$ catalyzed ^{18}O labeling of (a) catechol, (b) 4-chlorocatechol, (c) 4-methylcatechol, (d) 3,4-DHBA, and (e) dopamine. Relative intensities of unlabeled, singly ^{18}O-labeled, and doubly ^{18}O-labeled catechols are shown in black, red, and blue, respectively. In absence of $FeCl_3$, no labeling was observed as indicated in (f) for 4-chlorocatechol. Note: the relative intensities shown are not corrected for the fact that 97% ^{18}O-labeled water was used as solvent.

Table 1 Labeling yields and recoveries of the five catechols after equimolar incubations (0.1 mM) with $FeCl_3$ at 37 °C, as determined by RP-UHPLC-PDA-ESI-MS. Results are shown as the average and standard deviation of two independent incubations. For the 1 h incubation of dopamine and incubations of 4-methoxycatechol no duplicates were included. The labeling percentage has been corrected for the fact that the $H_2{}^{18}O$ contained 97% ^{18}O. Recovery represents relative concentration of the compound (sum of labeled and unlabeled) compared to the initial concentration. Chromatograms and mass spectra are shown in Fig. S5†

Compound	Time (h)	Doubly labeled (%)	Recovery (%)
Catechol	0.5	100 ± 0.1	101 ± 1
Catechol	1	100 ± 0.1	102 ± 5
Catechol	24	100 ± 1	88 ± 5
4-Chlorocatechol	0.5	100 ± 1	95 ± 4
4-Chlorocatechol	1	100 ± 0.5	91 ± 1
4-Chlorocatechol	24	100 ± 0.1	70 ± 3
4-Methylcatechol	1	84 ± 1	69 ± 1
4-Methylcatechol	4	100 ± 0.2	79 ± 2
4-Methylcatechol	24	100 ± 0.1	70 ± 1
3,4-DHBA	1	91 ± 10	86 ± 13
3,4-DHBA	4	94 ± 8	88 ± 15
3,4-DHBA[a]	4	98 ± 2	49 ± 3
3,4-DHBA	24	95 ± 6	80 ± 0.3
Dopamine	1	92	98
Dopamine	4	96 ± 1	92 ± 11
Dopamine[a]	4	97 ± 2	59 ± 3
Dopamine	24	95 ± 2	72 ± 13
4-Nitrocatechol	24	0.2 ± 0.04	65 ± 13
4-Nitrocatechol[a]	24	0.7 ± 0.03	60 ± 4
3,4-DHBZ	24	9 ± 1	87 ± 9
3,4-DHBZ[a]	24	24 ± 9	59 ± 6
4-Methoxycatechol	24	66	2
4-Methoxycatechol[a]	24	N.D.	0

[a] These incubations were performed at a catechol : $FeCl_3$ ratio of 1 : 3.

3,4-DHBA and dopamine, incubations of 4 h were required to achieve >90% doubly ^{18}O labeled products. The double labeling yields of 3,4-DBHA and dopamine stagnated around 95%, and addition of extra iron or incubating at 1 : 3 catechol : Fe ratio did not substantially increase the labeling yield, but mainly resulted in a decreased recovery. Significantly lower labeling yields were obtained for 4-nitrocatechol and 3,4-dihydroxybenzaldehyde (3,4-DHBZ), even though both compounds possess an electron withdrawing substituent. No general trend could be observed on the effect of electron withdrawing and electron donating substituents on the labeling yield. The reason for the poor yields of the $^{16}O/^{18}O$ exchange of 4-nitrocatechol and 3,4-DHBZ remains to be investigated. For 4-methoxycatechol, 66% ^{18}O labeling was observed after 24 h, along with a remarkably low recovery. Various new peaks corresponding to compounds of decreased molecular weight were observed in the RP-UHPLC-PDA-ESI-MS chromatograms, suggesting extensive oxidative degradation of 4-methoxycatechol in the presence of $FeCl_3$ (Fig. S5i†).

Table 2 Labeling yields and recoveries of catechol at various concentrations after equimolar incubations with $FeCl_3$ at 37 °C, as determined by RP-UHPLC-PDA-ESI-MS. Results at 0.1, 1 and 5 mM are shown as the average and standard deviation of two independent incubations. The labeling percentage has been corrected for the fact that the $H_2{}^{18}O$ contained 97% ^{18}O. Recovery presents relative concentration of the compound (sum of labeled and unlabeled) compared to the initial concentration. For incubations at 50 mM no duplicates were included

Concentration (mM)	Time (h)	Doubly labeled (%)	Recovery (%)
0.1	0.5	100 ± 1	101 ± 1
1	0.5	100 ± 0.8	101 ± 8
5	0.5	95 ± 2	90 ± 15
50	4	8	100
50	24	16	85

Results in Table 1 show that $FeCl_3$ is an excellent catalyst for the ^{18}O labeling of various catechols. Although isotopically labeled compounds are mainly used in mass spectrometric studies, requiring low concentrations, the above described experiments (at 0.1 mM) would only allow synthesis of ~10–15 μg of labeled compound per 1 mL of solvent. Therefore, we investigated whether the ^{18}O labeling of catechol could be upscaled to concentrations that are more meaningful for lab-scale synthesis. We note that at increased concentrations the catecholato-complexes partly precipitated, but could be completely resolubilized in ethanol after incubation. RP-UHPLC-PDA-ESI-MS analysis of the resolubilized samples showed that using 10 and 50-fold higher concentrations (1 and 5 mM) still resulted in complete and near-to complete labeling, respectively, within 0.5 h (Table 2). Further upscaling to 50 mM, however, showed a drop in the labeling yield. Presumably this is caused by the poor water solubility of the catechol–iron mixtures at increased concentrations. We are currently investigating approaches to circumvent this. Nonetheless, even partially ^{18}O-labeled compounds can be highly valuable as mechanistic probes and quantification standards.

In summary, we showed that Fe(III), Cu(II), Zn(II) and Mn(II) cations can catalyze $^{16}O/^{18}O$ exchange of catechol motifs with $H_2{}^{18}O$. This may provide a new route for facile production of ^{18}O labeled catechol derivatives. At the same time, our findings indicate that extra caution is required when drawing conclusions from studies on the reactions of catechol derivatives that employ $H_2{}^{18}O$ labeling in the presence of transition metals.

Conflicts of interest

There are no conflicts to declare.

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