Mechanistic Investigation of the Dehydration Reaction of Lacticin 481 Synthetase by Using Site-Directed Mutagenesis

Supporting Information

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Table S1. Primer names and sequences used for site-directed mutagenesis of LctM.

Primer name	Sequences of primers
K159MFP	5` TTA ATT TAT ATG CCA AAG TCA TTA AGT AAT GAT GTG 3`
K159MRP	5` CAC ATC ATT ACT TAA TGA CTT TGG CAT ATA AAT TAA 3`
D242NFP	5’ AT ACT TTA AAT TTA ACT AAC TTA CAT TTT GAA AAT GTG 3’
D242NRP	5’ CAC ATT TTC AAA ATG TAA GTT AGT TAA ATT TAA AGT AT 3’
H244NFP	5` TTA ACT GAC TTA AAT TTT GAA AAT GTG ATC TCA C 3`
H244NRP	5` G TGA GAT CAC ATT TTC AAA ATT TAA GTC AGT TAA 3`
N247LFP	5’ GAC TTA CAT TTT GAA CTA GTG ATC TCA CAA GGA 3’
N247LRP	5’ TCC TTG TGA GAT CAC TAG TTC AAA ATG TAA GTC 3’
D259NFP	5’ CCT TGT ATT ATT AAC CTA GAG ACT ATG TTT AAC 3’
D259NRP	5’ GTT AAA CAT AGT CTC TAG GTT AAT AAT ACA AGG 3’
E261QFP	5’ CCT TGT ATT ATT GAC CTG CAG ACT ATG TTT AAC ATG CC 3’
E261QRP	5’ GG CAT GTT AAA CAT AGT CTG CAG GTC AAT AAT ACA AGG 3’
R399MFP	5` AGT AGT GTT ACC TGT ATG ATA TTA TTT AGA AAT ACG A-3`
R399MRP	5` T CGT ATT TCT AAA TAA TAT CAT ACA GGT AAC ACT ACT-3`
R399KFP	5’ GT AGT GTT ACC TGT AAA ATA TTA T TT AGA AAT ACG 3’
R399KRP	5’ CGT ATT TCT AAA TAA TAT TTT ACA GGT AAC ACT AC 3’
R399LFP	5’ CT AGT AGT GTT ACC TGT CTG ATA TTA TTT AGA AAT ACG 3’
R399LRP	5’ CGT ATT TCT AAA TAA TAT CAG ACA GGT AAC ACT ACT AG 3’
T405AFP	5’ TGT AGA ATA TTA TTT AGA AAT GCA ATG GAA TAC TCA GTT TTA 3’
T405ARP	5’ TAA AAC TGA GTA TTC CAT TGC ATT TCT AAA TAA TAT TCT ACA 3’
E446MFP	5’ AAT GAT ATT ATT AAA TCG ATG ATA AGT CAA ATA AAC ACT 3’
E446MRP	5’ AGT GTT TAT TTG ACT TAT CAT CGA TTT AAT AAT ATC ATT 3’
Y408FFP	5’ TTA GAA ATA CGA TGG AAT TCT CAG TTT TAT TAA ATG CAG C 3’
Y408FRP	5’ G CTG CAT TTA ATA AAA CTG AGA ATT CCA TCG TAT TTC TAA 3’
Nhe I -38	5’ CAT CAC AGC AGC GGC CTG G 3’
MfeIRP	5’ TAT TGT TTC TCC ATC CAT ATT CTT TAT TAA GTT TGA G 3’

Discussion of solubility of substrate in assays.

As mentioned in the main text, the LctA substrate has poor solubility at pH 7.5. At 10 µM concentration, the peptide usually is soluble at the start of the assay but the assay mixture rapidly becomes turbid over time. To date, it has not been possible to prevent this precipitation of the substrate even when starting at lower concentrations.

Because of these solubility issues, every assay was analyzed by two methods. In method A, the assay mixture was first centrifuged, then acidified to a final concentration of 0.5 % TFA and analyzed by MALDI-MS. In method B, the assay mixture was first acidified to a final concentration of 0.5% TFA (pH ~1) resulting in solubilization of all material in the assay tube, and then assayed by MS. This protocol consistently showed that the soluble fraction of a reaction assay is enriched in dehydration and phosphorylation products whereas the insoluble fraction consists predominantly of starting peptide. An example is shown in Figure S1. Panel A shows the soluble fraction of the assay with H244N after 15 min on the left (method A) and the same sample analyzed by method B on the right. Note that the starting peptide peak is significantly larger in the right panel. It is important to note that if assays are just analyzed by looking at the soluble fraction, the reaction of the H244N mutant would have been judged complete after 15 min. However, when using method B, it clearly takes about 30 min for complete consumption by this mutant.

This protocol of analyzing the dehydration assays also revealed that whereas the starting peptide is only sparingly soluble and often results in precipitation, the insoluble peptide is consumed over time. For instance, whereas after 1 h of incubation time the E446M mutant shows large amounts of starting material by method B, after 6 h all of the starting material is consumed, regardless whether method A or B was used (panel B, Figure S1).

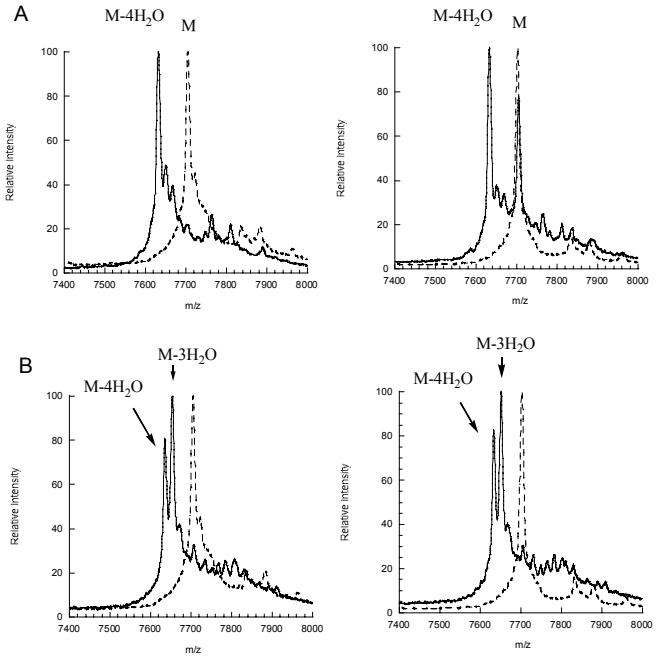

Figure S1.

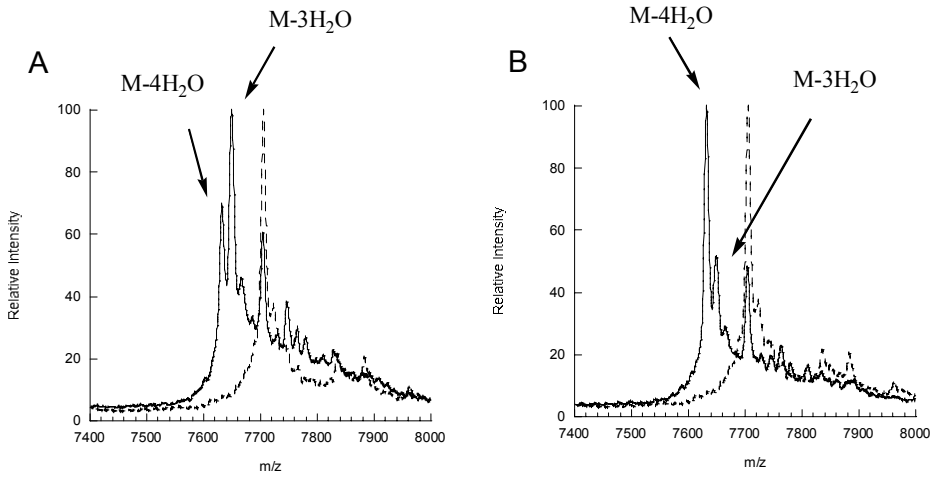

Figure S2. Dependence of the activity of E261Q-LctM on ATP and Mg^{2+} concentration. (A) 12 µM His_6-LctA was incubated with E261Q-LctM in the presence of 1 mM ATP and 10 mM $MgCl_2$ for 1 h. (B) The mutant enzyme was incubated with 12 µM His_6-LctA, 2 mM ATP, and 20 mM $MgCl_2$ for 1h.

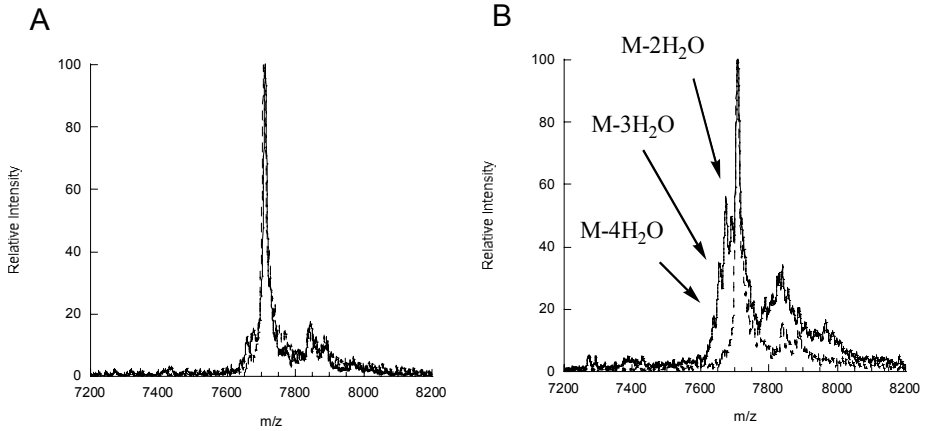

Figure S3. Incubation of wild-type His_6-LctA with N247L for 2 h (A) and for 6 h(B). All conditions as described in the Materials and Methods section.