Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Analysis of the bioactivity of magnetically immunoisolated peroxisomes

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Separation of BODIPY standards

To ensure the MEKC system can separate $B_{12}FA$ from potential β-oxidation products we optimized the MEKC-LIF separation using commercially available BODIPY fatty acid analogs that include B_3FA, B_5FA and $B_{11}FA$. At low concentrations of borate and SDS (10 mM each), B_3FA and B_5FA could not be separated, while at high concentrations of borate and SDS (30 mM), $B_{11}FA$ and $B_{12}FA$ comigrated. We therefore investigated the use of a cyclodextrin (CD) as an additive in separations of lipids.[1, 2]

CDs are not incorporated into micelles and provide an additional partition phase because hydrophobic compounds with features of similar dimensions to the cavity can partition into such cavities. When analytes partition into the CD cavity, their partition into micelles decreases.[3] Thus, highly hydrophobic compounds that tend to be totally incorporated into micelles can be separated due to their different affinity toward the CDs. The addition of 5 mM γCD to 30 mM borate, 30 mM SDS buffer (BS30) to form the BS30-γCD5 buffer system facilitated the separation of BODIPY fatty acid standards with different chain lengths (B_3FA and B_5FA) and structural isomers ($B_{11}FA$ and $B_{12}FA$) (Figure S1). The calculated and corrected electrophoretic mobilities, the peak areas, and limits of detection are shown in Table S1. The relative standard deviation (RSD) of mobility and peak area of the four standards in three consecutive runs is less than 1% and 5%, respectively. By using fluorescein as an internal standard [4], the reproducibility improves (RSD ~ 0.1-0.2%, Table S1). The limits of detection (LOD) of BODIPY fatty acid standards are at picomolar level.

Figure S1. Separation and detection of BODIPY fatty acid analogs using MEKC-LIF. The MEKC was performed in a 43.3 cm fused silica capillary. Trace a: Buffer control which contains fluorescein as the internal standard; Trace b: A mixture containing 2.5 10^{-10} M of (1) B_3FA, (2) B_5FA, (3) $B_{12}FA$, (4) $B_{11}FA$ and fluorescein (i) was injected at 10.8 kPa for 1 s. Other conditions were the same as described in Figure 3 of the main manuscript. Insert: structures of B_3FA (n=2), B_5FA (n=4) and $B_{11}FA$ (n=10).

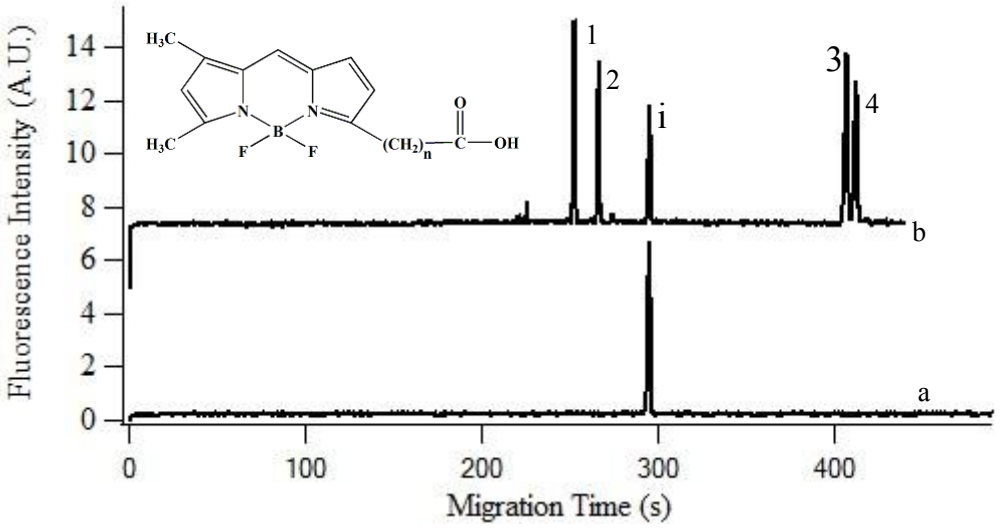


Table S1. Characterization of the separation system using BODIPY standards

	Peak area (A.U.)	Mobility ($10^{-4}cm^2 \cdot V \cdot s^{-1}$)	Corrected mobility* ($10^{-4}cm^2 \cdot V \cdot s^{-1}$)	LOD** (10^{-12} mole/L)
B_3FA	12.15±0.01	4.29±0.03	4.288±0.004	3.7±0.4
B_5FA	7.26±0.09	4.06±0.03	4.063±0.004	4.6±0.4
$B_{11}FA$	11.4±0.4	2.66±0.02	2.661±0.006	4.4±0.6
$B_{12}FA$	13.6±0.1	2.63±0.02	2.625±0.006	5.4±0.8

Results are the average ± standard deviation of three consecutive runs

* Mobility is corrected using fluorescein as the internal standard

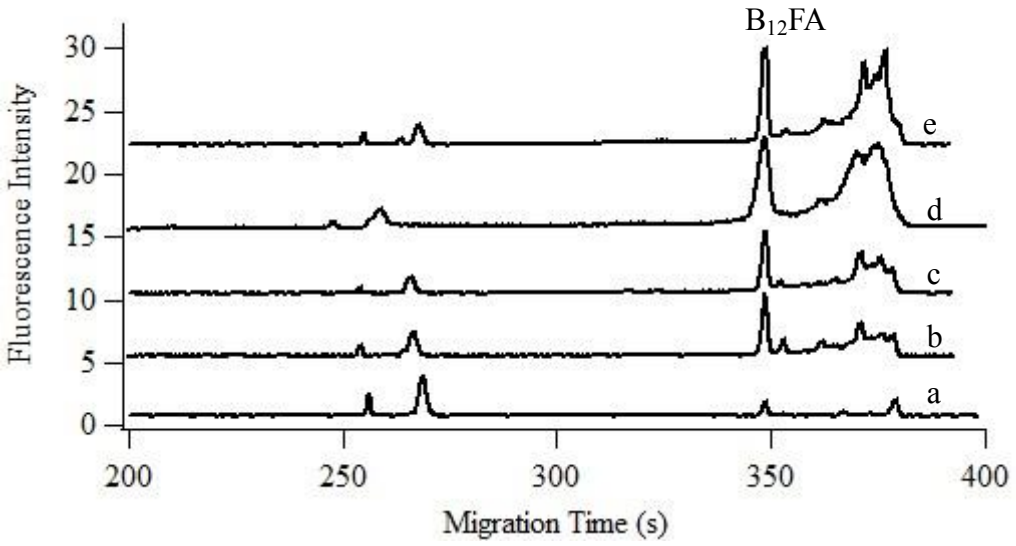
****** For each BODIPY fatty acid standard, the estimated LOD (defined as the concentration that gives a signal equal to 3 times the standard deviation of the background) was calculated from the peak intensity resulting from injecting 2.5 10^{-10} mole/L of the respective standard and the estimated injected volume.

Metabolism of $B_{12}FA$ in cultured L6 myoblasts

L6 myoblasts were cultured with 5 μM $B_{12}FA$ for different lengths of times (30 min, 2 h, 4 h and 6 h). After treatments, cell culture medium was removed and the cells were washed three times with PBS. The cells were then lysed in 10 mM borate and 10 mM SDS buffer (pH = 9.3) and diluted to an equivalent cell density of 2×10^6 cells/ml. The cell lysates were kept at -80 ºC until MEKC analysis.

The MEKC-LIF were performed in 30 mM borate, 30 mM SDS and 5 mM γ-cyclodextrin buffer (pH = 9.3). Figure S2 shows the electropherograms of L6 cells after being incubated with $B_{12}FA$ for different lengths of times. Multiple peaks were observed after the $B_{12}FA$ peak. These peaks have lower mobilities than $B_{12}FA$, which indicates that they are more hydrophobic. Based on the hydrophobicity, these peaks could be the triacylglycerol form of $B_{12}FA$. The intensities of these peaks increase with time because more $B_{12}FA$ were taken up by the cells with longer incubation time.

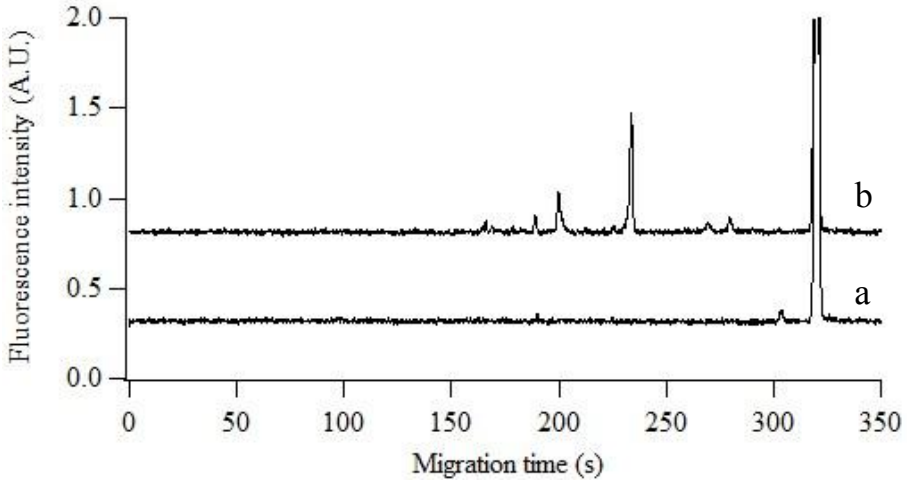
Figure S2. Electropherograms of L6 cells incubated with $B_{12}FA$ for different lengths of times. The MEKC were performed in a 45.5 cm fused silica capillary. Trace a is L6 cell lysate alone, and Traces b to e are L6 cells incubated with $B_{12}FA$ (5 μM) for 30 min, 2h, 4h and 6 h, respectively. Traces b to e were y-axis offset for clarity. Other conditions were the same as those described in Figure 3.



Reaction of DOX with concentrated H_2O_2

We observed evidence for the reaction of DOX with H_2O_2 in an *in vitro* system. After incubating DOX with 1 mM H_2O_2 for 3 h, several products that account for 20% of the total peak area were detected by MEKC-LIF (Figure S3). Indeed, we cannot exclude the possibility of formation of non-fluorescent products such as 3-methoxyphthalic acid or simple phthalates[5] that are undetectable by the LIF detector used in these studies.

Figure S3. DOX and products after reacting with H_2O_2 *in vitro*. The MEKC was performed in a 42.5 cm fused silica capillary under a 400 V/cm electric field in a buffer containing 10 mM borate and 10 mM SDS (pH = 9.3). Trace a is 10 μM DOX alone at 37 ºC for 3 h and trace b is 10 μM DOX incubated with 1 mM H_2O_2 for 3 h at 37 ºC. Trace b was y-axis offset for clarity. Other conditions were same as that described in Figure 3 except the 520+-17.5nm band-pass filter was substituted with a 635+-27.5 band pass filter



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