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Enzyme-like Regiodivergent Behavior of a Flavopeptide Catalyst in Aerobic Baeyer-Villiger Oxidation

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Abstract: We recently developed a flavopeptide immobilized on polystyrene resin, **Fl-Pep-PS**, that could realize the first *N*5-unmodified neutral flavin (**Fl**)-catalyzed aerobic oxygenation reactions under non-enzymatic conditions. Although a key active species is assumed to be the corresponding 4a-hydroperoxyflavin ($\mathbf{Fl}_{\mathbf{4aOOH}}$) from the unprecedented activity and unique chemoselectivity, further circumstantial support would be helpful to be sure since spectroscopic evidence is difficult to obtain due to the compound's insolubility. In this article, we report that the aerobic Baeyer-Villiger oxidation of a fused cyclobutanone, (±)-*cis*-bicyclo[3.2.0]hept-2-en-6-one (**1**), can be promoted with **Fl-Pep-PS** in a FMO-like chemoselectivity and regiodivergent manner *via* **Fl**-related catalytic intermediates, which delivers strong evidence of the involvement of $\mathbf{Fl}_{\mathbf{4aOOH}}$ as an active species in **Fl-Pep-PS**-catalyzed aerobic oxygenation reactions.

Keywords: Aerobic oxygenation · Baeyer-Villiger oxidation · Biomimetic catalyst · Flavin · Peptide

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1. Introduction

Enzymes are specific and efficient in native organic reactions; therefore, their catalytic functions have often served as guides for the design of highly active, selective, and green artificial catalysts and reactions. Among diverse classes of enzymes, oxidoreductases employing the isoalloxazine ring system **Fl** (Scheme 1), found in flavin cofactors as an active center, are called flavoenzymes, which are responsible for various oxidative metabolic processes in nature.[1] A notable series of flavoenzymes is flavin-containing monooxygenases (FMO) that metabolize xenobiotic substrates through the activation of molecular oxygen (O_2) followed by the donation of an oxygen atom to the substrate in mammalian liver. A key active species for the monooxygenation has been recognized to be 4a-hydroperoxy adducts of **Fl** ($\mathbf{Fl}_{\mathbf{4aOOH}}$) and the catalytic cycle has long been well understood (Scheme 1, lower cycle).[2] Nevertheless, **Fl** as a simple non-enzymatic organocatalyst had never been successfully employed for simulating the aerobic oxygenation ability of FMO due to the lability of $\mathbf{Fl}_{\mathbf{4aOOH}}$, which readily decomposes into **Fl** and H_2O_2 under apoenzyme-free conditions (Scheme 1, upper cycle).[3] Recently, this long-standing challenge was overcome at last using our designed catalyst, 3-FlC2-Pro-Tyr-Asp-Ado-NH-PS (**Fl-Pep-PS**; 3-FlC2 = lumiflavin-3-acetic acid residue, Scheme 1), consisting of **Fl**, a tripeptide linker, and polystyrene (PS) resin.[4] We calculated the lowest energy conformation of $\mathbf{Fl}_{\mathbf{4aOOH}}$ that could be stabilized by the conjugated peptide through intramolecular hydrogen bonds, and demonstrated that **Fl-Pep-PS** could efficiently catalyze the electrophilic sulfoxidation of thioanisole as well as the nucleophilic Baeyer-Villiger oxidation of 3-phenylcyclobutanone using O_2 as the terminal oxidant, in which the resin could play a crucial role probably as hydrophobic microenvironment in stabilizing the corresponding $\mathbf{Fl}_{\mathbf{4aOOH}}$. Although spectroscopic evidence is not available due to the insolubility of the resin, the involvement of $\mathbf{Fl}_{\mathbf{4aOOH}}$ as well as the non-involvement of a peracid as the active species were supported by the unique chemoselectivity of **Fl-Pep-PS**, which was similar to that of FMO. For example, 3-phenylcyclobutanone was exclusively oxidized into β-phenyl-γ-butyrolactone in the presence of other reactive substrates such as thioanisole and cyclooctene under suitable conditions with **Fl-Pep-PS**, whereas such FMO-like chemoselectivity was not observed under typical *m*CPBA-based oxidation conditions.[4]

In this brief communication, we describe how the aerobic Baeyer-Villiger reaction of a fused cyclobutanone, (±)-*cis*-bicyclo[3.2.0]hept-2-en-6-one (**1**), can be promoted with **Fl-Pep-PS** in a FMO-like chemoselectivity and regiodivergent manner, which can be strong evidence of the involvement of $\mathbf{Fl}_{\mathbf{4aOOH}}$ as a key active species in **Fl-Pep-PS**-catalyzed aerobic oxygenation reactions.

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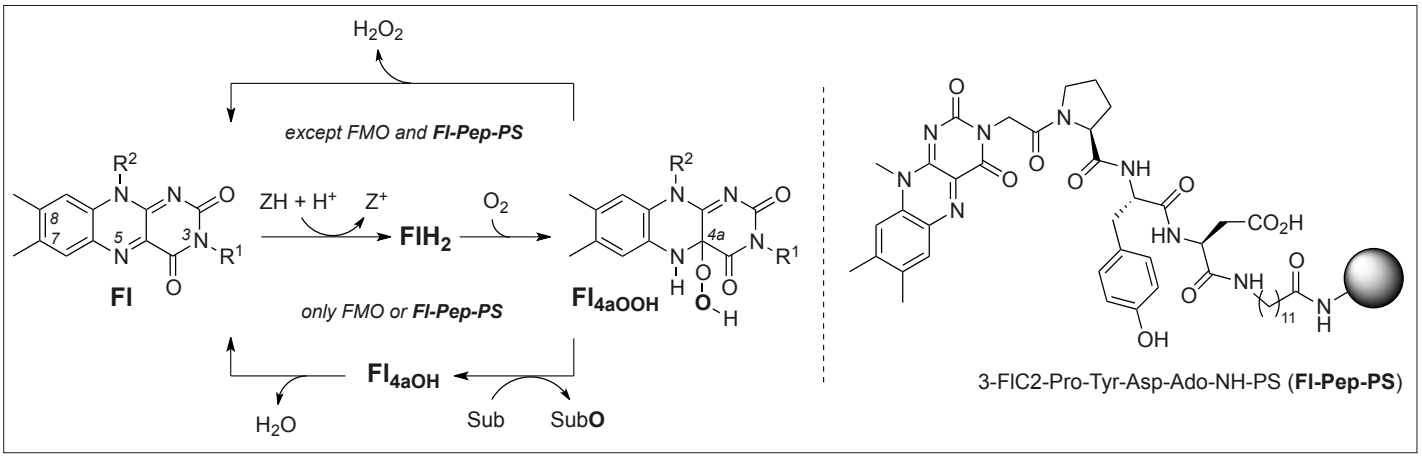


Scheme 1. General catalysis of neutral flavins (**Fl**) under aerobic oxygenation conditions.

2. Experimental

2.1 *Materials*

(Aminomethyl)polystyrene (70–90 mesh, 1% cross-linked, the N loading was determined by elemental analysis to be 1.24 mmol g^{-1}) was purchased from Sigma-Aldrich. 3-Methyllumiflavin (**LFl**),[5] lumiflavin-3-acetic acid,[6] 5-ethyl-3-methyllumiflavinium perchlorate (**LFlEt$^+$ClO$_4^-$**),[5] and 3-FlC2-NH-PS[4] were prepared according to the literature procedures. Zinc dust was treated with 2N HCl aq. under ultrasonication for 15 minutes, washed with H_2O and acetone, and dried in *vacuo* to activate prior to use. All other reagents were purchased from commercial supplies and used without purification.

2.2 *Synthesis of Fl-Pep-PS*

To (aminomethyl)polystyrene (NH_2-PS, 250 mg, 0.31 mmol) pre-swollen in DMF was added a solution of Boc-Ado-OH (244 mg, 0.78 mmol), *O*-(*1H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU, 321 mg, 0.78 mmol), and *N*-ethyldiisopropylamine (DIPEA, 301 mg, 2.3 mmol) and the heterogeneous mixture was shaken for 1.5 h. The insoluble resin (Boc-Ado-NH-PS) was washed with DMF (5×), DMF/CH_2Cl_2 (4:1) (5×), and CH_2Cl_2 (3×), and then treated with a mixture of TFA/CH_2Cl_2 (2:1) twice (the first time: 1 h, the second time: 20 min) to remove the Boc group, and washed with CH_2Cl_2 (3×), 5% v/v DIPEA in CH_2Cl_2 (3×), and CH_2Cl_2 (6×). The resulting resin, H-Ado-NH-PS, was mixed with a solution (prepared with a minimum amount of DMF) of Fmoc-Asp(O*t*Bu)-OH (319 mg, 0.78 mmol), HCTU (321 mg, 0.78 mmol), and DIPEA (301 mg, 2.3 mmol), which was agitated for 1.5 h for coupling, washed with DMF (5×), DMF/CH_2Cl_2 (4:1) (5×), and CH_2Cl_2 (3×), then treated with a 20% v/v solution of piperidine in DMF for 15 min twice for Fmoc-deprotection, and washed with DMF (5×), DMF/CH_2Cl_2 (4:1) (5×), and CH_2Cl_2 (3×) to give H-Asp(O*t*Bu)-Ado-NH-PS. According to the coupling and Fmoc-deprotection procedures, H-Asp(O*t*Bu)-Ado-NH-PS was further converted into H-Pro-Tyr(*t*Bu)-Asp(O*t*Bu)-Ado-NH-PS *via* H-Tyr(*t*Bu)-Asp(O*t*Bu)-Ado-NH-PS. Subsequently, a solution (prepared with a minimum amount of DMF) of lumiflavin-3-acetic acid (244 mg, 0.78 mmol), HCTU (321 mg, 0.78 mmol), and DIPEA (301 mg, 2.3 mmol) was added to H-Pro-Tyr(*t*Bu)-Asp(O*t*Bu)-Ado-NH-PS pre-swollen in DMF, and the mixture was agitated for 2 h. The suspension was washed with DMF repeatedly until the solution layer becomes colorless, then with DMF/CH_2Cl_2 (4:1) (5×), and with CH_2Cl_2 (3×) to give 3-FlC2-Pro-Tyr(*t*Bu)-Asp(O*t*Bu)-Ado-NH-PS. Finally, 3-FlC2-Pro-Tyr(*t*Bu)-Asp(O*t*Bu)-Ado-NH-PS pre-swollen in CH_2Cl_2 was treated with a mixture of TFA/CH_2Cl_2 (2:1) twice (the first time: 1 h, the second time: 20 min), and washed with CH_2Cl_2 by means of Soxhlet extractor and dried in *vacuo* to give 453 mg of 3-FlC2-Pro-Tyr-Asp-Ado-NH-PS (**Fl-Pep-PS**) as an orange-colored resin. The coupling reactions were monitored by qualitative Kaiser test[7a] and chloranil test (secondary amine).[7b] The **Fl** loading of **Fl-Pep-PS** used in this study was determined as previously reported[4] to be 0.50 mmol g^{-1}.

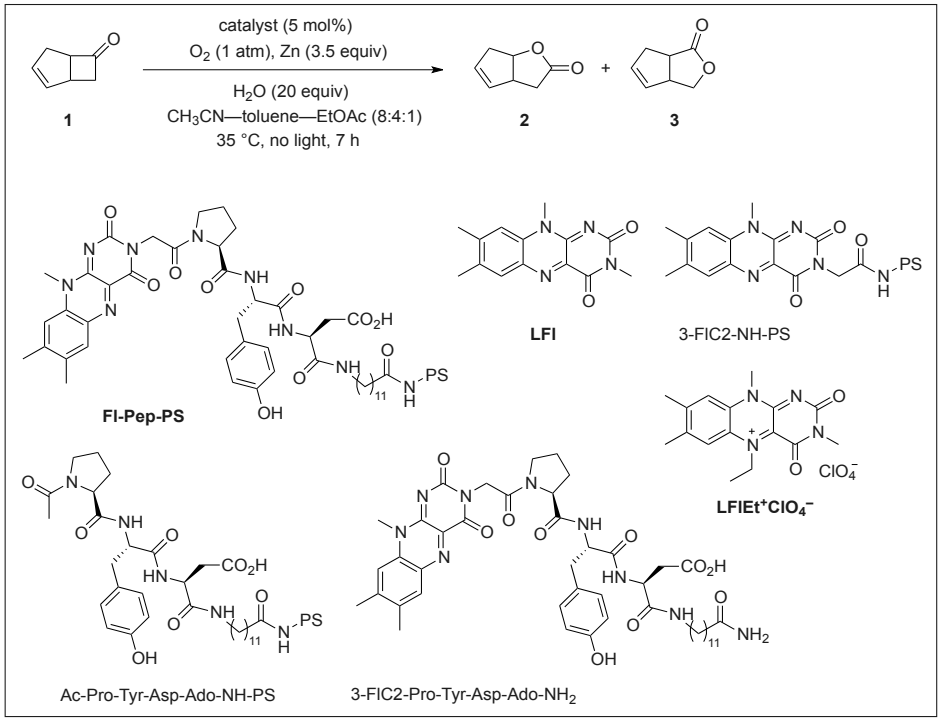
2.3 *Aerobic Baeyer-Villiger Oxidation of 1 Catalyzed by Fl-Pep-PS*

To an acetonitrile–toluene–ethyl acetate mixed solvent (8:4:1, 0.9 ml) was added **Fl-Pep-PS** (10 mg, 5 μmol) and zinc dust (22.9 mg, 0.35 mmol), and the mixture was sonicated for 2 min before adding H_2O (36 μl) and a 1 M stock solution of **1** (0.1 ml, 0.1 mmol) in the same mixed solvent containing 10 mol% of dodecane as an internal standard, which was stirred at 35 °C for 7 h under an atmosphere of oxygen while protected from light. The reaction was evaluated by ^{1}H NMR spectroscopy of the crude mixture with reference to the published spectral data of **2** and **3**,[8] in which the yields of **2** and **3** were estimated from the integration of peaks assignable to methyl protons of dodecane at 0.88 ppm and that assignable to a proton of the olefin moiety either at 5.58 ppm (for **2**) or at 5.66 ppm (for **3**).

3. Results and Discussion

We explored the aerobic Baeyer-Villiger oxidation of the fused cyclobutanone **1** by means of **Fl-Pep-PS** as a catalyst under conditions that were previously developed by our group for the oxidation of 3-phenylcyclobutanone.[4] In the presence of 5 mol% of **Fl-Pep-PS**, 1 atm of O_2, 20 equivalents of H_2O, and 3.5 equivalents of zinc dust in a mixed solvent of acetonitrile, toluene, and ethyl acetate (8:4:1), the desired oxidation reactions were found to proceed smoothly with 76% conversion of **1** in 7 h to afford the corresponding *cis*-lactones **2** and **3** in 35% yield and 29% yield, respectively, without undesired oxidation to epoxides (Table 1, entry 1). By contrast, the use of **LFl** instead of **Fl-Pep-PS** as a catalyst under identical conditions resulted in no conversion of **1** (entry 2), indicating that, as expected, such a simple **Fl** molecule has no catalytic activity due to the general lability of the corresponding **Fl$_{4aOOH}$**. The same result, no conversion of **1**, was obtained with 3-FlC2-NH-PS as a catalyst (entry 3), and Ac-Pro-Tyr-Asp-Ado-NH-PS containing no **Fl** was also totally inactive (entry 4) even in the presence of **LFl** (entry 5). In addition, only trace amounts of the products were formed when the reaction was performed with 3-FlC2-Pro-Tyr-Asp-Ado-NH_2 having no PS as a catalyst (entry 6). Naturally, no reaction occurred without any catalysts (entry 7). These results show that all components of **Fl-Pep-PS** are essential for its catalytic

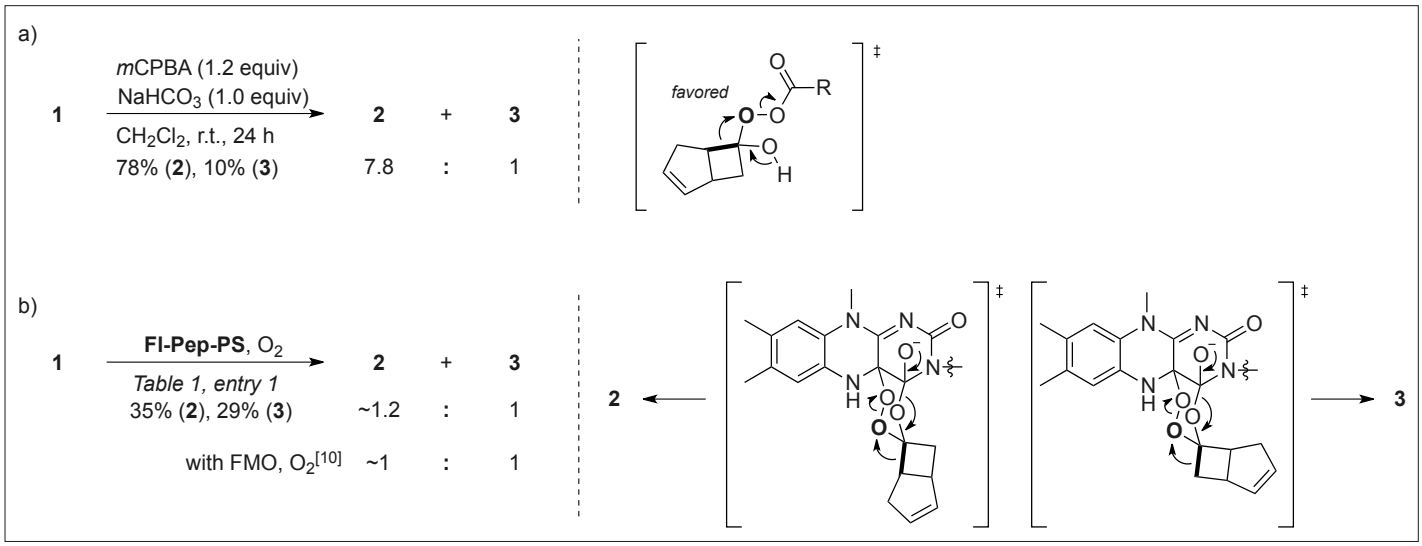
Table 1. Aerobic Baeyer-Villiger oxidation of **1** with flavin catalysts.



entry	catalyst	conversion [%]	yield [%]	
			2	**3**
1	**Fl-Pep-PS**	76	35	29
2	**LFl**	0	0	0
3	3-FlC2-NH-PS	0	0	0
4	Ac-Pro-Tyr-Asp-Ado-NH-PS	0	0	0
5	Ac-Pro-Tyr-Asp-Ado-NH-PS + 5 mol% **LFl**	0	0	0
6	3-FlC2-Pro-Tyr-Asp-Ado-NH_2	4	<1	<1
7	none	0	0	0
8	**LFlEt$^+$ClO$_4^-$**	66	36	27

activity and they should be arranged properly with each other. On the other hand, **LFlEt$^+$ClO$_4^-$**, one of the most common conventional pseudo-flavin catalysts,[3] promoted the reaction as efficiently as **Fl-Pep-PS** to furnish **2** and **3** in 36% yield and 27% yield, respectively (entry 8), as expected from our previous reports on the Baeyer-Villiger oxidation with such artificial cationic flavins.[9]

Provided that the above reaction is carried out under the typical Baeyer-Villiger oxidation conditions, the formation of **2** *via* migration of the adjacent more substituted carbon in the Criegee intermediate should be kinetically favored. Indeed, the normal lactone **2** was preferentially obtained under *m*CPBA-based oxidation conditions in 78% yield along with the abnormal lactone **3** as well as an epoxidized by-product in 10% and 12% yield, respectively (Scheme 2a). Interestingly, such electronic limitations can be overcome under FMO-mediated enzymatic conditions, providing 'normal' and 'abnormal' lactones in a ratio of nearly 1:1,[10] and this regiodivergent behavior can be rationalized by fixation of Criegee intermediates as the corresponding 4-hydroxy-1,2,5-trioxane adducts formed from the ketone and **Fl$_{4aOOH}$**.[11] Thus we propose that the regioselectivity of **Fl-Pep-PS** (Table 1, entry 1) may also come from such FMO-like cyclic transition states including one that gives rise to abnormal migration (Scheme 2, the right transition state model) as much as normal migration (Scheme 2, the left transition state model), in which the corresponding **Fl$_{4aOOH}$** should be necessarily involved as a key precursor. In other words, the present study provides strong evidence for the effective use of **Fl$_{4aOOH}$** as the active species in the **Fl-Pep-PS**-catalyzed aerobic catalytic oxygenations (Scheme 1, lower cycle).



Scheme 2. The aerobic Baeyer-Villiger oxidation of **1** under conditions with (a) *m*CPBA and (b) **Fl-Pep-PS**, and plausible origins of each regioselectivity.

4. Conclusion

A flavopeptide catalyst, **Fl-Pep-PS**, recently designed by our group[4] was found to be effective for the aerobic Baeyer-Villiger oxidation of the fused cyclobutanone **1**, which could provide the normal lactone **2** and the abnormal lactone **3** in a nearly equal ratio *via* FMO-like **Fl**-related catalytic intermediates. All components of **Fl-Pep-PS** were demonstrated to be essential for the catalysis by some control experiments. These brief but significant results have led us to conclude that $\mathbf{Fl}_{\mathbf{4aOOH}}$ is the active species in the **Fl-Pep-PS**-catalyzed aerobic oxygenation system.

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