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Biomaterials

Modeling Fungal Melanin Buildup: Biomimetic Polymerization of 1,8-Dihydroxynaphthalene Mapped by Mass Spectrometry

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Abstract: Due to the emerging biomedical relevance and technological potential of fungal melanins, and prompted by the virtual lack of information about their structural arrangement, an optimized synthetic protocol has been devised for a potential structural model of *Ascomyces* allomelanin through enzyme-catalyzed oxidative polymerization of 1,8-dihydroxynaphthalene (1,8-DHN). Electrospray ionization mass spectrometry (ESI-MS) measurements of freshly synthesized DHN-polymer recorded in the negative ion mode allowed detection of oligomers up to *m/z* 4000, separated by 158 Da, corresponding to the in-chain DHN-unit. The dominant peaks were assigned to singly-charged distribution, up to 23 repeating units, whereas a doubly charged polymer distribution was also detectable. Chemical derivatization, ultra-performance liquid chromatography (UPLC)-ESI MS, and MS/MS data confirmed that oxidative polymerization of 1,8-DHN proceeds through C–C coupling of the naphthalene rings. The new insights reported here into synthetic 1,8-DHN oligomers/polymers as a mimic of fungal melanins may guide novel interesting advances and applications in the field of biomimetic functional materials.

Introduction

Melanins include a heterogeneous group of black insoluble phenolic polymers widespread in animals, plants, bacteria, and fungi, and accounting for the variety of skin and hair coloration in man and mammals. During the last decade, eumelanins have become the focus of growing interest because of their remarkable antioxidant,[1] photoprotective,[2] and optoelectronic properties,[3] suggesting a wide range of biomedical and technological applications,[4] including, for example, organic electronics, bioelectronics, and sensing.[5–7] Despite significant advances in the chemistry of nitrogenous eumelanin polymers of animal origin and their synthetic mimics from DOPA, dopamine, and 5,6-dihydroxyindoles,[8] relatively little is currently known about fungal melanins, heterogeneous non-nitrogenous phenolic pigments often referred to as allomelanins.[9] Interest in fungal melanins is growing steadily owing to their biotechnological importance and their key role in the virulence of plant and human pathogenic fungi.[10,11] Like their nitrogenous counterparts, fungal melanins display marked insolubility in all solvents, an amorphous character, and unusual structural complexity arising from both molecular and electronic disorder, which makes their direct investigation a difficult task. In consequence, a clear-cut definition of structure-property-function relationship, which is crucial for application purposes, is still missing. Although the natural pentaketide intermediates leading to 1,8-dihydroxynaphthalene (1,8-DHN) have been characterized,[12] the chemical elucidation of the biosynthetic steps involving oxidative polymerization of the 1,8-DHN precursor that finally lead to the *Ascomyces* black pigment is still a desirable goal. Soft ionization mass spectrometry (MS) such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) have proven powerful tools for structural elucidation of melanin polymers,[13a,b] as well as to gain new insights in their mechanism of formation.[14a,b] Very recently Lianrong Wang, Wan Chan et al.[15] proposed an interesting ESI-MS approach revealing a novel structural model of DOPA-melanin based on a self-aggregation mechanism. The non-covalent supramolecular aggregates of L-DOPA are linked through hydrogen bonds, stacking interactions, and ionic bonds, showing that L-DOPA might be a possible building block of eumelanin together with the well-known 5,6-dihydroxyindole (5,6-DHI) and 5,6-dihydroxyindole-2-carboxylic acid (5,6-DHICA). Beltrán-García, Di Mascio et al.[16] reported a linear MALDI-TOF mass spectrometric study on the natural pigment secreted by the fungal pathogen *Mycosphaerella fijiensis*.

Motivated by the emerging biomedical relevance and technological potential of fungal melanins, and the virtual lack of information about their structural arrangement, we undertook an in-depth mass spectrometric investigation of 1,8-DHN-melanin synthesized by means of horseradish peroxidase- (HRP-)

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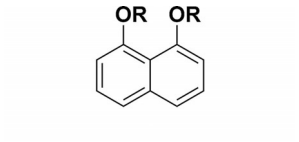
and laccase-catalyzed polymerization (Scheme 1). Insight into coupling of DHN units that form the black fungal pigment is expected to lead to a more precise determination of physical-chemical properties that may guide a rationale design of bio-inspired melanin polymers.



1: R = H, DHN (MW 160)
2: R = $COCH_3$, DAN (MW 244)

Scheme 1. 1,8-DHN (R = H) building block of fungal melanins, and the acetylated analogue DAN (R = $COCH_3$). DAN = 1,8-diacetoxynaphthalene.

Results and Discussion

DHN-polymerization has been investigated using two different enzymes. Horseradish peroxidase (HRP) is the most commonly used biocatalyst in the polymerization of phenols and their derivatives.[17–19] HRP is active in buffer solutions as well as solvent mixtures, and reactions are normally initiated by addition of hydrogen peroxide as oxidant reagent. Laccase is involved in the degradation of lignin and is widely used in the enzyme-mediated polymerization, given the increasing demand of greener technologies for polymer synthesis.[20] Furthermore, only oxygen/air is required as co-substrate to activate the oxidative process. Although the enzyme involved in the final biosynthetic step of DHN-based melanin is not yet identified, laccase seems to be the main candidate that takes part in the process.[10] Thus, both enzymatic systems have been used to reproduce in vitro the synthesis of DHN melanin. HRP allowed access to a DHN melanin with a higher degree of polymerization (see below), whereas laccase was used to induce oligomerization with the aim to isolate and characterize lower molecular weight oligomers, such as the dimeric and trimeric intermediates.

Analysis of HRP-mediated polymerization of 1,8-DHN by ESI-QToF MS

Despite the well-known heterogeneity and insolubility of the melanin pigments in ordinary organic solvents, optimization of the synthetic procedure with the HRP/H_2O_2 system led to a polymer perfectly soluble in methanol and acetonitrile, and thus amenable to electrospray analysis. Polymerization of 1,8-DHN was thus carried out by dissolving the substrate in phosphate buffer and adding the enzyme. Polymerization is started by rapidly adding H_2O_2 to the solution, at room temperature. After 30 seconds the reaction was quenched by sodium dithionite and extracted by ethyl acetate to give a brownish precipitate eventually. The ESI MS spectra of the freshly synthesized DHN-polymer were recorded in the negative ion mode (Figure 1), allowing detection of oligomers up to *m/z* 4000 (Figure 1 C). The peaks displayed the typical Gaussian shape

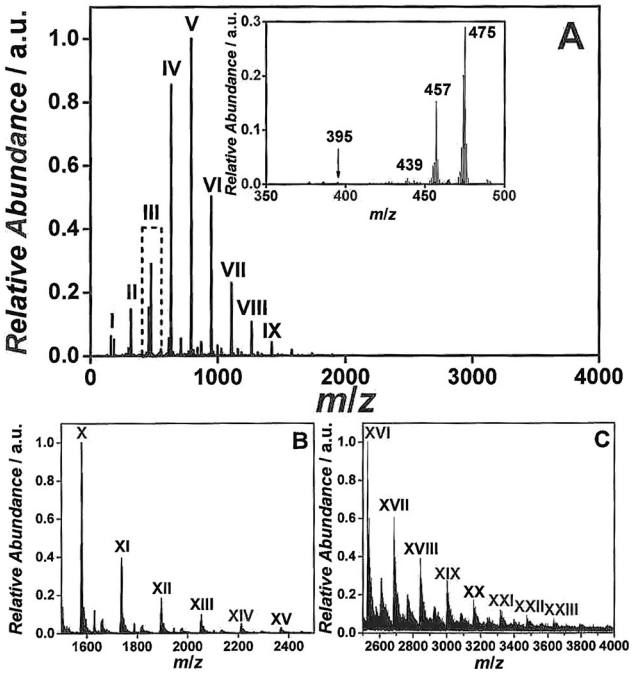


Figure 1. ESI-QToF mass spectra in negative ionization mode of non-acetylated 1,8-DHN melanin. Boxes B and C represent zooms of the mass regions between *m/z* 1400 and *m/z* 4000.

distribution of a polymer (Figure 1 A) and were separated by 158 Da, corresponding to the "in-chain" DHN-unit. This pattern of well-defined spacing between the peaks suggests a significant degree of regularity in the polymer chain. The dominant peaks were assigned to singly-charged distribution, up to 23 repeating units. A closer examination of the fine structure of the peak clusters suggested the coexistence of a doubly charged polymer distribution. In the inset (Figure 1 A), the ion at *m/z* 475 was assigned to the singly charged trimer [**III**-H]$^-$ (the MW of the trimer is 476 Da), whereas the much less abundant peak at *m/z* 395 corresponded to the doubly charged pentamer [**V**-2H]$^{2-}$. Spotting of the negative double charged ions revealed the polymer growth up to 46 repeating units, thus confirming the upper levels of oligomer detection reported in the literature.[16] The peaks at *m/z* 457 and *m/z* 439 are clearly related to dehydration of the trimer. The peaks at two or three mass units lower than the trimer **III** can be in principle assigned to the radical anion of the dehydrogenated trimer [**III**-2H]$^{\bullet-}$ (*m/z* 474), and to its deprotonated congener [**III**-3H]$^-$ (*m/z* 473), respectively. They might be produced both by oxidation during the ionization process, due to the phenolic nature of the polymer. For a complete structural determination of the oligomers, tandem mass spectrometry experiments proved to be crucial. In Figure 2, the ESI MS/MS spectrum of the dimer [**II**-H]$^-$ at *m/z* 317 is reported. The fragmentation points out the product ion at *m/z* 299 as a result of the dehydration process. The fragment at *m/z* 158 may be explained by the neutral loss of a radical DHN-unit of 159 Da from the molecular ion at *m/z* 317. This would suggest a σ-bond linkage between two repeating units of the dimer, whose homolytic cleavage provides the ion at *m/z* 158 as a [*M*−H]$^{\bullet-}$ ion-type.

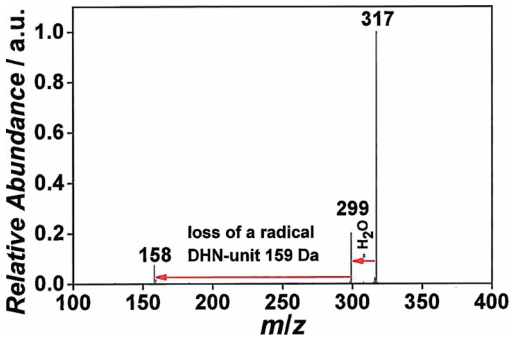


Figure 2. ESI-MS/MS spectrum in negative ionization mode of the dimer at *m/z* 317.

Tandem mass spectrometry experiments carried out on higher mass oligomers demonstrated that fragmentations occur through direct cleavage of the main linkages between the aromatic rings.

However, the mass of the dimer at *m/z* 317, corresponding to the coupling of two repeating units (a repeating unit is individually considered as consisting of [160 Da −1H]) less $1H^+$ for ionization, which fits perfectly with the formation of a C–C link-type as well as a C–O one during the polymerization process. To clarify this key point, the freshly synthesized DHN-polymer was acetylated with acetic anhydride with a large excess of pyridine to replace the hydroxyl groups with acetoxy moieties. In the C–O linking case, in fact, the coupling mechanism that builds up the DHN-melanin should result in the formation of oxygen bridges in the main structural arrangement of the polymer, which would cap the hydroxyl groups. The acetylated 1,8-DHN-polymer (see Figure S1 in the Supporting Information for the UPLC-ESI-MS chromatogram) was then analyzed using ESI-MS in the positive ion mode, and oligomers were detected up to the level of *m/z* 5000, corresponding to 20 repeating units (Figure 3). The di-acetylated building block 1,8-DAN **2** depicted in Scheme 1 has a molecular mass of 244 Da, providing an "in-chain" mass value of 242 Da. The predominant singly charged distribution reveals oligomeric species as Na^+ adducts. The mass value of each Na^+-charged ion perfectly fits with isotopic simulations, considering the phenolic groups of the 1,8-DHN "in-chain" unit functionalized by two acetyl moieties. Thus, the ESI-MS spectra provided clear-cut evidence that the hydroxyl groups are not implicated in the coupling of DHN units. This means that DHN polymer is built upon C–C bonds, with exclusion of ether linkages.

To support this assumption, as a proof of concept a detailed structural investigation of the acetylated dimer $[\mathbf{II}+Na]^+$ at *m/z* 509 was carried out by tandem MS experiments. The ion at *m/z* 509 accounts for three putative isomeric C–C linked 1,8-DHN dimers (2-2′, 2-4′, and 4-4′), each acetylated at the four free hydroxyl groups.

The other possible dimers formed across C-3 of the naphthol ring have been tentatively ruled out (see below), as dictated by the regiochemistry of the phenolic oxidative coupling reaction.[21] The MS/MS spectrum of the ion evidenced two different fragmentation pathways, as reported in Figure 4. The first

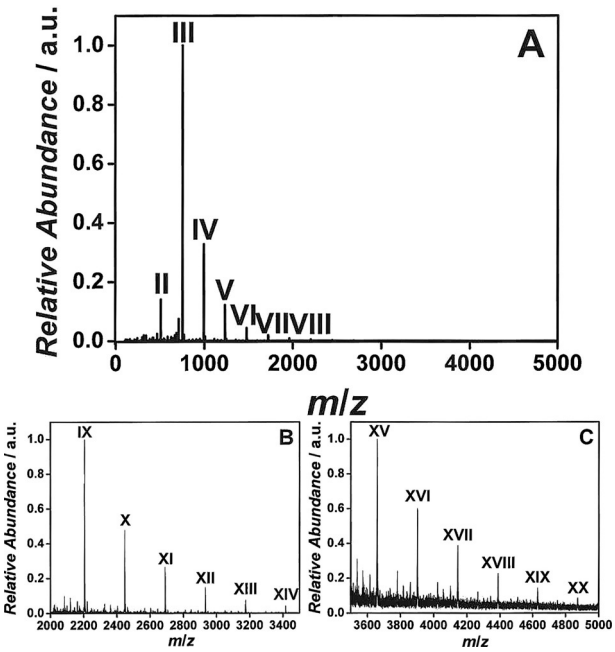


Figure 3. ESI-QToF mass spectra in positive ionization mode of acetylated 1,8-DHN melanin. Boxes **B** and **C** represent zooms of the mass regions between *m/z* 2000 and *m/z* 5000.

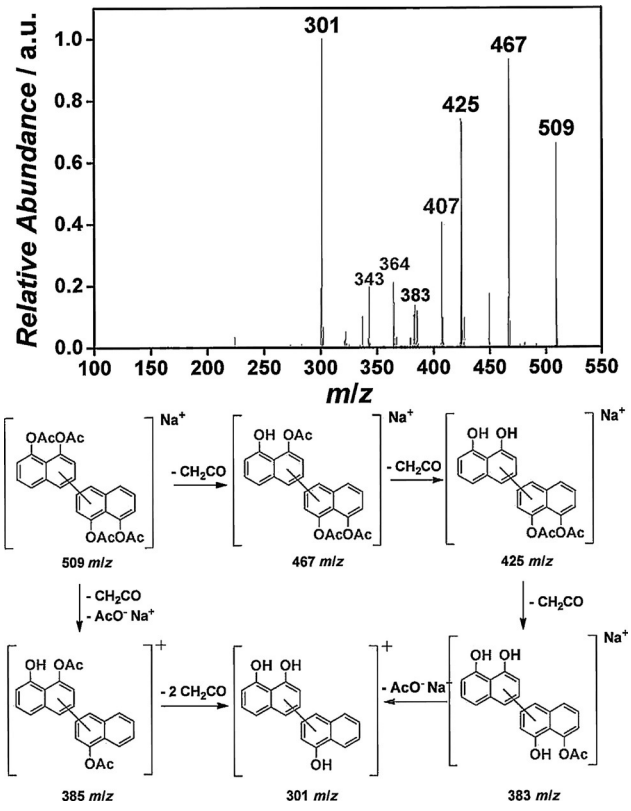


Figure 4. Top: ESI-MS/MS in positive ionization mode of the acetylated dimer at *m/z* 509; bottom: the proposed fragmentation pattern of the ion at *m/z* 509.

step involves elimination of a neutral ketene molecule (42 Da) from the molecular ion, consistent with the known behavior of

phenolic acetyl esters of phenols,[22] affording the product ion at *m/z* 467. Two more consecutive losses of ketene units account for peaks at *m/z* 425 and *m/z* 383. The product ion at *m/z* 383 finally delivers the fragment ion at *m/z* 301 by release of the last acetyl group as sodium acetate (82 Da). An alternative fragmentation pathway can be outlined. After cleavage of the first ketene molecule, direct elimination of sodium acetate (82 Da) justifies the formation of the fragment at *m/z* 385. Such a process continues with the loss of the two remaining ketene molecules, taking to the fragment ions at *m/z* 343 and *m/z* 301 respectively. In summary, the two convergent fragmentation pathways leading to the product ion at *m/z* 301 can only be explained by four acetoxy substituents placed onto the naphthalene scaffold. Such fragmentation pathways, with consecutive losses of the acetyl/acetoxy moieties, are common to the higher mass oligomers. This confirms the C–C linking nature of the 1,8-DHN-polymer.

As a side observation, ^{1}H NMR spectroscopy measurements performed on the fully acetylated 1,8-DHN melanin polymer also suggest predominance of C–C networks through C-2/7 and C-4/5 atoms of the naphthalene ring. The integral of the signal related to H-3/H-6 atoms is approximately twice the integral of H-2/H-7, and four times that of H-4/H-5 (Figures S2 and S3 in the Supporting Information), compared with the ^{1}H NMR spectrum of the monomer 1,8-DAN (Figure S4). This confirms that C-4 and C-5 are most involved in the inter nuclei bridges, followed by C-2 and C-7, then C-3 and C-6.

Analysis of laccase-mediated polymerization of 1,8-DHN: structural investigation of the dimers and trimers by UPLC-ESI-MS

Though the tandem MS experiment of the HRP-mediated polymerization clearly supported C–C coupling steps, whether or not this encompasses *ortho*-, *meta*-, or *para*-like positions (i.e., C-2, C-3, and C-4 of the naphthol ring) still remained an open issue. To this aim, DHN was also polymerized by laccase enzyme, with the presumption that this enzyme would lead to low molecular weight oligomers.[23] A solution of 1,8-DHN in acetonitrile was added to sodium acetate buffer. Laccase from *Trametes versicolor* was added, and the reaction stirred at room temperature over a period of 24 h. The reaction was quenched by sodium dithionite and extracted by ethyl acetate to give a brownish powder, which was then subjected to the acetylation reaction. From ESI-MS analysis, the acetylated polymer showed essentially the presence of dimeric, trimeric, and tetrameric derivatives. Acetylated dimers and trimers, obtained as regioisomer mixtures by preparative layer chromatography (PLC) separation, were finally investigated by UPLC-ESI-MS.

The UPLC-ESI-MS chromatogram (Figure 5) indicated a dimer composed of a mixture of isomers that split the chromatographic signal into three different peaks, $\mathbf{II}_a$, $\mathbf{II}_b$, and $\mathbf{II}_c$ at a 20:24:56 ratio, respectively. They all displayed the same molecular ion clusters featuring the most abundant adduct $[\mathbf{II}+Na]^+$ at *m/z* 509 (Figure S5 in the Supporting Information), and shared similar MS/MS fragmentation pathways, according to the scheme proposed in Figure 4.

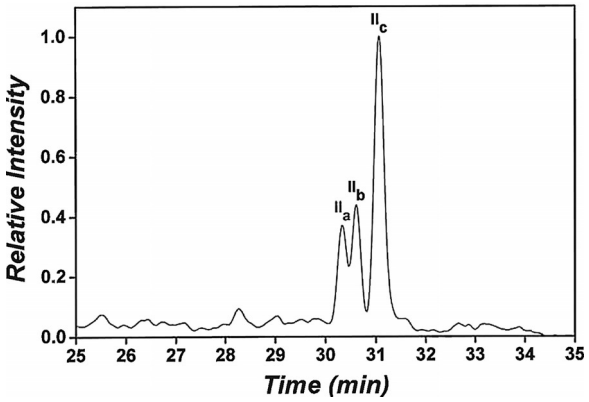
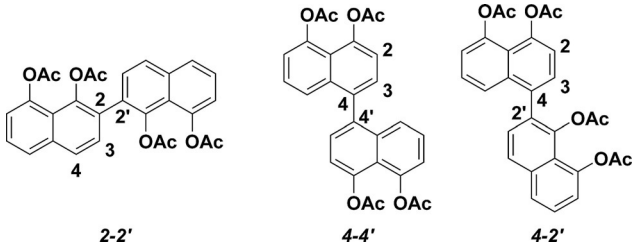


Figure 5. UPLC-ESI-MS chromatogram of the isolated acetylated dimers mixture (all at *m/z* 509).

By forcing the collision energy, cleavage of the σ-bond between the two naphthalene rings does not occur. Nevertheless, the presence of three products is in agreement with the involvement of only two positions (namely C-2 and C-4) of the naphthalene rings, leading to three dimers linked through 2-2′, 2-4′, and 4-4′ bonds (Scheme 2). It therefore confirmed the formation of regioisomeric dimers coupled through C–C links between the naphthalene rings, in accordance with the mode of polymerization of 1,8-DHN through C–C coupling of naphthalene rings. Recently, Hertweck and co-workers[24] reported a detailed investigation of different polyketide congeners from a fungal orchid endophyte, and speculated on an interesting model of biosynthetic pathway of binaphthyl-based compounds starting from 1,8-DHN as a precursor. The model describes the *ortho-para* (*hinnulin D*) and *para-para* ([1,1′-binaphthalene]-4,4′,5,5′-tetrol, BNT) coupling of two units of 1,8-DHN, corresponding to the 4-2′ and 4-4′ couplings reported in Scheme 2, respectively. The same approach was used for the structural investigation of the acetylated trimers. In this case the "*ortho*/*para* coupling" paradigm postulated for the dimeric derivatives might in principle generate a number of isomeric possibilities. The UPLC-ESI-MS chromatogram (Figure S6 in the Supporting Information) shows five peaks, two of which appear as unresolved peaks. They all shared the same mass spectra, with the most abundant adduct being $[\mathbf{III}+NH_4]^+$ at *m/z* 746, and showed the same fragmentation pattern, as evi-



Scheme 2. Proposed dimeric structures linked through C–C bonds, assignable to the ion at *m/z* 509, related to the three chromatographic peaks shown in Figure 5.

denced by the UPLC-ESI-MS/MS analysis performed on the ammoniated molecular ion. Also, in this case, the mass at 746 Da denotes three C–C linked DHN units, plus six acetyl groups.

Enzyme-mediated polymerization of 2,7-dihydroxynaphthalene (2,7-DHN)

In a final set of experiments the enzymatic polymerization of 2,7-dihydroxynaphthalene (2,7-DHN) was set up, with the aim of investigating the effect of the different location of the hydroxyl groups on the naphthalene ring with respect to the possible formation of O–C inter-ring bonds. A pattern of oligomer distribution up to *m/z* 2000 (8-mer) for the fully acetylated poly(2,7-DHN) similar to that obtained for the 1,8-congener was determined both with HRP/H_2O_2 and laccase/air systems. UPLC-ESI MS and MS/MS analysis of the fully acetylated mixture revealed instead significant differences in the mode of polymerization of the 2,7-precursor. The 2,7-DHN oligomers, in fact, proved to possess both C–C and C–O inter-units nature, as shown by the UPLC-ESI MS chromatogram (Figure 6). The ESI mass spectrum of the chromatographic peak labeled $\mathbf{II_{C\text{-}C}}$ at 7.3 min (Figure S7 in the Supporting Information) corresponds to the fully acetylated C–C dimer (molecular ions at *m/z* 509 and *m/z* 487, as $[\mathbf{II_{C\text{-}C}}+Na]^+$ and $[\mathbf{II_{C\text{-}C}}+H]^+$ adducts, respectively). The next chromatographic peak at 8.0 min also labeled $\mathbf{II_{C\text{-}C}}$ shows a mass spectrum with identical ion composition as the previous species, thus being attributed to a different regioisomer. Conversely, the chromatographic peak labeled as $\mathbf{II_{C\text{-}O}}$ at 8.9 min shows two molecular ions (Figure S8 in the Supporting Information) at *m/z* 467 and *m/z* 445, corresponding to $[\mathbf{II_{C\text{-}O}}+Na]^+$ and $[\mathbf{II_{C\text{-}O}}+H]^+$ adducts, respectively. These mass peaks would match, in fact, with an acetylated dimeric structure devoid of one acetyl group with respect to the other two, one oxygen atom being thus involved in a C–O inter-ring coupling. This implies that the 2,7-DHN-polymer grows by using the phenolic oxygen as well as the aromatic carbons, unlike the 1,8-DHN congener. It is confirmed that the position of the phenolic groups on the naphthalene rings determines the mode of polymerization.[25–27]

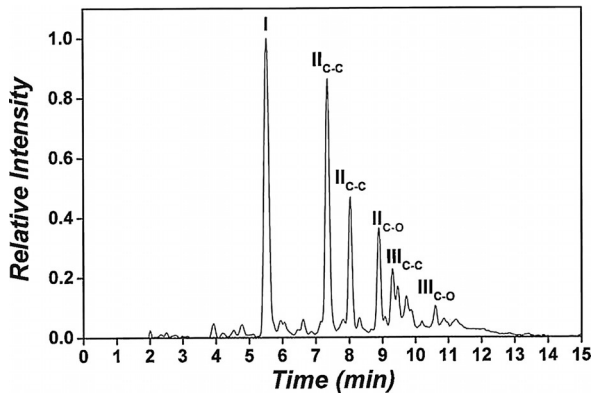


Figure 6. UPLC-ESI MS chromatogram of acetylated poly(2,7-DHN) obtained through laccase-mediated polymerization.

Conclusion

We reported here an optimized synthetic protocol for a potential structural model of *Ascomyces* allomelanins through enzyme-catalyzed oxidative polymerization of 1,8-DHN. Whereas HRP-mediated polymerization led to products with a high degree of polymerization, laccase-mediated polymerization induced low level oligomerization of 1,8-DHN, thus allowing the structural investigation of low molecular weight oligomer populations. The in-depth UPLC-ESI-MS (and MS/MS) investigation showed that the oxidative polymerization of 1,8-DHN proceeds through C–C coupling of the naphthalene rings. The new insights reported here into synthetic 1,8-DHN oligomers/polymers as a mimic of fungal melanins may guide novel interesting advances and applications in the field of biomimetic functional materials.

Experimental Section

HRP-mediated polymerization of 1,8-DHN

The oxidative polymerization of 1,8-dihydroxynaphthalene (1,8-DHN) was carried out by dissolving 30 mg of substrate in a phosphate buffer solution 0.1 M (pH 7.0, 17 mL) with 2 mL of acetonitrile and adding 9 EU/(mg DHN) of horseradish peroxidase (HRP, Type II, 150–250 EU mg^{-1}) dissolved in 1 mL of phosphate buffer. The polymerization starts rapidly at room temperature by adding 20 μL of 30% H_2O_2 to the solution. After 30 s the reaction was quenched by 60 μL of sodium dithionite saturated solution (per 3 mL of polymer solution) followed by a solution of HCl (1 M) until acid pH was reached. The reaction mixture was extracted by ethyl acetate and the organic layer washed with sodium chloride saturated solution and dried over sodium sulphate. The organic solvent was then removed under vacuum to obtain a brownish precipitate.

Laccase-mediated polymerization of 1,8-DHN

The oxidative polymerization of 1,8-dihydroxynaphthalene (1,8-DHN) was carried out dissolving the substrate in a sodium acetate buffer solution 0.1 M (pH 5.0, 17 mL) with 2 mL of acetonitrile and adding Laccase from *Trimetes versicolor* (0.66 EU mg^{-1}) dissolved in 1 mL of NaOAc buffer. Air was bubbled into the solution for the first 5 min of reaction and carried out under air over a period of 24 h. Then the reaction was quenched by 60 μL of sodium dithionite saturated solution (per 3 mL of polymer solution) followed by a solution of HCl (1 M) until acid pH was reached. The reaction mixture was extracted by ethyl acetate and the organic layer washed with sodium chloride saturated solution and dried over sodium sulphate. The organic solvent was then removed under vacuum to obtain a brownish precipitate.

Acetylation procedure

The obtained DHN-polymer was solubilized in pyridine, and acetic anhydride was added slowly under stirring. The stoichiometric ratio between reagents was as follows: for 10 mg of polymer, 300 μL of pyridine and 150 μL of acetic anhydride have been used. The reaction mixture was stirred over a period of 24 h at room temperature and then HCl (1 M) was added until pH 3 was reached. The reaction mixture was extracted by ethyl acetate as above, to recover quantitatively the acetylated DHN-polymer as a reddish powder. The acetylated mixture from laccase-mediated polymeri-

zation of 1,8-DHN was subjected to preparative layer chromatographic separation on PLC plates, by using $CHCl_3$:MeOH 99:1 as mobile phase, to afford the acetylated dimers and trimers as regioisomer mixtures.

ESI-QTof-MS

ESI-MS measurements were performed on a quadrupole time of flight mass spectrometer instrument (Xevo® G2 QTof; Waters Corporation, Milford, USA), equipped with a high performance Z Spray™ source and operating with MassLynx™ Software package (Version 4.1, Waters Corporation). The instrument was calibrated using a 5 mM sodium formate solution in 90:10 2-propanol:water, in the *m/z* range 100–5000 Th. The spectra were recorded in negative polarity for the DHN-polymer and in positive polarity for the acetylated DHN-polymer, choosing the sensitivity mode according to the MassLynx™ software; the *m/z* range 100–5000 Th was set to detect the polymeric distributions. The following setting parameters have been used: source temperature at 150 °C; desolvation temperature at 280 °C; capillary voltage of 3.5 kV. The cone voltage was adjusted, according to the MassLynx™ software, to 80 and 100 V (depending on the experiment; the value of 100 V was used for the in-source fragmentation experiments and to perform the MS/MS experiments of product ions of the oligomeric intermediates of the melanin polymer) and the extraction cone to 2.0 V. The desolvation gas flow rate was 800 L h^{-1}. Confirmation of the ion structure was obtained from exact mass calculations, isotopic simulations, and tandem mass spectrometry experiments. The collision energy was adjusted ranging from 15 to 35 eV, according to the molecular mass values of the oligomeric species.

UPLC-ESI-QTof-MS

UPLC-ESI-MS analyses have been performed with an ACQUITY UPLC® BEH C18-1.7 μm column. The acetylated polymer was eluted with 0.1% formic acid (A) and acetonitrile (B) using 0.3 mL min^{-1} of flow rate. The polymeric mixture was separated by the following gradient: from 60% A and 40% B as initial condition to 10% A and 90% B as final condition over a period of 15 min. The column oven temperature was set at 40 °C. The mass spectrometry analysis was performed using positive ion mode and the following parameters: capillary voltage at 3.0 kV, sampling cone at 20 V, extraction cone at 2.0, source temperature at 150 °C, desolvation temperature 200 °C, and desolvation gas flow rate at 800 L h^{-1}. The dimer sample was eluted with 0.1% formic acid (A) and acetonitrile (B) using 0.2 mL min^{-1} of flow rate and the following gradient: from 95% A and 5% B as initial condition to 40% A and 60% B after 45 min. The trimer sample was eluted with 0.1% formic acid (A) and acetonitrile (B) using 0.2 mL min^{-1} of flow rate and the following gradient: from 98% A and 2% B as initial condition to 40% A and 60% B after 38 min. The mass spectrometric parameters were set as follows for both the chromatographic experiments: capillary voltage at 0.6 kV, sampling cone at 30 V, extraction cone at 2.0, source temperature at 150 °C, desolvation temperature 280 °C, and desolvation gas flow rate at 800 L h^{-1}.

Synthesis and characterization of poly(2,7-DHN)

The poly(2,7-DHN) was synthesized and characterized following the same experimental procedures and analytical conditions of those used for the preparation and analysis of poly(1,8-DHN), to carry out a reasonable comparison between the two different polymers.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: 1,8-dihydroxynaphthalene · biomimetic polymerization · fungal melanins · mass spectrometry · structural modeling

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