Advanced NMR characterisation of novel fluorinated cellulose-like materials

Juan C. Muñoz García,[1] Peterson de Andrade,[2] Giulia Pergolizzi,[2] Valeria Gabrielli,[1] Sergey Nepogodiev,[2] László Fábián,[1] Rinat Nigmatullin[3], Marcus A. Johns[3], Robert Harniman,[3] Dinu Iuga,[4] Steve Eichhorn,[3] Jesús Angulo[1], Robert A. Field,[2] Yaroslav Z. Khimyak[1]

[1]School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, United Kingdom
[2]John Innes Centre, Norwich Research Park, Colney Ln, Norwich NR4 7UH, United Kingdom
[3] Bristol Composites Institute (ACCIS), University of Bristol, Bristol BS8 1TR, United Kingdom
[4]The UK 850 MHz Solid-State NMR Facility, Department of Physics, University of Warwick, Coventry CV4 7AL, United Kingdom

Abstract

The application of ^{19}F NMR to probe chemical and biological interactions is constantly increasing due to the 100% isotopic abundance of ^{19}F, the extremely high sensitivity of ^{19}F to changes in local environment avoiding overlapping signals, and its almost total absence in biological receptors in nature.

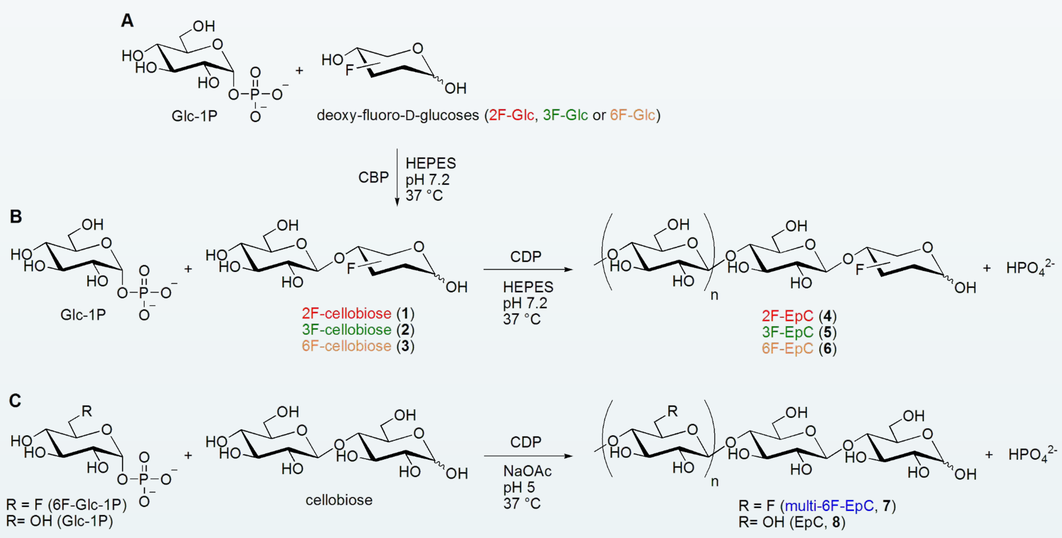
Therefore, the introduction of ^{19}F in molecules represent a very powerful strategy to monitor conformational changes, ligand-receptor interactions and assembly processes occurring in complex and heterogeneous systems such as cellulose. We have accomplished the cellodextrin phosphorylase synthesis of (i) multiply 6-fluorinated cellulose oligomers and (ii) a series of cellulose oligomers containing a single deoxy-fluoro glucose per cellulose chain (at the reducing end), all yielding a degree of polymerization of about 7-9 glucose units. We have characterised their structure by advanced solid-state and solution NMR experiments, x-ray diffraction and microscopy methods.

Our results show that single 2-, 3- and 6-monofluorination at the reducing end does not significantly impact either the long (crystallinity) nor the short-range (inter-chain interactions) order of enzymatically produced cellulose (EpC) oligomers, thus representing a powerful chemical strategy to probe self-assembly. Interestingly, the presence of multiple 6-fluorination per oligosaccharide chain gave rise to morphological and crystallinity features and inter-chain interactions that are unprecedented for cellulose-like materials.

This work demonstrates that phosphorylases can facilitate the "bottom-up" assembly of novel biomaterials from a fluorinated donor substrate, which might lead to potential applications across different industries (food, health, cosmetics, pharmaceuticals, etc).

Enzymatic synthesis of fluorinated celluloses

Enzymatic synthesis of single 2-, 3- and 6-monofluorinated and multiple 6-monofluorinated cellodextrins



Scheme 1. Enzymatic synthesis of fluorinated cellodextrins. (**A**) Cellobiose phosphorylase (CBP) catalysed reaction of α D glucose 1 phosphate (Glc 1P) and deoxy fluoro D glucose (2F Glc, 3F Glc or 6F Glc), followed by (**B**) cellodextrin phosphorylase (CDP) catalysed oligomerisation with Glc 1P and monofluorinated cellobioses, to afford enzymatically produced fluorinated cellodextrins (2F-EpC, 4; 3F-EpC, 5; and 6F-EpC, 6). (**C**) CDP catalysed reaction of 6 deoxy 6 fluoro α D glucose 1 phosphate (6F-Glc-1P) or Glc 1P and cellobiose as acceptor, to produce multiply 6-fluorinated cellodextrin (multi-6F-EpC, 7) or the parent enzymatically produced cellodextrin (EpC, 8), respectively.

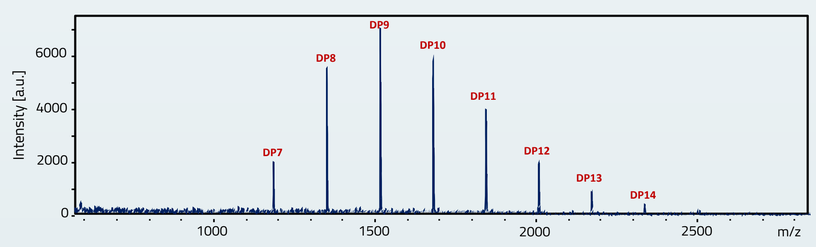


Figure 1. Representative MALDI spectrum of the four fluorinated cellodextrin materials. The degree of polymerization (DP) corresponding to each m/z peak is indicated above each.

Raman spectroscopy

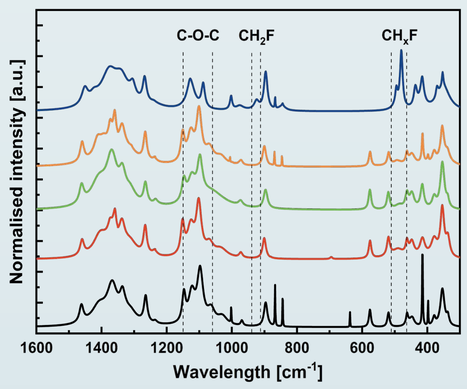


Figure 2. Deconvoluted and normalised Raman spectra for EpC (black), 2F-EpC (red), 3F-EpC (green), 6F-EpC (orange) and multi-6F-EpC (blue). Dashed lines correspond to boundaries of bands associated with C-O-C stretching (C-O-C) and presence of fluorinated carbon groups (CH2F and CHxF). Each Raman spectrum represents the average of three Lorentzian-deconvoluted spectra upon noise removal.

Powder x-ray diffraction (PXRD)

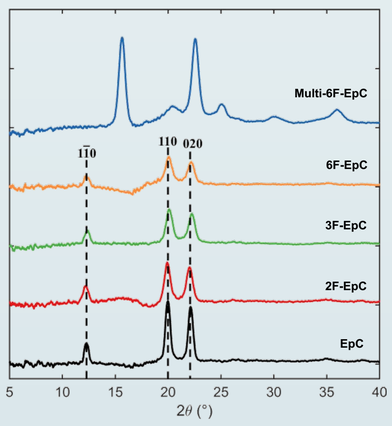


Figure 3. Powder x-ray diffraction patterns of non-substituted EpC (black), 2F-EpC (red), 3F-EpC (green), 6F-EpC (orange) and multi-6F-EpC (blue). The diffraction pattern of non-substituted and single fluorinated EpCs (black, red, green and orange) is characteristic of cellulose type II. Note that the pattern of multi-6F-EpC (blue) does not correspond to any know allomorph previously reported for cellulose.

Nano and microscale characterisation

Solid state nuclear magnetic resonance (SSNMR)

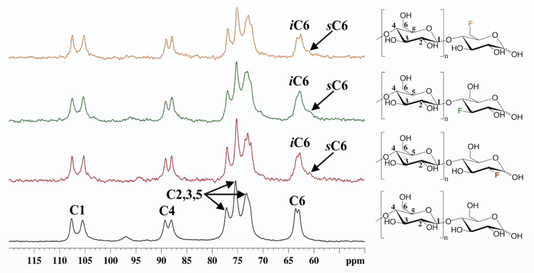


Figure 4. ^{1}H-^{13}C CP/MAS NMR spectra of EpC powder acquired at 10 kHz MAS rate (black), and 2F- (4, red), 3F- (5, green) and 6F-EpC (6, orange) 10 wt% dispersions acquired at 6 kHz MAS, and 100 MHz ^{13}C frequency

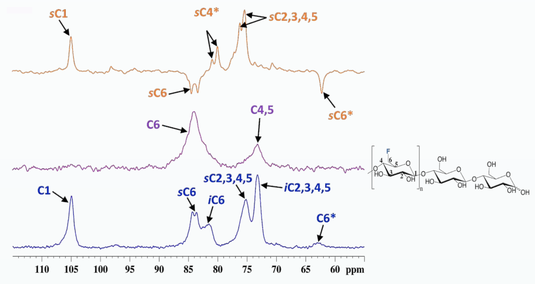


Figure 5. ^{1}H-^{13}C CP (blue) and ^{1}H,^{19}F-decoupled ^{19}F-^{13}C CP (purple) NMR spectra of multi 6F EpC (7) powder acquired at 60 and 15 kHz MAS spinning, respectively, and 212.5 MHz ^{13}C frequency. The ^{13}C DEPT135 spectrum of a 1 wt% dispersion of multi-6F-EpC (7) in D_2O is shown for comparison (orange). *Low intensity peaks corresponding to the non fluorinated glucose units of 7 at the reducing terminal of each cellodextrin chain.

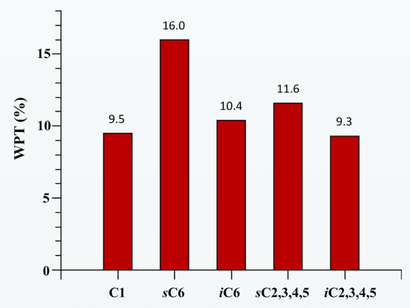


Figure 6. Bar graph showing the water polarisation transfer (WPT) factors, in percentage, determined for each carbon peak of multi-6F-EpC 25 wt% hydrogel using a mixing time of 16 ms. The higher WPT factor observed for *s*C6 and *s*C2,3,4,5 compared to *i*C6 and *i*C2,3,4,5 demonstrates the increased solvation of the former, hence being assigned to surface domains. The individual WPT values appear on top of each bar.

Transmission electron microscopy (TEM)

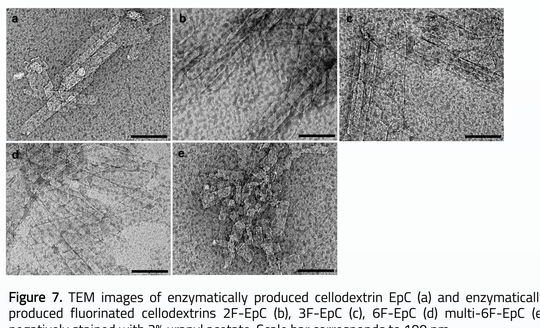


Figure 7. TEM images of enzymatically produced cellodextrin EpC (a) and enzymatically produced fluorinated cellodextrins 2F-EpC (b), 3F-EpC (c), 6F-EpC (d) multi-6F-EpC (e) negatively stained with 2% uranyl acetate. Scale bar corresponds to 100 nm

Scanning electron microscopy (TEM)

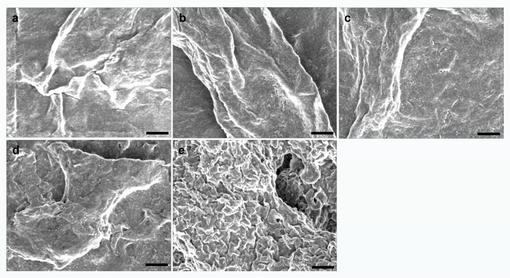


Figure 8. SEM images of enzymatically produced cellodextrin EpC (a) and enzymatically produced fluorinated cellodextrins 2F-EpC (b), 3F-EpC (c), 6F-EpC (d) per-6F-EpC (e) coated with platinum. Scale bar corresponds to 1 μm.

Atomic force microscopy (AFM)

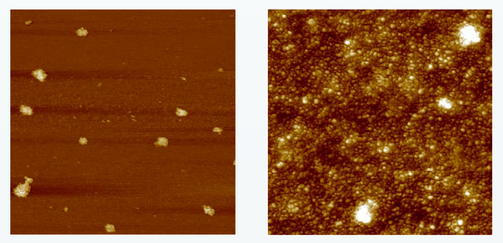


Figure 9. AFM images obtained at 0.9 μm magnification for single 3F-EpC (left) and multi-6F-EpC (right) diluted suspensions (0.04 mg/ml) deposited on freshly cleaved mica. Note that 3F-EpC deposited in quite regular aggregates leaving an empty surface between them, while multi-6F-EpC forms a film-like coating with a grainy structure.

Conclusions

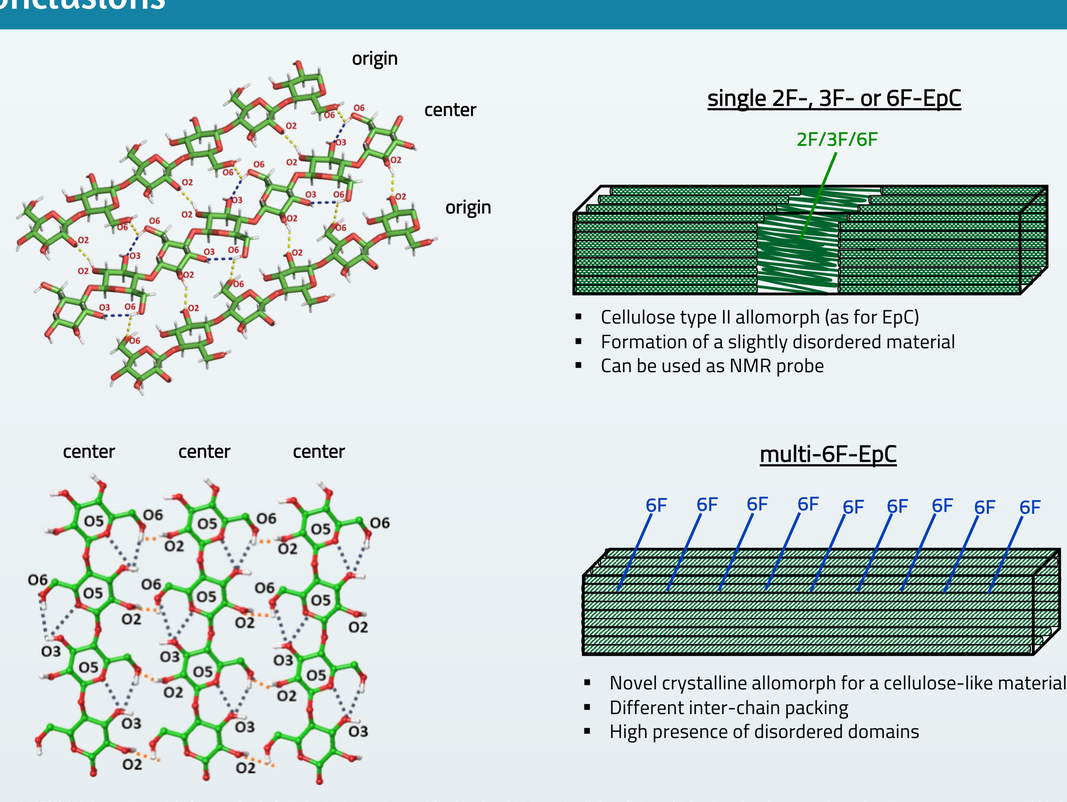


Figure 10. (Right) Schematic model of a nanofibril of single 2F-, 3F- or 6F-EpC (top) and multi-6F-EpC cellulose (bottom). The disordered or amorphous domains appear as green scribbled lines, while crystalline regions are represented as rectangles with an specific drawing pattern. Note that the pattern of multi-6F-EpC is different from the other two, indicating a different crystalline order or allomorph. **(Left)** 3D models showing the inter-chain interactions present in non-modified EpC.

- The presence of only 1 fluorine atom per oligosaccharide chain at either position 2, 3 or 6 of glucose (2F-EpC, 3F-EpC and 6F-EpC) produces cellulose oligomers resembling the native assembly of unsubstituted EpC, with a small population of disordered domains. Importantly, these materials can be used to probe interactions between EpC and other particle networks

- The enzymatic synthesis of cellodextrin using 6-fluorinated glucose-1-phosphate to form multi-6F-EpC gives rise to an unprecedented morphology, crystalline allormorph and inter-chain interactions, as shown by microscopy, PXRD and NMR, respectively

- Phosphorylases can facilitate the "bottom-up" assembly of novel biomaterials from a fluorinated donor substrate

Acknowledgements

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