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COMMUNICATION

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Aerobic Baeyer-Villiger Oxidation Catalyzed by a Flavin-Containing Enzyme Mimic in Water

Yoan Chevalier,[a] Yvette Lock Toy Ki,[b] Didier le Nouen,[b] Jean-Pierre Mahy,[a] Jean-Philippe Goddard,[b]* Frédéric Avenier[a]*

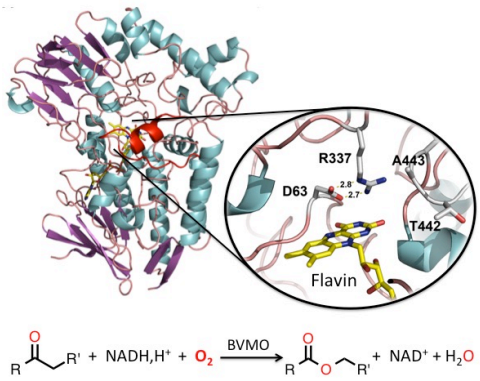
Abstract: Direct incorporation of molecular oxygen into small organic molecules is attracting much attention for the development of environmentally friendly new oxidation processes. In this line, bio-inspired systems mimicking enzymes activities are of particular interest since they may perform catalysis in aqueous media. Herein, we demonstrate that the incorporation of a natural flavin cofactor (FMN) into the specific microenvironment of a water-soluble polymer allows the efficient reduction of the FMN by NADH in aqueous solution. Once reduced, this artificial flavoenzyme can then activate molecular dioxygen under aerobic conditions and perform the Baeyer-Villiger reaction at room temperature in water.

Selective transformation of ketones into the corresponding esters or lactones, also known as the Baeyer-Villiger (BV) reaction, prevails as a major reaction in organic synthesis since its discovery in 1899.[1] It was originally performed using persulfate in concentrated sulfuric acid and evolved towards the use of other strong oxidants such as *m*CPBA or H_2O_2 activated by Lewis acids.[2–4] In the present context of sustainable growth, the use of those hazardous materials, combined to the use of organic solvents, has now become a major issue for these transformations and historical chemical processes need to be renewed. Therefore, performing selective oxidation reactions by direct incorporation of an oxygen atom coming from molecular dioxygen into organic molecules under mild condition has become a priority, not only for the Baeyer-Villiger reaction, but also for other oxidation processes.[5,6]

Baeyer-Villiger monooxygenases (BVMO) can activate dioxygen thanks to the flavin cofactor located into their active site (Scheme 1) and stand as an interesting solution to reduce the impact on the environment.[3,7] However, biochemical issues such as cloning, protein expression and substrate selectivity reduce their scope of applications for organic synthesis and chemical alternatives are still highly needed. In this sense, flavin derivatives have been developed as bioinspired catalysts and flavinium salts have demonstrated interesting oxidation activities using H_2O_2 or even O_2 in combination with a reductant, notably for sulfoxidation reactions.[8,9] In the particular case of the BV reaction, examples involving both a flavin derivative and H_2O_2 are very scarce.[10–12] So far, only two systems were described using O_2, the first one involving an N5-alkylated flavinium salt,[13] and the second one a flavin unmodified at the N5 position but substituted by a peptide at the N3 position.[14] In both cases, zinc dust was added as a source of electrons and reactions were performed in a mixture of organic solvents. The next challenge is therefore to develop new systems using readily available unmodified flavins performing catalysis using dioxygen in water.

Modified polyethyleneimines (PEI) are water soluble polymers known as good mimics for the locally hydrophobic microenvironment of enzyme's active sites.[15–27] Using PEI modified with guanidinium and octyl groups, we previously showed that the incorporation of flavin mononucleotide (FMN) into such a microenvironment generated artificial flavo-enzymes capable of collecting electrons from nicotinamide adenine dinucleotide (NADH) and then of reducing metallic cofactors under anaerobic conditions.[26,27] Here, we demonstrate that under aerobic conditions, similar artificial flavo-enzymes made of FMN incorporated into PEI modified with guanidinium and octyl groups (FMN-$PEI_{guan-oct}$; Schemes 2) catalyze the oxidation of NADH and perform aerobic BV reaction in water. To the best of our knowledge, this is the first example of non-enzymatic BV reaction performed in water using natural flavin cofactor and dioxygen from the air.

Scheme 1. Example of natural BVMO bearing a flavin cofactor in its active site[28]



Before preparing the artificial flavoenzyme, PEI was randomly modified by the covalent incorporation of guanidinium and octyl groups and purified by dialysis as previously described.[24] After lyophilization from water, the so-formed polymer was characterized by ^{1}H NMR, which allowed the determination of the guanidinium/octyl group ratio (Figure S1, S.I. section). The FMN cofactor was then incorporated into the polymer thanks to the specific electrostatic interaction between the negatively charged phosphate groups and the guanidinium moieties. This incorporation of the FMN into the confined hydrophobic environment of the polymer was monitored by UV-Vis absorption spectroscopy, showing a characteristic 10 nm hypsochromic shift of the FMN band at 370 nm (Figure 1).[26,29,30] Importantly, we also measured the absorption spectrum of FMN-PEIguan-oct in the presence of an excess of bicylo[3.2.0]hept-2-en-6-one as

[a] Yoan Chevalier, Prof. Jean-Pierre Mahy, Dr. Frédéric Avenier
Univ Paris Sud, Université Paris Saclay
LCBB, ICMMO, (UMR CNRS 8182), 91405 Orsay, France.
E-mail: frederic.avenier@u-psud.fr
[b] Yvette Lock Toy Ki, Didier le Nouen, Prof. Jean-Philippe Goddard
Université de Haute-Alsace, Université de Strasbourg, CNRS
LIMA UMR 7042, F_68100 Mulhouse, France.
E-mail: jean-philippe.goddard@uha.fr

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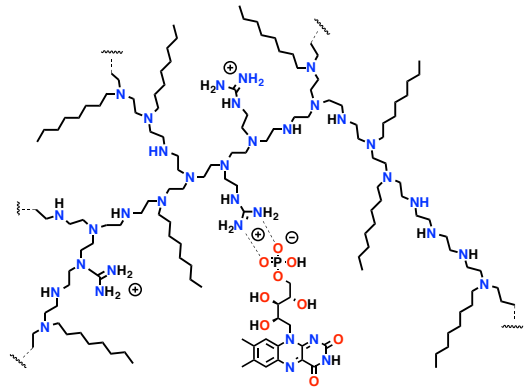
potential substrate, and the same 10 nm shift was observed (Figure 1, red trace). This indicated that the excess of substrate did not exclude the FMN from the hydrophobic environment of the polymer and that the artificial flavoenzyme was not altered by the presence of substrate. It is also worth to note that previous fluorescence spectroscopy studies showed that about 90% of the FMN was incorporated inside the local microenvironment of the polymer.[26]

Scheme 2. Artificial flavoenzyme (FMN-PEIguan-oct) obtained by incorporation of FMN into a 25 kDa multi-branched PEI modified with guanidinium and octyl groups.



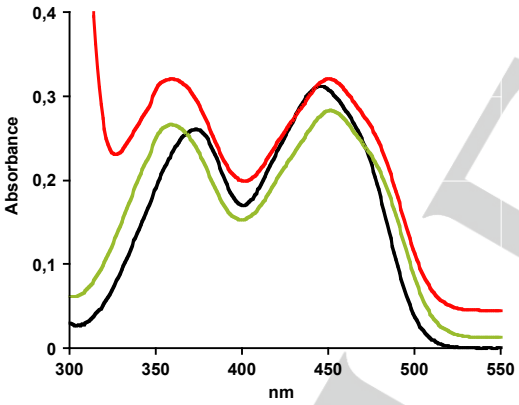


Figure 1. UV-Visible absorption spectra of a 25 µM aqueous solution of FMN: alone (black trace), in the presence of modified PEI (25 mM in monomer) (green trace), and in the presence of both PEI (25 mM in monomer) and bicylo[3.2.0]hept-2-en-6-one (50 mM) (red trace).

FMN-PEIguan-oct was then studied in the presence of NADH and Figure 2 shows the consumption of NADH when it was added either to a solution of FMN, or to a solution of FMN-PEIguan-oct. In the absence of polymer, the concentration of NADH remained stable over time, meaning that NADH was not able to reduce efficiently the FMN in solution. Advantageously, when the FMN was located inside the modified PEI, the kinetics of NADH consumption was improving with the concentration of PEIguan-oct, reaching a maximum for a ratio of 1000 equivalents of PEIguan-oct (in monomer) with respect to the FMN cofactor. For such a ratio, the 200 equivalents of NADH were fully consumed over 10 hours. This clearly indicated that the FMN could consume NADH thanks to its incorporation inside the hydrophobic environment of the polymer. This result is in agreement with previous observations made with FMN-PEIguan-oct under anaerobic conditions using stoichiometric amounts of NADH.[26] Here, the consumption of 200 equivalents of NADH with respect to the FMN demonstrates the catalytic behavior of the system, suggesting that during the process, the FMN is successively reduced to $FMNH_2$ and reoxidized by molecular dioxygen. During such a process, the FMN cofactor should therefore generate a transient peroxo species, which is reminiscent of the active intermediate formed within the catalytic cycle of BVMO.[3,7]

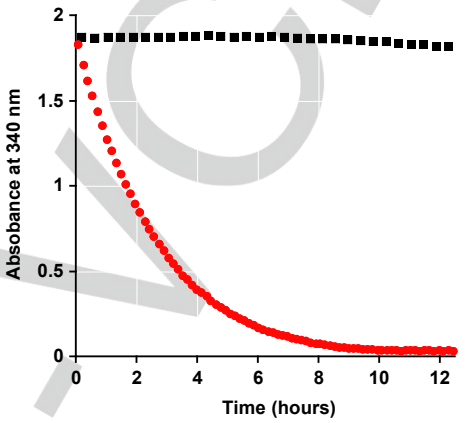


Figure 2. Evolution of the absorbance of NADH (400 µM) at 340 nm upon addition either to an aqueous solution of FMN (2 µM) (black squares) or to an aqueous solution of FMN-PEIguan-oct (red circles) (FMN (2 µM) and PEIguan-oct (2 mM in monomer).

These observations prompted us to study the system for BV reaction catalysis and NADH was added to an aqueous solution of FMN-PEIguan-oct in the presence of bicylo[3.2.0]hept-2-en-6-one as substrate (Table 1). After 12 hours stirring at room temperature under ambient atmosphere in deionized water, the solution was analyzed by GC-MS and ^{1}H NMR. It is worth to note that the FMN being light sensitive, all reactions were performed in tinted glassware in order to avoid any side photocatalytic reaction during the process. GC-MS analysis revealed the formation of one major product with a retention time corresponding to the 2-oxabicyclo[3.3.0]oct-6-en-3-one (lactone **1**), as well as a minor product with close retention time, 3-oxabicyclo[3.3.0]oct-6-en-2-one (lactone **2**) (Figure S2, S.I. section). In both cases, the mass detection allowed to clearly identify lactones **1** and **2** (Table 1), which was confirmed by ^{1}H NMR analysis (Fig. S3 and S4, S.I. section). In addition, no signal of epoxide was observed, neither by GC-MS nor by ^{1}H NMR analysis, clearly indicating the exclusive formation of the Baeyer-Villiger products. Once the lactones identified, the reaction was followed over time by GC (Figure 3) in 20 mM HEPES buffer, pH 8.5. The concentration of lactones reached a plateau after 10 h of reaction with a maximum yield of 70 % with respect to NADH, together with an excellent lactone **1**:lactone **2** ratio of 87:13. This global yield corresponds to 140 turnovers and nicely correlates with the consumption of NADH observed in Figure 2. In the absence of polymer, the FMN only yielded 3 % of lactone, which is also in good agreement with the very low consumption of NADH observed in Figure 2. Other controls in absence of dioxygen, or simply using PEIguan-oct or NADH on their own, did not yield any product. When the reaction was performed under stoichiometric conditions (10 mM both in substrate and NADH) a 42 % conversion of the substrate was obtained, corresponding to 840 turnovers.

In order to gain more insight into this catalytic system, the pH dependency was also investigated (Figure S5, S.I. section). Below pH = 7, only the lactone **1** was obtained with a maximum yield of 47 % at pH = 6.7. For higher pH(s), a mixture of lactones **1** and **2** was obtained reaching a maximum global yield of 72 % at pH 9 (Table 1). This trend is in good agreement with the mechanism of natural BVMO involving the nucleophilic attack of a deprotonated 4a-peroxo-flavin intermediate on the carbonyl group of the substrate. However, the selectivity for the formation of lactone **1** at acidic pH is more difficult to rationalize. One may suggest that the 4a-hydroperoxo-flavin intermediate is not reactive enough to form the less favored lactone **2**.

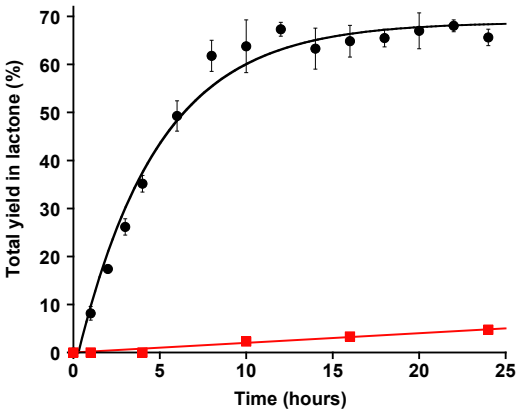
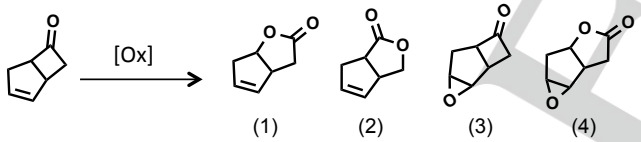


Figure 3. Kinetics for the total formation of lactones **1** and **2** catalyzed by FMN-PEIguan-oct (FMN: 5 µM; PEIguan-oct: 5 mM) (black circles) or by FMN only (FMN: 5 µM) (red squares) 20 mM HEPES, pH 8.5 with NADH: 1 mM; substrate: 10 mM; at room temperature, under ambient atmosphere.

Table 1. Chemical and biomimetic oxidation of bicylo[3.2.0]hept-2-en-6-one.



Oxidation Conditions	Products yields (%) (1)	(2)	(3)	(4)	Selec.[d] (%)	Ref.
FMN-PEIguan-oct						
O_2/NADH **(pH 6.7)**[a]	47	-	-	-	100	This work
O_2/NADH **(pH 8.0)**[a]	56	8	-	-	87	This work
O_2/NADH **(pH 9.0)**[a]	60	12	-	-	83	This work
H_2O_2 / acetic acid[b]	77	14	-	-	85	(31)
***m*CPBA**[c]	28	-	32	37	29	(32)

[a]GC yields using [FMN] = 5 µM; [PEIguan-oct] = 5 mM in monomer ; [substrate] = 10 mM; [NADH] = 1 mM; Atmospheric O_2.; room temp. [b]Isolated yield using conditions from ref. (31) with specific yields calculated by NMR (see Supp. Info.). [c]Data from ref. (32). [d]Selectivity for lactone 1.

For the sake of comparison, the chemical BV reaction was also performed using over-stoichiometric amounts of hydrogen peroxide in acetic acid,[31] which afforded a mixture of lactone **1** and **2** in 91% yield and 85% selectivity. The biomimetic reaction using dioxygen appears therefore competitive with the use of hydrogen peroxide. Interestingly, it also compares well with the use of *m*CPBA, which was previously reported with only 29 % selectivity for lactone **1** (Table 1).[32]

This artificial enzyme is the first catalytic system utilizing unmodified natural flavin cofactor to activate dioxygen and perform the B.V. reaction in water. Only one catalyst using unmodified flavin at the N5 position and bearing an oligopeptide at the N3 position was described so far.[14] In this case, Imada and collaborators showed that efficient catalysis was dependent on the presence of a carboxylic acid at the C-terminus of the oligopeptide, and DFT calculations demonstrated that this carboxylic acid could actually stabilize a putative flavin-4a-hydroperoxo intermediate by hydrogen bonding. Based on these calculations, one may suggest that similar flavin-4a-hydroperoxo intermediate could be stabilized via hydrogen bonding with the amine groups present in the microenvironment of the FMN-PEIguan-oct system. This would explain the good catalytic activity of such systems. However, neither Imada's group, nor ours, have yet been able to experimentally observe such flavin-4a-hydroperoxo intermediate with N5 unmodified flavin. Finally, apart from the stabilization of a flavin-4a-hydroperoxo species, catalysis could also be explain by the in situ formation of hydrogen peroxide, which was indeed observed during catalysis. However, the control experiment using hydrogen peroxide in the absence of FMN and dioxygen, only yielded 27% of lactone, suggesting that both phenomenon were probably involved in the production of lactone.

Overall, we have shown that the incorporation of the natural FMN cofactors into the locally hydrophobic microenvironment of a modified polyethyleneimine allows the flavin cofactors to collect electrons from NADH, activate dioxygen and perform the BV reaction in good yield and high selectivity. Such an artificial flavo-enzyme stands therefore as potential alternative to the use hazardous materials such as peroxides or peracids in organic solvents and paves the way to the development of new environmentally friendly catalysts capable of using dioxygen in water.

Experimental Section

For experimental data, please see the supporting information section.

Acknowledgements

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Keywords: Dioxygen activation • Baeyer-Villiger reaction • Catalysis • Artificial enzyme • Sustainable chemistry

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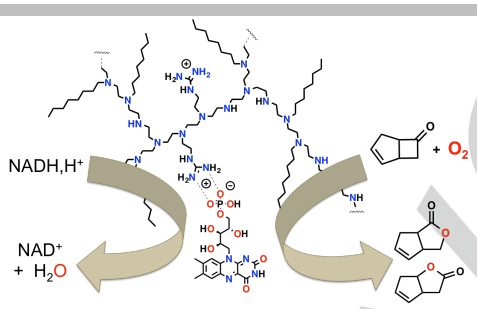
Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Incorporation of flavin cofactors (FMN) within the locally hydrophobic microenvironment of a modified polyethyleneimine allows to activate dioxygen in the presence of NADH and to perform the Baeyer-Villiger reaction in good yield, under ambient atmosphere, at room temperature in water.



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Aerobic Baeyer-Villiger Oxidation Catalyzed by a Flavin-Containing Enzyme Mimic in Water