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Enzyme-triggered Radical Reactions: Another Approach For Tin-free Radical Chemistry

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Abstract: The system laccase/mediator/dioxygen is able to trigger radical reactions with radical precursors which are not natural substrates of this enzyme. The radical generation has been accomplished by single electron transfer oxidation of a 1,3-dicarboxyl precursor. The process is exemplified with a radical cascade.

Keywords: 1,3-Dicarboxyl compounds • Laccase • Oxidoreductase • Radical mediator • Radical reaction

Over the last few years, our research group has explored the possibility to broaden the synthetic potential of radical reactions by combining them with an enzymatic transformation. This led us to report the first switchable dynamic kinetic resolution (DKR) process allowing the synthesis of either (R)- or (S)-amides from racemic amines depending on the nature of the enzyme, lipase or protease.\textsuperscript{[1]} This fruitful adventure in the world of hydrolases led us to think about the possibility to use an enzyme to initiate radical reactions. The latter can be initiated through a redox reaction.\textsuperscript{[2]} This second approach might be achieved with the assistance of an oxidoreductase. Indeed, numerous redox reactions, such as oxidation of alcohol, aldehyde or ketone, hydroxylation of aromatic or non-activated carbon atoms, can be catalyzed by isolated enzymes.\textsuperscript{[3,4]} Oxidase, a subclass of the oxidoreductases, is constituted of any enzyme that catalyzes an oxidation-reduction reaction involving molecular oxygen as the final electron acceptor.\textsuperscript{[5]} In these reactions, oxygen is reduced to water or to hydrogen peroxide. In order to avoid the formation of hydrogen peroxide in the reaction medium that might induce side reactions, laccases have been selected for this preliminary study since, with these copper-containing oxidases,\textsuperscript{[5b,6]} the only side-product formed during the redox process is water (Scheme 1).

Laccases have a low redox potential (0.5–0.8 V/NHE)\textsuperscript{[6,7]} which explains why their natural substrates are mainly electron-rich phenols.\textsuperscript{[6a]} To extend the scope of applications to non-phenolic substrates, a redox mediator can be used (Fig. 1). The role of mediators in laccase oxidations is depicted in Scheme 2.\textsuperscript{[8]} They act as ‘electron shuttles’; after being oxidized by the enzyme, the oxidized form of the mediator oxidizes any substrate that could not enter in the enzymatic pocket.

Moreover, the wide structural variety of oxidized mediators enables the reaction to evolve through oxidation mechanisms that cannot be catalyzed by the enzyme alone, and thus to broaden the range of potential substrates (Scheme 2). Thus PT and ABTS radical cations lead to single electron transfer (SET) reactions (Fig. 1).\textsuperscript{[9]} N-Hydroxy mediator-derived radicals (e.g. HBT, HPI) enable the abstraction of a hydrogen atom from benzylidic positions.\textsuperscript{[9a,9b]}

In the present paper, we describe our recent results on the use of the laccase-mediator system to initiate a radical reaction on radical precursors which are not laccase classical substrates.

SET oxidative processes that enable the generation of radicals are interesting...
alternatives to tin-based methodologies.\(^{[11]}\)

Carbon-centered radicals have been
generated from neutral carbonyl deriva-
tives, carbanions, enamines or enolates
by employing, among other oxidants,
Mn(OAc)\(_3\),\(^{[12]}\) ceric ammonium nitrate
(CAN),\(^{[13]}\) copper(II)\(^{[14]}\) or iron(II)\(^{[14a,b,15]}\)
complexes. The main drawback of these
methodologies is that the oxidant is often
used in excess and it is difficult to get rid
of metal traces. This problem may be eas-
ily overcome by the enzymatic approach.
Indeed, laccases enable the use of a cataly-
ic amount of enzymes since the co-oxidant
of the reaction is dioxygen. Moreover, the
copper atoms in charge of the oxidation
are strongly liganded to the enzyme and
from this point of view the medium can be
considered as metal-free at the end of the
process.

The goal of this preliminary study was
to trigger, with a laccase, a radical reaction
from a substrate which is not a classical
electrophile, between the enzyme and the
substrate, during the reaction. Thus ABTS and
Meldrum’s acid were selected as mediator and radical pre-
cursor respectively. The strong acidity of
this 1,3-dicarbonyl compound enables the
use of the corresponding sodium salt, as
substrate in the buffer aqueous medium
required to maintain the enzyme active.
The commercially available laccase
Polyergus pinistus 504 (Pp. 504)\(^{[16]}\) from
Novo Nordisk was selected so that the method-
ology could easily be reproduced or
extended to other substrates.

When Meldrum’s acid (1) or its sodium
salt (Na-1) was reacted with the radical
cation of ABTS (ABTS\(^{**}\))\(^{[17]}\) (1 equiv.),
generated in the presence of Pp. 504 in a
pH 5 succinate buffer solution, no evolution,
like dimerization, was detected and the
dark blue color of ABTS\(^{**}\) remained
unchanged. Another mediator, N-ethyl-
phenothiazine (EPT),\(^{[18,19]}\) was used under
the same conditions. The light orange
color of EPT\(^{**}\) became violet instantaneously
after addition of Na-1 before turning again
light orange at the end of the reaction (24 h).
The analysis of the mixture showed that
ylide 2\(^{[20]}\) together with sulfoxide 3 were
formed in 59 and 16% yield respectively
(Scheme 3). The same result was observed
starting from 1, showing that at pH 5 the

equilibrium between both forms enabled
the reaction to proceed.

The formation of 2 might be explained
on the grounds of the mechanism proposed
in Scheme 4. After a first electron trans-
fer (ET) step from EPT to the enzyme,
EPT\(^{**}\) oxidized Na-1/I through a second
ET generating radical A. The latter was
then trapped by the persistent EPT\(^{**}\) (B)\(^{[21]}\)
which evolved to 2 after deprotonation.
The concomitant formation of 3 resulted
from the reaction of EPT\(^{**}\) with water. The
structure of 3 was confirmed by comparison
with an authentic sample produced by
a direct oxidation of EPT with m-CPBA,\(^{[22]}\)
In this case the corresponding sulfone was
also produced. The latter was not detected
during the enzyme-catalyzed reaction.

This transformation was applied to
other acidic 1,3-dicarbonyl compounds.
Acetylacetone and dimesone led to 4 and 5
in 58 and 78% yield (Fig. 2) whereas a less
acidic substrate like ethyl cyanoacetate did
not undergo any transformation. It must be
underlined that the formation of sulfoxide
3 could not be avoided.

This first approach showed that a com-
mercially available laccase could be used
as catalyst to generate a carbon-centered
radical. However, the nature of the couple
laccase/mediator had to be optimized in or-
der to preclude any interference of the me-
diator with the fate of the radical species.
In spite of the rather disappointing pre-
liminary results obtained with ABTS, we
decided to reinvestigate its reactivity. The
absence of evolution, when the reaction
was performed with 1, did not necessarily
mean that the oxidation of the dicarbonyl
compound did not occur. The interesting
point was that if the oxidation proceeded
the resulting radical did not react with the
mediator.

With this purpose in mind, Meldrum’s
acid was functionalized with an efficient
internal radical acceptor,\(^{[23]}\) i.e. a styrene
moiety (6). No transformation occurred in
the presence of EPT contrary to the reac-
tivity previously observed with unsubstit-
tuted 1,3-dicarbonyl compounds.

On the other hand, when a solution of
6 in toluene was added to the dark blue
buffered solution of ABTS\(^{**}\), a slow dis-
coloration of the solution was observed.
ABTS was added by portion until the
blue color became persistent (0.6 equiv.).
After consumption of the substrate (TLC
monitoring, 3 h), the reaction was stopped
and analyzed. Cyclopropane 7 was char-
acterized as a mixture of two diastereomers
(1:1). Its formation might be explained by
the mechanism proposed in Scheme 5. The
α-dicarbonyl radical C, resulting from the
oxidation of 6, added reversibly to the dou-
ble bond. Equilibrium was then displaced
by the oxidation of radical D with ABTS\(^{**}\)
leading to the benzylic cation E which was
trapped by a molecule of water to form 7
in 38% yield. This radical-polar crossover
cascade showed that ABTS was able to act
first as ‘electron shuttle’ between the lac-
case and the radical precursor and second
as final oxidant. However the modest yield
and the quantity of mediator used in the
process, 0.6 equiv., confirmed previous ob-
servations that ABTS might give rise to by-
products and therefore it was not the best
mediator for a selective reaction.\(^{[24]}\)

In summary, this preliminary study
showed that the couple laccase/mediator,
with dioxygen as co-oxidant, is a viable
alternative to initiate a radical reaction in-
volving a redox process. This new method
of radical generation enables the forma-
tion, at 30 °C, of C–C bond and subsequent
functionalization. This system might be
improved by the design of new mediators
which will not interfere with the radical
cascade. These results will be reported in
due course.

**Experimental Section**

Solvents are commercially available,
they were used as purchased, without fur-
ther purification. Purifications were per-

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**Scheme 3.**

**Scheme 4.**

**Fig. 2.**
formed on Macherey Nagel silica gel 60 A (70–230 mesh). Analytical thin layer chromatography was performed on pre-coated silica gel plates. Visualization was accomplished by UV (254 nm) and with phosphomolybdic acid in ethanol. 4H NMR, 13C NMR spectra were recorded at 400 MHz and 100 MHz, respectively. Chemical shifts (δ) are reported in ppm. Signals due to residual undeuterated solvent (1H NMR) or to the solvent (13C NMR) served as internal standards: CDCl3 (7.26 ppm and 77.0 ppm). Multiplicity is indicated by one or more of the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). The lists of coupling constants (J) correspond to the order of multiplicity assignment and are reported in Hertz (Hz). APT was used for 13C spectro assignment.

**Enzyme Assay**

The crude laccase from *Polyporus phosphitinus* (Pp. 504) from Novo Nordisk (170 mg) was diluted in 2 mL of pH 5 succinate buffer (0.2 M) and corresponds to Pp. 504 enzymatic extract in the following. Enzyme activity (1.93 pm/min/mg) was measured by monitoring the oxidation of syringaldazine[23] to the corresponding quinone (e20 = 65 000 M-1 cm-1) at 525 nm in succinate buffer 0.1 M, pH 4.0 on a spectrophotometer UVikon XL. One unit (U) of laccase activity is defined as the amount of enzyme that oxidizes 1 µmol of the substrate per minute per g.

**General Procedure for Ylide Synthesis**

To 10-ethylphenothiazine[18] (91.2 mg, 0.4 mmol) in a mixture of acetone (3.2 mL) and pH 5 succinate buffer (32 mL) at 30 °C was added Pp. 504 enzymatic extract (100 µL). The solution was stirred 30 min before addition of 1,3-dicarbonyl compound (0.4 mmol). After 24 h stirring, the reaction medium was extracted with CH2Cl2 (3x), the combined organic phases were dried over MgSO4. After concentration, the crude product was purified by flash chromatography on silica gel (5/95 to 100/0 AcOEt/ pentane) leading to the title compound.

5-(10-Ethyl-10H-phenothiazin-5-ylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (2)

5-(10H-Phenothiazin-5-ylidene)pentane-2,4-dione (4)

3-(10-Ethyl-10H-phenothiazin-5-ylidene)-5,5-dimethylcyclohexane-1,3-dione (5)

**Cyclization of 6**

To ABTS (22.1 mg, 0.04 mmol) in pH 5 succinate buffer (32 mL) at 30 °C were added Pp. 504 enzymatic extract (400 µL), the color of the solution becomes dark blue. Then (E)-2,2-dimethyl-5-(3'-phenylallyl)-1,3-dioxane-4,6-dione[23] (205 mg, 0.8 mmol) in toluene (20 mL) was added. The solution was stirred and each time the color turned yellow ABTS was added by portion (7 x 22 mg) at 5°C, 3h. 38%

Scheme 5.

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