F-*O*-G Ring Formation in Glycopeptide Antibiotic Biosynthesis is Catalysed by OxyE

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Supplementary Information

SI Table 1. Results of OxyE and coupled OxyB/OxyE turnover reactions

Oxy enzymes	peptide	carrier	OxyB activity [%][a]	OxyE activity [%][a]
OxyE	T7P(D/L-Hpg$_7$)	PCP	-	4 ± 1
OxyB/OxyE	T7P(D/L-Hpg$_7$)	PCP	31 ± 1	1 ± 1
OxyE	T7P(D/L-Hpg$_7$)	PCP-X	-	5 ± 1
OxyB/ OxyE	T7P(D/L-Hpg$_7$)	PCP-X	71 ± 2	42 ± 1
OxyB/ OxyE	T7P(L-Hpg$_7$)	PCP-X	70 ± 3	72 ± 1
OxyB/ OxyE	T7P(D-Hpg$_7$)	PCP-X	82 ± 1	57 ± 1

[a] The Oxy activities expressed as the percentage of cyclized peptide relative to the respective substrate. Results are obtained from triplicate experiments, ± standard deviation.

SI Table 2. Results of OxyB/OxyE competition experiments

Reaction[a]	OxyB activity [%][b]
1 (OxyB only)	56 ± 7 [1]
2 (OxyB only)	51 ± 4 [1]
1	37 ± 5
2	28 ± 4
3	22 ± 5

[a] Assignment of the reactions according to Figure 6; reaction 1 addition of OxyB before OxyE, reaction 2 simultaneous addition of OxyB and OxyE and reaction 3 addition of OxyE before OxyB.

[b] Data are expressed as the percentage of the monocyclic peptide relative to the total amount of detected peptide. Results obtained from triplicate experiments, ± standard deviation.

References

1 Peschke, M., Haslinger, K., Brieke, C., Reinstein, J. & Cryle, M. Regulation of the P450 oxygenation cascade involved in glycopeptide antibiotic biosynthesis. *J. Am. Chem. Soc.* **138**, 6746-6753 (2016).