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DNA damage induced by bare and loaded microporous coordination polymers from their ground and electronic excited states†

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We report on interactions of cell free double-stranded DNA (dsDNA) with a selected subgroup of Microporous Coordination Polymers (MCPs). In particular, we have studied the influence of different metal ion constituents and chemically modified linkers using a set of five benzene carboxylate-based MCPs. Our results suggest that the DNA moiety can be structurally modified in two different ways: by direct MCPs–dsDNA interaction and/or through photosensitized processes. The extent of the observed damage was found to be strongly dependent on the charge density of the material. The potential use of the MCPs tested as inert carriers of photosensitizers was demonstrated by analyzing the interaction between dsDNA and harmine-loaded Cr-based materials, both in the absence of light and upon UVA irradiation.

Introduction

In the last twenty years, a relatively new class of microporous polymeric hybrid materials, lying between the classic organic (carbon materials) and inorganic (zeolites) materials, has emerged. The structure of these organic–inorganic hybrid polymers can be described in terms of a combination between a metal ion (or a metal ion cluster) coordinated with organic linkers. These materials are also commonly referred to as Metal Organic Frameworks (MOFs).[1] Their main features are: high surface areas, wide range of pore sizes and shapes, and a great variety of chemical functionalities exposed in the pore walls.[2] Such features are possible due to an overwhelming number of organic linkers and metal cluster combinations, yielding thus a very versatile tunable microporous material appealing for diverse applications such as separation,[3,4] adsorption,[5] gas storage,[6] and heterogeneous catalysis.[7,8]

MCPs have potential biological-related uses, *e.g.*, in drug delivery[9,10] or as agents for imaging contrast.[11] However, in order to achieve these goals (aside from the requirements of water stability,[12] and drug affinity[9]), biocompatibility must be ensured. Additionally, given the different dimensions of biomolecules such as DNA, and the pore window size ranges of MCPs, interactions are expected to occur at the external surfaces of MCP particles rather than at the inner porous surface. Hence, particle size distribution should be controlled for a meaningful quantification. Special attention must be paid to interactions both in the dark[13] (*e.g.*, through adsorption on Lewis acid sites present on the microporous matrix), and in the presence of light[14,15] of MCPs with biological target molecules such as DNA and proteins. Studies on interactions of MCPs aqueous suspensions with genetic materials are scarce. Recently, Zhang *et al.* reported the possible use of the Zr-based materials UiO-66 and UiO-66-NH_2 as fluorescent sensing platforms for single-stranded DNA (ss-DNA) detection.[16] However, there should be differences in the interaction between MCPs and DNA particles when using double-stranded DNA (dsDNA), due to the lack of unpaired bases available for hydrogen bonding.

Motivated by the discussion above, and searching for a biocompatible MCP, we explored as a starting point, the interaction between cell free dsDNA and five different MCPs on aqueous suspensions. In particular, we used commercially available Fe^{3+} and Cu^{2+} trimesates, Basolite F300 or Fe(BTC) and Basolite C300 or Cu(BTC) (with BTC = 1,3,5-benzenetricarboxylate); both synthesized chromium (Cr^{3+}) terephthalates: MIL-101 or Cr(BDC) (with BDC = 1,4-benzenedicarboxylate) and MIL-101-SO_3H or Cr(BDC-SO_3H) (where BDC-SO_3H is the sulphonic acid substituted 1,4-benzenedicarboxylate); and also synthesized amino substituted UiO-66-NH_2,

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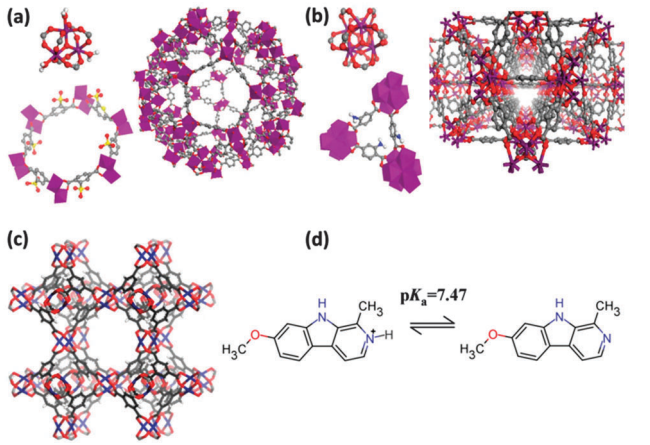

Fig. 1 Structures of the selected MCPs, together with the corresponding metallic clusters and pore windows. (a) Metallic cluster and the cage structure (upper left and right panels, respectively) corresponding to Cr-based MIL-101 (BDC^{2-} linker). The pore window structure for MIL-101-SO_3H modified MCP used is also shown in the left bottom panel. (b) The corresponding metallic cluster and the cage structure for UiO-66-NH_2. (c) Cage structure for Basolite C300. (d) Chemical structure of the harmine moiety and its acid–base equilibrium.

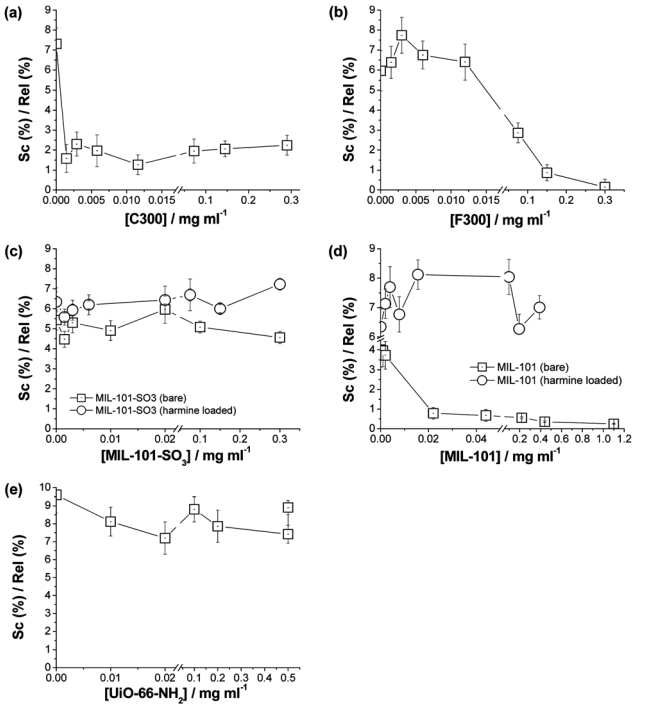

Fig. 2 Dose-dependence response on the PM2 relaxation as a function of MCPs concentration. Results are shown as the ratio between the relative intensity of supercoiled (Sc) to relaxed (Rel) DNA for each MCP concentration used. Plots from (a) to (e) correspond to the interaction between PM2 with five different bare MOFs: C300, F300, MIL-101-SO_3H, MIL-101 and UiO-66-NH_2, respectively. Also, in plots (c) and (d), the results obtained using harmine-loaded MIL-101-SO_3H and MIL-101 materials are presented. The corresponding electrophoretic gels are shown in the ESI† (Fig. S5).

featuring Zr^{4+} metal ion centers and BDC-NH_2 linkers. In this way, we assessed both the influence of different metal ions, as well as the addition of chemically modified linkers that provide different net charges and/or electronic densities on MCP pore walls. Moreover, searching for a biocompatible MCP suitable for its use in photodynamic therapy (as an inert support of a photosensitizer), we extended our studies to include dsDNA interactions with Cr-based MCP loaded with harmine, a well-known photosensitizer.[17,18] Fig. 1 shows the structures of the above mentioned MCPs, and both protonated and neutral species of harmine.

Results and discussions

The long term water stability of a given MCP depends on the tendency of the metal–linker bond to undergo hydrolysis.[12] Even as this issue is still under debate for a vast number of recently reported MCPs, the time range employed in our experiments (4 h maximum) allows us to assume stability and, therefore, to assess the influence of the metal ion and surface chemical composition on the interaction of the considered MCPs with dsDNA, with or without UVA irradiation.

The influence of increasingly higher concentrations of bare C300, F300, MIL-101, MIL-101-SO_3H and UiO-66-NH_2 materials on the extracellular dsDNA (PM2) structure was explored using a DNA relaxation assay. The ratio between the intensity of the band corresponding to supercoiled (Sc) and relaxed forms of DNA is displayed in Fig. 2. A higher value of this ratio means a lower extent of DNA relaxation induced by the considered MCP. The results obtained for C300, F300 and MIL-101 demonstrate that these materials provoke a considerable DNA relaxation, even at very low concentrations. In contrast, MIL-101-SO_3H and UiO-66-NH_2 cause a negligible or null dsDNA relaxation in the whole MCP concentration range investigated. We further tested the behavior of the system described above using a different source of dsDNA (*i.e.*, Hsp28-YFP plasmid), and results obtained were found to be reproducible (see ESI,† Fig. S6).

Care must be taken if a precise quantitative comparison of the activity of different MCPs is desired. The reason is that differences in particle size distributions would also yield differences in the external surface areas exposed per unit weight, which might bias the results obtained. However, the broad concentration range studied here allows for a meaningful qualitative comparison of the intensity of interactions between DNA and the various chemically different MCPs used. Thus, the results discussed above demonstrate that, aside from putative particle-size effects, the polar character of the MCP considered has a decisive role on the interaction observed with biomolecules such as DNA, *i.e.*, for an increase in either negative net charge or electronic density, damage to a lesser extent was observed due to repulsive coulombic interactions with the negatively charged DNA moiety.

The photosensitizing properties of the five investigated bare MCPs, upon UVA excitation, were also evaluated. In particular, two types of plasmid DNA (Hsp28-YFP and PM2) were irradiated in the presence of the materials considered herein. The results depicted in Fig. 3 show a visible dsDNA relaxation, photosensitized by all the non-substituted MCPs investigated (*i.e.*, C300, F300 and MIL-101), leading to an overall decrease in the initial Sc

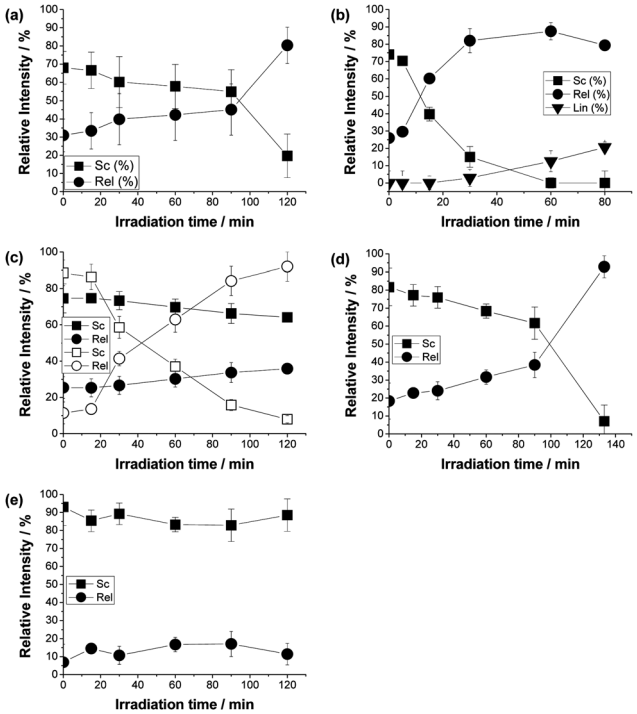

Fig. 3 Evolution of the relative intensity of supercoiled (Sc), relaxed (Rel) and linear (Lin) forms of DNA as a function of the irradiation time in the presence of (a) 0.3 mg ml^{-1} of C300, (b) 0.3 mg ml^{-1} of F300, (c) 0.5 mg ml^{-1} of bare and harmine-loaded MIL-101-SO_3H (full and open symbols, respectively), (d) 0.5 mg ml^{-1} of MIL-101 and (e) 0.5 mg ml^{-1} of UiO-66-NH_2. The corresponding electrophoretic gels are shown in the ESI† (Fig. S7).

concentration of ≈90 to 100%. However, the extent was clearly different for each material. Upon irradiation, the iron based material F300 was found to produce a much higher measurable structural modification on dsDNA than C300 (note that the cumulative damage photoinduced by F300 produces the appearance of the linear form of dsDNA, Fig. 3b).§ Aside from the different structures of the MCPs, the behavior observed upon irradiation can be traced back to the different metal ion center, since both materials display the same organic linker (BTC). We speculate on the mechanism of dsDNA damage to be highly dependent on the efficiency of the metal ion center to photosensitize reactive oxygen species and/or to photoinduce redox processes. It was recently proposed that the empty 3d orbitals of MCP metal ion centers would combine with the LUMOs of the organic linkers giving rise to a conduction band. Upon light irradiation, electron excitation would take place followed by subsequent electron transfer. Therefore, MCPs can be expected to display photocatalytic and/or photosensitizing activity. In this regard, a chemically similar material to F300 (MIL-53(Fe) or Fe-BDC) was found to be active for methylene blue dye degradation under UV light irradiation.[14] Nevertheless, to fully elucidate this point further experiments are needed and are part of an ongoing project in our lab.

In contrast, MIL-101-SO_3H and UiO-66-NH_2 showed low dsDNA photosensitization when subjected to irradiation, leading to a decrease in the initial Sc concentration of ≈10% after 120 min of UVA exposure (Fig. 3c and e, respectively). This fact suggests that MIL-101-SO_3H and UiO-66-NH_2 would be appealing candidates for its use in photodynamic therapy, *i.e.*, as inert carriers of photoactive molecules.

As a proof of concept, both Cr-based materials (MIL-101 and MIL-101-SO_3H) were loaded with harmine, a well-known photosensitizer (see below). These two particular MCPs were selected, over UiO-66-NH_2, due to their higher specific surface areas (see ESI,† Section S1). Harmine is a heterocyclic compound that belongs to the family of the β-carboline alkaloids widely found in nature. The photosensitizing properties of these alkaloids against biomolecules such as dsDNA[17,19,20] and its components (*i.e.*, deoxynucleotides)[21,22] have recently been well studied and documented. Briefly, both the extra- and intracellular DNA moiety is seriously injured by photoexcited β-carbolines inducing a broad spectrum of modifications (single strand-breaks, 8-oxo-7,8-dihydroguanine, pyrimidine dimers, *etc.*).

As mentioned above, harmine was adsorbed on both Cr-based materials MIL-101 and MIL-101-SO_3H, yielding different final loadings. The maximum absolute loading achieved was higher for the SO_3H, compared to the non-substituted material (180 mg harmine g^{-1} MIL-101-SO_3H and 70 mg harmine g^{-1} MIL-101, respectively). This difference can be traced back to the presence of the sulphonic acid group, which leads to the protonation of harmine, causing a stronger electrostatic attraction for MIL-101-SO_3H and, consequently, an increase on the absolute loading. Results obtained for the interaction of ds-DNA with harmine-loaded materials, MIL-101 and MIL-101-SO_3H, can be observed in Fig. 2c and d (see the open circles). The harmine loaded MCPs have no effect on dsDNA structure. This shows a clear difference from what was observed for bare materials, with a stronger effect for MIL-101. Different from the dsDNA molecule, harmine can access both external surface sites and intraporous surface sites on the MCP particles. We speculate on the above discussed activity change to be related to a reduction in the number of available external sites for dsDNA on the substrate particles caused by harmine adsorption.

To further evaluate the photodynamic effect of the photosensitizer when loaded onto a suitable microporous carrier, dsDNA was irradiated in the presence of harmine-loaded MIL-101-SO_3H (Fig. 3c). The latter MCP was selected, instead of MIL-101, because of its higher harmine loading capacity and because it was found to be inert both in the presence and absence of irradiation. The increased damage extent photoinduced by harmine-loaded material demonstrates that the already proved photosensitizing properties of harmine in solution[20,21] are maintained, *i.e.*, MIL-101-SO_3H does not deactivate the electronic excited states of harmine, at least, in a significant way. The latter fact was also explored by monitoring the capabilities of harmine loaded material to produce hydrogen peroxide (H_2O_2) upon photoexcitation (through electron transfer reactions),[23,24] see ESI† (Fig. S8). Once more, these results indicate that the capability of harmine to photosensitize reactive oxygen species is not significantly deactivated by the MCP material retaining, at least to a large extent, its photosensitizing properties.

Conclusions

MCPs allow for a great deal of structure and chemical variability, and can be tuned to meet the requirements of a wide range of applications. In the examples studied herein, we showed that interactions with biomolecules such as DNA constitute a crucial property to be evaluated if the materials are to be used in biological applications. In particular, we demonstrate that properties such as surface charge (provided by modified linkers on the MCPs considered) or available adsorption sites greatly modify the extent of damage observed when dsDNA is exposed to a given MCP in the absence of light. Upon irradiation, the photosensitizing properties of MCPs were found to be highly sensitive to the chemical identity of the metal ion center present. Moreover, our results suggest that some MCPs can provide a suitable platform for its use in photodynamic therapy.

We envision that the insight gained into dsDNA interactions with bare, as well as harmine-loaded MCPs, both in the absence of light and under irradiation will have strong implications on the selection of a particular material for a biological purpose (*e.g.*, drug delivery, photodynamic therapy, photocatalysis, *etc.*), introducing a new property to be taken into account besides stability and affinity for the desired drug.

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Notes and references

§ This result is an indication of the possibility for application of the considered MCP as a photosensitizing agent without the need for any further addition of a guest photoactive molecule.

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