Cystine-based MBioF for Maintaining the Antioxidant–Oxidant Balance in Airway Diseases

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S Supporting Information

ABSTRACT: Reactive oxygen species, contributing to oxidant–antioxidant imbalance, initiate damage to the airways cells, inflammatory processes, and further pathophysiological effects. Enhancing antioxidant properties is the main prophylactic and therapeutic challenge. In this work, a newly synthesized and biocompatible structure of the metal–biomolecule frameworks (MBioF) harnessing cystine as a linker and magnesium as metal nodes is presented. This structure provides crucial sulfhydryl groups of cysteine, with antioxidant activity, released stepwise in the site of delivery. We prove that once released, the compounds of MBioF increase the intracellular level of cysteine and total antioxidative capability of airway cells. Presented MBioF structures offer new perspectives for clinical applications as therapeutics or preventatives maintaining the antioxidant–oxidant balance.

KEYWORDS: Airway diseases, metal–organic framework, MBioF, stepwise release, drug delivery system, cystine

In the last decades we witnessed exceptional rapid changes in the environment as a consequence of intense anthropogenic activity. Air pollution, and more precisely particulate matter, is both an urgent environmental concern and a public health problem. Several studies carried out in cell culture, in animal models, and in humans have established that, once inhaled and deposited into the lungs, fine and ultrafine particles can be a source of reactive oxygen species (ROS) and trigger pro-inflammatory responses.¹ Therefore, air pollution has been associated with increased respiratory mucosal symptoms, exacerbation of asthma, chronic obstructive pulmonary disease, and cardiovascular diseases and mortality.²

Several studies have shown that ROS play a key role in initiation as well as amplification of inflammation in airways. The airways of asthmatic patients are further subjected to increased levels of reactive oxygen and nitrogen species produced by inflammatory and epithelial cells. Excessive ROS production in asthma coincides with alterations in key enzymatic and nonenzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glutathione, vitamins C and E, uric acid, and thioredoxin, leading to severe oxidant–antioxidant imbalance.^{3,4} Oxidant–antioxidant imbalance initiates further pathophysiological effects associated with

asthma such as vascular permeability, mucus hypersecretion, smooth muscle contraction, and epithelial shedding.⁴

Inhaled corticosteroids are the support of anti-inflammatory therapy in many chronic inflammatory diseases. Due to their numerous side-effects, there is the need to develop novel anti-inflammatory therapies, either as add-on therapy to corticosteroids or even as a replacement.⁵ There have been also a vast number of clinical studies to evaluate different antioxidants, including inhaled *N*-acetylcysteine (NAC), for the treatment of airway inflammation-based diseases, but these have produced conflicting results.^{6,7} Moreover, the *in vivo* test results prove the adverse effect of chronic NAC administration mimicking the chronic hypoxia effects and increasing protein level of hypoxia-inducible factor-1 α .⁸ Harnessing the novel structures with intrinsic antioxidative particles seems to be a simple solution to face the above problems.

In the last 20 years, metal–organic frameworks (MOFs) have been a truly explosive area of research. MOFs are considered to be promising materials for a wide range of

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applications, including gas purification and storage, catalysis, sensing, etc. MOF materials can be one-, two-, or three-dimensional structures^{9,10} containing nodes formed by inorganic units or clusters of metal cations, connected with organic ligands.^{10,11} Since the coordination bonding between metal and ligand is relatively strong, well-defined porous crystal structures are formed.¹² Transition metals such as Zn, Cu, Fe, Cr are the most commonly used elements in nodes, but also alkaline earth metals (for example Mg, Ca, Sr, Ba, Ra), basic metals of the periodic table main groups (for example, Sn or Al), and/or rare earth metals (such as Lanthanides) have been employed.¹³

The unique properties of MOFs have been attracting attention not only in the chemistry field but also for chemical engineering applications, nanotechnology, environmental engineering, biology, and medicine.¹⁴ One of the strategies used to incorporate active (inorganic or organic) compounds into a MOF is to make an active agent a part of the material structure—the so-called bioactive MOF (Bio-MOF) can be obtained.¹⁵ The application of such prepared materials as drug carriers can be restricted due to the problems with toxicity of components released during their decomposition in tissues. Toxicity limits also the use of organic ligands. Fortunately, various strategies of the synthesis of biocompatible MOF materials have been proposed. First, the concept of Manton et al.¹⁶ proposed so-called bioinspired MOFs, as metal–peptide frameworks based on oligovaline. Imaz et al.¹⁷ extended the idea to the metal–biomolecule frameworks (MBioFs). In this concept, different biomolecules (for example, amino acids, peptides, nitrogen bases, etc.) could be incorporated into a MOF structure. This significantly overcomes the problem of toxicity.¹⁸

Due to employing cystine as a linker and magnesium as metal nodes, the obtained Mg-MBioFs were supposed to exhibit desired antioxidant properties. We have analyzed these newly developed MBioF structures in terms of their physicochemical characteristics, antioxidative properties, and potential to maintain the oxidant–antioxidant balance in lung epithelial cells cultured *in vitro*.

The results from structure determination are presented in Figure 1. Based on PXRD and HRTEM analysis (see Supporting Information) one can conclude that no crystallinity at macroscale is present in the structure. Already in nanoscale, the nanocrystalline units, in the range of 5–10 nm, are able to be observed. The material synthesized in basic water/methanol environment leads to the creation of crystals in an orthorhombic system, similarly as the one reported by Ferrer et al.¹⁹ The nanocrystallinity prevents the cells from mechanical damage and thus enables the material to be successfully used as drug or drug carrier.

The characteristic signals for amino acids hydrogen bonds are visible in the FTIR spectrum of cystine as a broad and mutually overlapping band in the OH/NH stretching spectral range below 3400 cm^{−1} as well as in the bending range ~1600–1500 cm^{−1} (Figure 2). As one could expect, those bands are very low in intensity in the Raman spectrum. These phenomena are due to fact that, for formed here hydrogen bonds, a change in polarizability with respect to the vibrational coordinate is small.

Therefore, the polarizability cannot be a function of the coordinates of the normal vibration. In contrast, the low polarity and high polarizability of CH bonds causes a low FTIR

Figure 1. (a) Final Rietveld refinement plot, showing the experimental (red circles), calculated (black line), and difference profiles (blue line); green tick marks indicate reflection positions; (b) obtained elemental cell; (c) HRTEM pictures of obtained Mg-MBioF. Long-range crystallinity in the material is not present, and the observed nanocrystalline units are in the range of 5–10 nm. For further details, see Supporting Information.

intensity of $\nu(\text{CH})$ vibrations and high intensity in the Raman spectrum.

The formation of the MOF causes the structure to lose the hydrogen bonds, revealing the $\nu(\text{NH})$ asymmetric and symmetric signals at 3350 and 3250 cm^{−1} and $\delta(\text{NH}_2)$ at 1564 cm^{−1}, respectively. Note that there are no $\nu(\text{OH})$ in the IR as well as in the Raman spectrum, meaning that, in the obtained MBioF, carboxyl groups are involved in the metal ion nodes.

The absence of $\nu(\text{OH})$ as well as the presence of sole and isolated $\nu(\text{NH})$ indicates a stiffness of the $-\text{NH}_2$ group due to $\text{H}_2\text{N}-\text{Mg}$ coordination. Thus, in the case of MBioF the formation of metal coordination sphere through O (from COO^- group)– Mg^{2+} –N (from NH_2 group) could be assumed.

The formation of Mg–cystine bonds is proved also as the appearance of the band at 680 cm^{−1} and ~15 cm^{−1} blue-shift of 498 cm^{−1}, i.e., $\nu(\text{SS})$, signal in the Raman spectrum. The former is responsible for the O–Mg–O bonds formation. It is much higher in intensity in the Raman than in the FTIR, contrary to the $\nu(\text{C}-\text{O})$ from COOH groups visible only in FTIR at 872 cm^{−1}. The S–S Raman signal will be used further

Figure 2. Comparison of Raman and FTIR vibrational spectra for Mg-MBioF and cystine.

to characterize the SS bond in cystine or in the Mg-MBioF structure.

Note that neither in the Raman nor in the FTIR spectra the characteristic signals for MgO/Mg(OH)₂ exist.

In the Raman spectrum in the range 1800–400 cm^{−1}, the SS stretching band dominates. Interestingly, formation of the MBioF causes drastic relative increase in the intensity of the other bands, meaning the rise in the polarizability of the bonds.

The cytotoxicity evaluation of the Mg-MBioF was performed in A549 cell culture during 24 h (Figure 3a). In the wide range of concentration, the material proved to be nontoxic and well tolerated by cells, meaning that it could be safely used in further *in vitro* experiments. Application of cystine molecules as organic linkers suggest existence of potential antioxidative properties of the whole MBioF.

Mg-MBioFs themselves exhibited the antioxidative potential determined as a capacity to reduce Cu²⁺ ions and measured in comparison to Trolox a water-soluble analogue of vitamin E, which serves as a standard.²⁰ The antioxidant capacity of tested material in the concentration 500 μg mL^{−1} was equivalent to 0.2 μmol mL^{−1} of Trolox. Thus, MOF's antioxidative capacity is similar to flavonoids and phenolic antioxidants.²¹ Admittedly, the amount of cystine, equivalent with this MBioFs, has closely two times higher reducing capacity, but bearing in mind the effect of NAC mimicking the chronic hypoxia, one has to

Figure 3. Mg-MBioFs influence on the A549 cells physiology: (a) cell viability; (b) antioxidative capacity of cells.

remember that strong reductive environment can be also detrimental.

The most interesting result is that Mg-MBioFs enhanced the innate antioxidative capacity of A549 cells (Figure 3b). We have determined the total antioxidative capacity (TAC) of A549 cells treated with 125, 500, and 2000 μg mL^{−1} MOFs for 24 h, then lysed and subjected to the test.

It is noteworthy that the nonenzymatic antioxidative capacity gains the bigger share the higher the MBioFs concentration. We suggest that it is due to the increase in internal reductive capability derived from small particles like cysteine or glutathione. To confirm that, we have determined the concentrations of cysteine and glutathione inside the A549 cells; Table 1.

It is of evidence that rising concentration of Mg-MBioF influences only intracellular cysteine concentration. Constant concentration of total and reduced glutathione means that liberated cysteine does not serve as a substrate in glutathione biosynthesis.

Table 1. Intracellular Concentration of the Main Thiol-Derived Compounds

sample	total glutathione [mM]	reduced glutathione [mM]	cysteine [mM]
A549 control	8.61 (0.39) ^a	3.03 (0.26)	0.32 (0.01)
Mg-MBioF [0.5 mM]	7.56 (0.47)	2.40 (0.35)	1.16 (0.03)
Mg-MBioF [2.0 mM]	7.52 (0.36)	3.14 (0.29)	2.04 (0.09)

^aValues in parentheses indicate the standard error of mean.

GSH is the predominant intracellular thiol. Within cells, the majority of GSH is found in the cytosol, the primary site of GSH synthesis, with concentrations ranging from 1 to 11 mM.²² However, administration of GSH is not considered optimal because of its poor bioavailability and limited ability to cross the phospholipid bilayer of cells. Likewise, delivery of cysteine suffers from rapid oxidation to its disulfide, cystine, which has poor solubility and renders the crucial sulfhydryl functional group at least temporarily inaccessible. As a result, other means for increasing concentration of sulfhydryl compounds must be developed to circumvent this problem.²³ Thereafter, the stepwise and controlled increase in intracellular cysteine concentration limiting its toxic effects is expected. The harnessing of MBioF is a beneficial alternative solution in anti-inflammatory therapies of pulmonary system diseases.

We realize that the comprehensive mechanism of enhancing antioxidative defense and maintaining the balance between the oxidative and antioxidative processes needs to be precisely elucidated. The scheme presented below summarizes the possible pathways that should be taken into consideration.

The initial degradation path in the scheme proposed is based on UV-vis results (Figure 4b). From previously reported

Administration of Mg-MBioF, despite of the above changes in thiol compounds composition, leads to an increase in the intracellular concentration of Mg^{2+} . It is noteworthy that enzymes engaged in glutathione synthesis (gamma-glutamyl-cysteine ligase and GSH synthetase) require magnesium ions for proper activity. Two magnesium ions function to stabilize the acylphosphate intermediate and facilitate binding of ATP.³⁰

Summing up, the proposed Mg-MBioF seems to be beneficial in maintaining the oxidative–antioxidative balance in the cells. Undoubtedly, the Mg-MBioF pharmacodynamics and pharmacokinetics require further examinations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00468.

Experimental procedures for the biological experiments and synthetic procedures and characterization data for the compounds (PDF)

Accession Codes

The Mg-MBioF structure was deposited in the Cambridge Crystallographic Data Centre, deposition number 1822425.

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K.R. and M.W.: designing the study. M.W. and A.P.T.: Mg-MBioF characterization. P.B. and A.B.: Mg-MBioF synthesis. K.R. and J.C.: *in vitro* experiments and HPLC assays. P.F. and I.S.: Mg-MBioF crystal structure determination and Rietveld refinement. The manuscript was written through contributions of all authors.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

MOF, metal–organic frameworks; MBioF, metal–biomolecule frameworks; BioMOF, bioactive MOF; ROS, reactive oxygen species; NAC, N-acetylcysteine

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Figure 4. (a) Proposed pathways of Mg-MBioF degradation and activity; (b) UV-vis spectrum of dissolved Mg-MBioF (concentration: red, 0.1 mg/mL; blue, 1 mg/mL); inset, structure of Mg-MBioF.

work,²⁴ the absorption band at ca. 390 nm was identified as the disulfide radical anion ($\text{RSSR}^{\bullet-}$), which is elongated with respect to a neutral disulfide bond.²⁵ This peak was recognized in samples of the disulfide-containing proteins: lysozyme, bovine trypsin, and N9 neuraminidase.²⁶ Also, the disulfide radical anion peak was observed in the solution of cystine.²⁷

Due to the charge transfer, proved by ab initio calculations, and the hydrolysis of such elongated S–S bond, the formation of sulfenic acids and cysteinyl radicals is expected. The radical compound preferentially coordinates Mg^{2+} cations.

The sulfenic acid molecules are able to modify cysteine (or selenocysteine) functionalities in different proteins, thus resulting in the influence on the enzymatic antioxidative activity in intracellular catalytic cycles.^{28,29} Presence of thioredoxine inside the cells causes reduction of the sulfenic acid molecules to cysteine as a final product, which was summarized in Table 1.

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