Diverse DNA-cleaving Capacitiesof the Jadomycins through Precursor-directed Biosynthesis

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Jadomycins were isolated from cultures of *S. venezuelae* ISP5230 according to published procedures.[1,2] Purification was accomplished using a Biotage SP1 HPFC purification system using both Biotage KP-C18-HS reversed-phase and Biotage KP-SIL normal-phase columns. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE 500 or Bruker DRX 500 spectrometers in MeOD. Low resolution electrospray mass spectra were recorded by Dalhousie University Mass Spectrometry Service using a VG/Micromass Quattro triple quadrupole spectrometer. High resolution accurate mass spectra were recorded at the University of British Columbia Mass Spectrometry Service on a Water/Micromass LCT spectrometer using pentalysine as a reference compound.

Supercoiled plasmid (Form I) was a derivative of pET28a containing the *desR* gene[3] and was used in the present study due to its ready availability in-house and increased GC content.[4] Larger quantities of the plasmid were prepared by transformation of NovaBlue cells (Novagen) followed by purification using the QIAprep Spin Miniprep Kit (Qiagen) to yield approximately 62 μg of plasmid DNA per 20-mL culture. The following reagents (Aldrich) were obtained commercially and used without further purification: copper acetate ($Cu(OAc)_2$), 4-morpholinepropanesulfonic acid (MOPS), trishydroxymethylaminomethane (Tris), ethylenediaminetetraacetic acid (EDTA), ethidium bromide, ethanol (EtOH), and acetic acid.

Reaction mixtures (20-μL total volume) for DNA cleavage assays contained 400 ng Form I DNA in 10 mM MOPS buffer and 100 mM NaCl, pH 7.4. DNA (2–5 μL) was delivered to the assay tubes as a solution in 10 mM Tris-Cl (pH 8.5) and diluted with MOPS (pH 7.5, final concentration 10 mM) and NaCl (final concentration 100 mM). Solutions of jadomycin (pre-mixed 1:1 with $Cu(OAc)_2$ where appropriate) were added to give the desired concentration, and the reaction mixtures were diluted to a final volume of 20 μL, when necessary, with distilled, deionized H_2O. All jadomycins were dissolved initially in 95% EtOH except for jadomycin G, which was dissolved in distilled, deionized H_2O. Subsequent dilutions were made with distilled, deionized H_2O where final assay tubes contained $<$1% EtOH. Reaction mixtures were incubated at 37 °C for 4 hours except for the photocleavage assays, where cold samples were irradiated for 15 minutes with 313–nm excitation inside a photoreactor (Luzchem LZC-4X). All samples were quenched by the addition of gel loading buffer (4 μL), loaded onto 1% agarose gels containing ethidium bromide (0.75 μg mL^{-1}), and electrophoresed for 30 minutes at 10–12 V cm^{-1} in 1X TAE (tris-acetate-EDTA). The bands were visualized with UV illumination and quantified using the Gel Doc-It Imaging system (UVP) or GNU Image Manipulation Program (GIMP).

References

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