Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary information

Synthesis of lipase nano-bio-conjugates as an efficient biocatalyst: Characterization and activity-stability studies with potential biocatalytic applications

Kirtikumar C. Badgujar,[a] Takehiko Sasaki,[b] Bhalchandra M. Bhanage[a]*

[a]Department of Chemistry, Institute of Chemical Technology, Matunga, Mumbai 400 019, India

[b]Department of Complexity Science and Engineering, Graduate School of Frontier Sciences, The University of Tokyo, Chiba 277-8561, Japan

*Corresponding author (B. M. Bhanage): Tel.: +91-22-3361-2601/2222; Fax: +91-22-2414-5614; Email address: bm.bhanage@ictmumbai.edu.in bm.bhanage@gmail.com bhalchandra_bhanage@yahoo.com

Supporting Characterization:

Materials and Methods:

1. XPS analysis

X-ray photoelectron spectra (XPS) were determined for support MCNT, free PFL and immobilized MCNT:lipase by using a PHI 5000 versaprobe scanning ESCA microprobe which is operated at the pressure of 2×10^{-9} Pa using Mg·Ka X-ray as the excitation source. The binding energies were standardized against the Au (4f) emission at 86 eV.

2. TGA analysis

The thermo gravimetric analysis (TGA) was carried out using Perkin Elmer STA 6000 analyzer. The support MCNT, free PFL and immobilized MCNT:lipase sample (5-8 mg) was kept in a ceramic crucible and analysis run was examined from 30 to 630 ^{o}C with 10 ^{o}C/min rise in temperature, under the 99.99 % pure nitrogen atmosphere with flow of 100 mL /min. The reference control run was performed with an empty sample crucible pan.

3. XRD analysis

The MCNT, MCNT:lipase and free lipase were characterised using X-ray diffractometer (XRD: Shimadzu XRD-6100 using Cu Kα radiation = 1.5405 Å) with a scanning rate of 2^{o}/min. The 2θ angle was ranging from 10 to 70^{o} at current 30 mA and voltage 40 kV.

Results and Discussion:

1. XPS analysis study

XPS measurement was carried out to investigate the presence of the various chemical species/ elements present after immobilization of lipase PFL on the MCNT.[11,15] Figure S1B indicating the XPS analysis of MCNT, MCNT-PFL lipase and free PFL. The MCNT showed peak of Carbon (C1s) only, while immobilized lipase on nano-tube (MCNT-lipase) indicating presence of the Carbon (C1s), Nitrogen (N1s) as well as Oxygen (O1s). Peak at 398.8-399.3 eV was attributed mainly to nitrogen (N1s) of the amide bond while the peak at 531-532.2 eV was attributed to oxygen (O1s) of carbonyl bond present in amide functionality.[11] The peak at 284.5-285.4 eV was attributed to presence of carbon (C1s). This observation confirmed immobilization of lipase bio-molecules on the MWNTs and synthesis of nano-bio-

conjugates.[15] Thus, the peak of Carbon, Oxygen and Nitrogen was attributed due to presence of amide (-$CONH_2$) functionality of enzymes.[11,15]

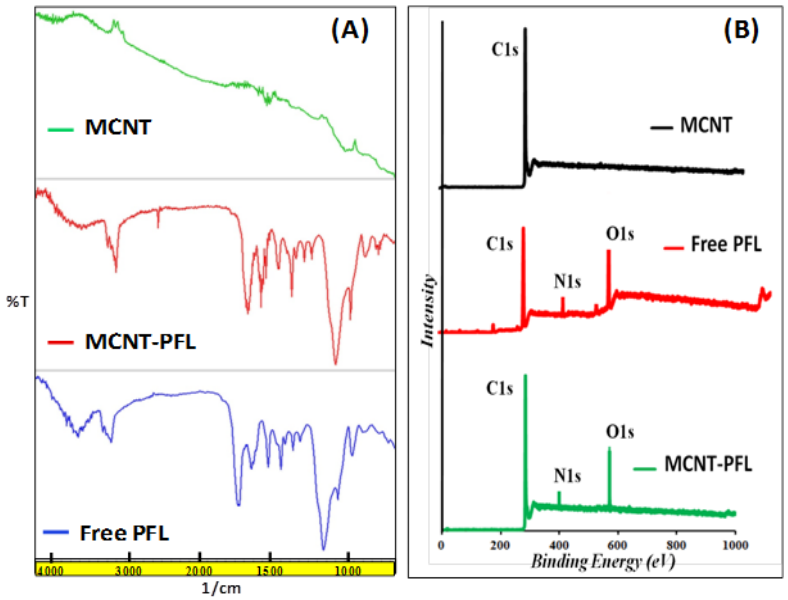

Fig. S1: FTIR and XPS analysis study

2. XRD analysis study

The XRD patterns of the native free lipase, MCNTs, and MCNT-immobilized lipase are illustrated in Figure S2A. The diffraction pattern of MCNTs (first-red colour spectra) contains sharp and prominent signature peaks at 25, 44 and 64^o. These XRD pattern peaks are matched according to the JCPDS chemical spectra data bank, where the prominent peak near to 25 was attributed to the (002) reflection of carbon. The free lipase PFL (third-green colour spectra) represented peak around 27, 31, 46, 56 and 66^o. Moreover, immobilized lipase (second-black colour spectra) showed peak at 25, 27, 31, 44, 46, 56 and 64^o. These results of XRD patterns signifying that structural morphology did not change after immobilization of lipase PFL on the MCNTs. Moreover, the XRD pattern of MCNT-immobilized lipase consist of all peaks of the free PFL and MCNTs which indicating only physical immobilization interaction of the MCNTs and lipase.

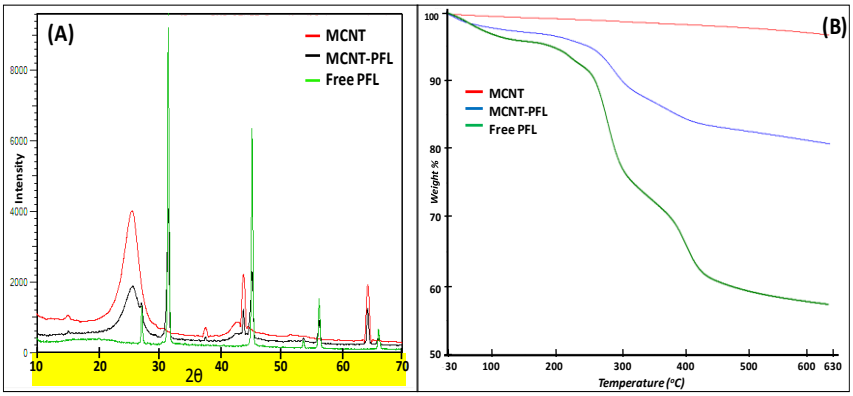

Fig. S2: XRD and TGA analysis study

3. TGA analysis

The thermo gravimetric analysis (TGA) was performed to investigate the thermal stability of the lipase (Figure S2B).[32] The TGA analysis showed insignificant weight loss for the MCNT, the weight loss was hardly 2-3 % at 600 ^{o}C. Whereas, native lipase PFL and immobilized MCNT:PFL lipase showed 4-5 % weight loss at nearly 100 ^{o}C due to the elimination of physically bound water while weight loss at the 210-220 ^{o}C was attributed due to elimination of the tightly bound water.[32] Compared to the support MCNTs, the free lipase PFL and immobilized MCNT-PFL showed faster decomposition which was attributed to denaturation of proteinaceous linked structure of lipase.[32] The TGA curve of free PFL showed 26 % weight loss at 350 ^{o}C while immobilized MCNT-PFL showed 13 % weight loss at 350 ^{o}C. Thus, above observations indicated higher/ improved stability of immobilized MCNT:PFL lipase compared to free PFL. Moreover at 600 ^{o}C, free lipase PFL showed 48 % weight loss while MCNT-PFL showed nearly 17-20 % weight loss, which indicating higher decomposition of free lipase PFL compared to immobilized MCNT:PFL

References Note:

All the numbered references cited in the supplementary information are included in the main manuscript.

Characterization of products

Characterization of the products by ^{1}HNMR and ^{13}C NMR

Cinnamyl acetate (^{1}HNMR, 400MHz, $CDCl_3$, using TMS as internal standard) (Table 7, entry 1): δ= 2.20 (3H, S); δ= 4.52 (1H, d); δ= 6.23 (1H, q); δ= 6.62 (1H, d); δ= 7.22-7.43 (5H, m)

Butyl acetate (^{1}HNMR, 400MHz, $CDCl_3$, using TMS as internal standard) (Table 7, entry 3): δ= 2.22 (3H, S); δ= 4.15 (2H, t); δ= 1.68 (2H, q); δ= 1.41 (2H, m); δ= 0.94 (3H, t)

Octyl acetate (^{1}HNMR, 400MHz, $CDCl_3$, using TMS as internal standard) (Table 7, entry 5): δ= 2.22 (3H, S); δ= 4.18 (2H, t); δ= 1.60 (2H, qn); δ= 1.40 (2H, qn); δ= 1.32 (4H, qn); δ= 1.30 (2H, qn); δ= 1.34 (2H, m); δ= 0.84 (3H, t)

Isobutyl acetate (400MHz, $CDCl_3$, using TMS as internal standard) (Table 7, entry 8): δ= 2.23 (3H, S); δ= 3.89 (2H, d); δ= 1.92 (1H, m); δ= 0.99 (6H, d)

Benzyl acetate (^{1}HNMR, 400MHz, $CDCl_3$, using TMS as internal standard) (Table 7, entry 11): δ= 2.21 (3H, S); δ= 5.23 (1H, s); δ= 7.35-7.49 (5H, m)

Cinnamyl acetate (^{13}CMR, 100MHz, $CDCl_3$) (Table 7, entry 1): δ= 20.4, 170.9, 64.5, 120.6, 133.1, 136, 2×127.9, 128.6, 127.1 129

Butyl acetate (^{13}CMR, 100MHz, $CDCl_3$) (Table 7, entry 3): δ= 20.9, 170, 64.8, 31.8, 18.2, 13.1

Octyl acetate (^{13}CMR, 100MHz, $CDCl_3$) (Table 7, entry 5): δ= 20.7, 170.8, 64.4, 28.5, 25.7, 29.8, 29.1, 31.5, 22.9, 14.7

Isobutyl acetate (^{13}CMR, 100MHz, $CDCl_3$) (Table 7, entry 8): δ= 20.4, 170.8, 70.9, 27.6, 19.9, 19

Benzyl acetate (^{13}CMR, 100MHz, $CDCl_3$) (Table 7, entry 11): δ= 20.9, 170.8, 66.7, 136.9, 127.4, 2×128.2, 127.1, 121.6

Characterization of the products by GCMS analysis

1. **Cinnamyl acetate** (Table 7, entry 1): MS (70 eV, EI) m/z (%): 176 (M^+) 133, 115, 105, 103, 92, 77, 63, 55, 43, 40.
2. **Prenyl acetate** (Table 7, entry 2): MS (70 eV, EI) m/z (%): 128 (M^+), 113, 86, 71, 67, 53, 43, 39.
3. **Butyl acetate** (Table 7, entry 3): MS (70 eV, EI) m/z (%): 116 (M^+), 101, 72, 61, 57, 56, 43
4. **Hexyl acetate** (Table 7, entry 4): MS (70 eV, EI) m/z (%): 144 (M^+), 129, 111, 84, 69, 61, 56, 43, 41.
5. **n-octyl acetate** (Table 7, entry 5): MS (70 eV, EI) m/z (%): 173 (M^+ +1), 129, 112, 111, 84, 83, 74, 70, 57, 55, 43.
6. **Cis-3-hexenyl acetate** (Table 7, entry 6): MS (70 eV, EI) m/z (%): 143, 127, 83, 72, 67, 55, 43
7. **Neryl acetate** (Table 7, entry 7): MS (70 eV, EI) m/z (%): 196 (M^+), 136, 121, 93, 69, 53, 41.
8. **Iso-butyl acetate** (Table 7, entry 8): MS (70 eV, EI) m/z (%): 116 (M^+), 86, 73, 71, 61, 57, 56, 55, 43
9. **Iso-amyl acetate** (Table 7, entry 9): MS (70 eV, EI) m/z (%): 131 (M^+ +1), 115, 101, 87, 73, 70, 61, 55, 43.
10. **2-ethyl-hexyl-1-acetate** (Table 7, entry 10): MS (70 eV, EI) m/z (%): 173 (M^+ +1), 112, 83, 74, 70, 57, 55, 43.
11. **Benzyl acetate** (Table 7, entry 11): MS (70 eV, EI) m/z (%): 150 (M^+), 108, 91, 79, 65, 43.
12. **3-Phenyl propyl acetate** (Table 7, entry 12): MS (70 eV, EI) m/z (%): 178 (M^+), 117, 105, 91, 77, 65, 57, 51, 43

13. **2-Phenyl ethyl acetate** (Table 7, entry 13): MS (70 eV, EI) m/z (%): 164 (M^+), 134, 121, 105, 104, 91, 78, 77, 66, 43.
14. **2-Methyl benzyl acetate** (Table 7, entry 14): MS (70 eV, EI) m/z (%): 164 (M^+), 122, 104, 105, 91, 78, 65, 43.
15. **4-Methoxy benzyl acetate** (Table 7, entry 15): MS (70 eV, EI) m/z (%): 180 (M^+), 138, 121, 109, 91, 77, 78, 43.
16. **2-Methoxy benzyl acetate** (Table 7, entry 16): MS (70 eV, EI) m/z (%): 180 (M^+), 137, 120, 107, 91, 77, 65, 51, 43.
17. **Piperonyl acetate** (Table 7, entry 17): MS (70 eV, EI) m/z (%): 194(M^+), 152, 135, 122, 105, 93, 77, 65.
18. **1-Phenyl ethyl acetate** (Table 7, entry 18): MS (70 eV, EI) m/z (%):164 (M^+), 122, 104, 77, 51, 43.
19. **Menthyl acetate** (Table 7, entry 19); MS (70 eV, EI) m/z (%): 198 (M^+), 141, 138, 123, 95, 81, 67, 55, 43.
20. **Cyclohexyl acetat**e (Table 7, entry 20): MS (70 eV, EI) m/z (%): 142 (M^+), 100, 82, 67, 54, 43, 41.
21. **Phenyl acetate** (Table 7, entry 21): MS (70 eV, EI) m/z (%): 136 (M^+), 94. 77, 66, 43, 40.
22. **P-Cresyl acetate** (Table 7, entry 22): MS (70 eV, EI) m/z (%): 150 (M^+), 108, 90, 77, 65, 43, 40.