FULLERENOL-BASED MIMICRY OF LarE-TYPE SULFUR INSERTASE ACTIVITY

V. Dolgun[1] · A. Marion[2] · S. Özçubukçu[1]

[1]Department of Chemistry, Middle East Technical University, Ankara, Türkiye
[2]Meddenovo Drug Design, Lyon, France
dvolkan@metu.edu.tr

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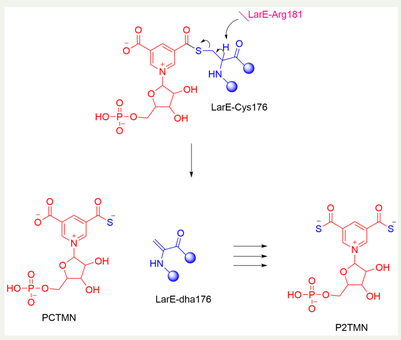
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INTRODUCTION

The development of synthetic systems capable of reproducing complex enzymatic transformations remains a central challenge in biomimetic catalysis. Enzymatic activity commonly depends not only on individual catalytic functional groups, but also on their precise spatial organization within a cooperative microenvironment.[1] LarE provides a particularly unusual model for such studies. It is a sacrificial sulfur insertase involved in the biosynthesis of the nickel-pincer cofactor of lactate racemase.[2] During sulfur insertion, an active-site cysteine residue acts as the sulfur donor and is subsequently converted into dehydroalanine (Dha), resulting in irreversible enzyme inactivation.[3] Thus, unlike conventional enzymes that undergo repeated catalytic turnover, LarE is consumed during the transformation it promotes. Reproducing this distinctive reactivity using a simplified synthetic platform would provide a useful model for investigating enzyme-inspired sulfur chemistry. Building on fullerene-based enzyme mimics ("fullerenzymes"), originally developed for hydrolase-like activity,[4] we therefore extended this concept to a non-enzymatic model of LarE-type reactivity.

Herein, arginine-functionalized fullerenol (F-Arg) was employed as a multifunctional and multivalent catalytic platform. The arginine-derived guanidinium groups distributed around the water-compatible fullerenol scaffold were expected to create a localized basic environment and facilitate cooperative interactions with the reacting substrates. The aim was to promote dehydroalanine formation from cysteine-derived substrates under mild, biologically relevant aqueous conditions. A series of cysteine derivatives was evaluated to establish a thioester-mediated biomimetic pathway in which thioester formation was followed by conversion of the cysteine residue into the corresponding Dha derivative. Among the investigated substrates, N-acetyl-L-cysteine-N′-benzylamide enabled productive conversion in the presence of F-Arg. The catalytic system was also compared with free arginine to examine the importance of presenting multiple arginine groups on the fullerenol scaffold. Reaction progress and product identity were evaluated by HPLC and LC–MS analysis, respectively.

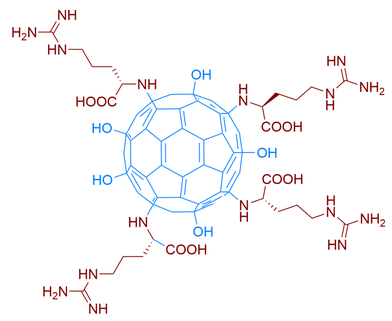


Native LarE: sulfur donation converts the active-site cysteine into dehydroalanine (Dha) and irreversibly inactivates the enzyme.

Our aim is to reproduce LarE-type sulfur chemistry with a cooperative F-Arg platform

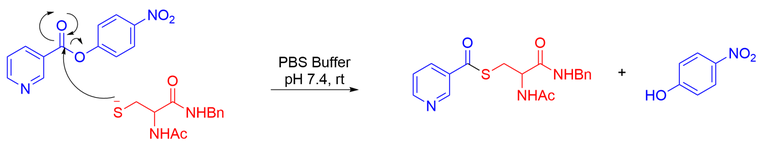
BIOMIMETIC DESIGN

F-Arg presents multiple guanidinium groups around a fullerenol core, creating a cooperative catalytic microenvironment.



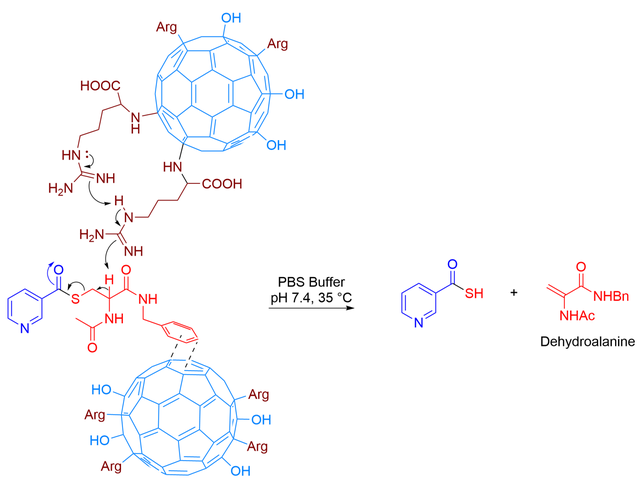
Arginine Functionalized Fullerenol
Arginine-functionalized fullerenol (F-Arg)

THIOESTER-MEDIATED PATHWAY



Substrate system: *p*-Nitrophenyl nicotinate was used as an activated nicotinic-acid-derived substrate analogue, while *N*-acetyl-L-cysteine-*N*′-benzylamide (2d) was selected as the cysteine-derived substrate. The corresponding dehydroalanine derivative is denoted 5d in the analytical data.

PROPOSED CATALYTIC MODEL



*Proposed **F**-Arg-assisted conversion of the thioester intermediate to the Dehydroalanine (Dha) product.*

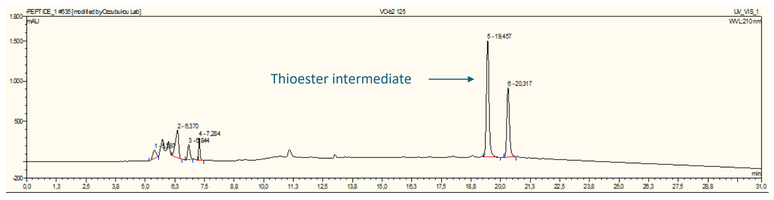
OPTIMIZED SEQUENCE

1. Pre-form the thioester in PBS (pH 7.4) for 1 h at room temperature.
2. Add F-Arg and incubate the reaction at 35 °C.
3. Withdraw timed aliquots and quench by acidification.
4. Quantify Dha formation by HPLC and confirm product identity by LC–MS.

RESULTS · HPLC AND LC–MS

STEP 1 Thioester Preformation

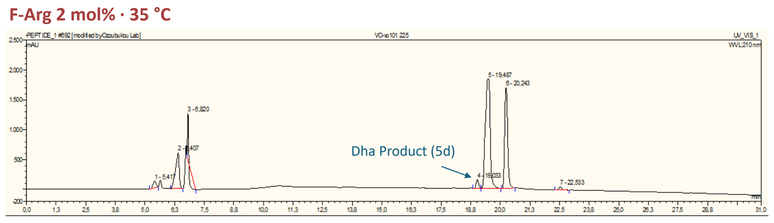
PBS, pH 7.4 · room temperature · 1 h · before F-Arg addition



Thioester formation was observed, with no detectable Dha product.

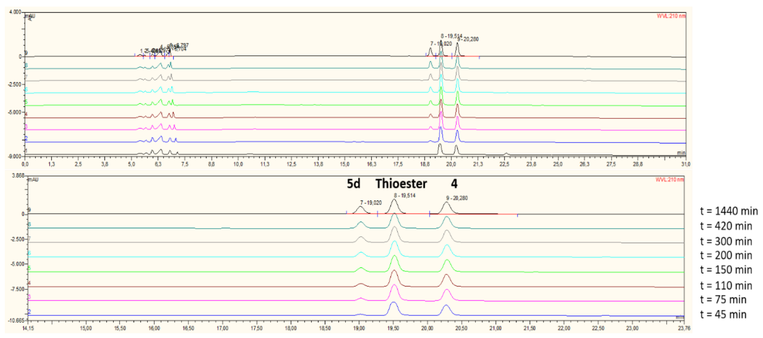
STEP 2 F-Arg-mediated Dha formation

F-Arg 2 mol% · 35 °C



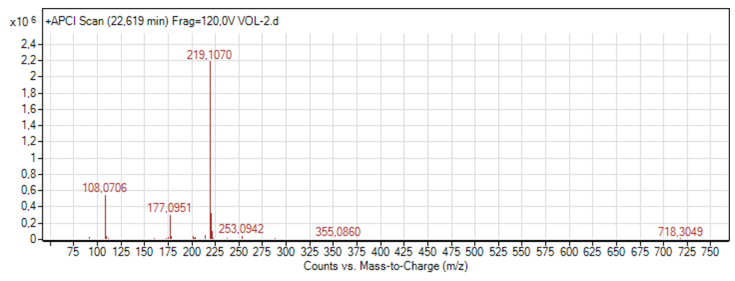
*A new peak assigned to Dha product 5d appeared after **F**-Arg addition.*

TIME-DEPENDENT PRODUCT FORMATION



The peak assigned to 5d increased progressively with reaction time.

LC–MS PRODUCT CONFIRMATION



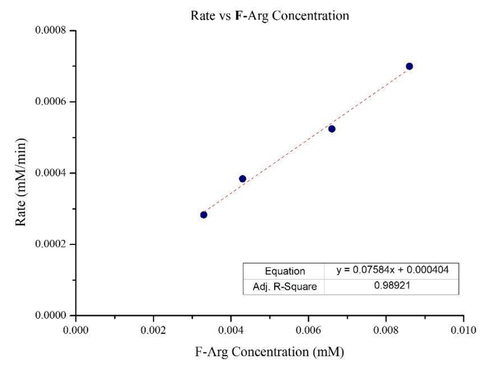
Dha product 5d: observed m/z 219.1070; calculated m/z 219.1089 for $[M+H]^+$.

CONTROLS ESTABLISH CATALYTIC ACTIVITY

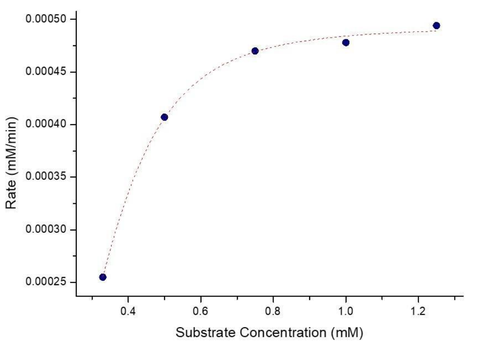
No F-Arg	**No Dha**
Free Arg · catalytic loading	**No Dha**
Free Arg · stoichiometric	**Dha formed**
F-Arg · catalytic loading	**Dha formed**

F-Arg promotes Dha formation at catalytic loading, whereas free arginine requires a stoichiometric amount. The controls support a cooperative effect of the multivalent F-Arg platform.

KINETIC CHARACTERIZATION



Linear catalyst dependence (R^2 = 0.989)



Substrate saturation is consistent with Michaelis–Menten-like behavior.

k_{cat} :	K_M:	k_{cat}/K_M:
0.0758 min^{-1}	0.33 mM	0.2297 $mM^{-1}\,min^{-1}$

K_M is a preliminary estimate; additional low-substrate points would improve precision.

CONCLUSIONS

- Screening of cysteine derivatives identified *N*-acetyl-L-cysteine-*N*′-benzylamide as a suitable substrate for the thioester-mediated formation of the corresponding Dha derivative.
- Under the optimized conditions (PBS, pH 7.4, 35 °C), Dha formation was observed by HPLC and the product identity was confirmed by LC–MS. No detectable Dha formation occurred in the absence of F-Arg.
- Free arginine required a stoichiometric amount to induce conversion, whereas F-Arg promoted Dha formation at catalytic loading. This difference supports a cooperative effect arising from the multivalent **F**-Arg platform.
- Reaction rate increased linearly with **F**-Arg concentration, giving k_{cat} = 0.0758 min^{-1}. Substrate-concentration analysis showed Michaelis–Menten-like saturation behavior and provided a preliminary K_M estimate of 0.33 mM.

KEY FINDING

The multivalent F-Arg platform provides a cooperative catalytic microenvironment that promotes LarE-inspired dehydroalanine formation under mild aqueous conditions. This study extends the fullerenzyme concept beyond hydrolase mimicry to sulfur-transfer chemistry.

ACKNOWLEDGEMENT

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REFERENCES

1. Yin, Y.; Dong, Z.; Luo, Q.; Liu, J. *Prog. Polym. Sci.* **2012**, *37*, 1476–1509.
2. Desguin, B.; Soumillion, P.; Hols, P.; Hausinger, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113*, 5598–5603.
3. Fellner, M.; Desguin, B.; Hausinger, R. P.; Hu, J. *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 9074–9079.
4. Gülseren, G.; Saylam, A.; Marion, A.; Özçubukçu, S. *ACS Appl. Mater. Interfaces* **2021**, *13*, 45854–45863.