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Supporting Information for

Microbial Resistance to Mitomycins Involves a Redox Relay Mechanism

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Protein Purification. E_3 was purified as previously described.[1] MCRA was purified from *Streptomyces lividans* containing pDHS3000.[2] Spores were cultured in YEME medium[3] supplemented with 1 μg/mL MC for 60 h at 30 °C. Mycelia were harvested by centrifugation at 4,600 *g* for 15 min and washed with protein extraction buffer (50 mM Tris-HCl, 2 mM EDTA, 10% glycerol, pH 8.0). The cells were resuspended in four volumes 20 mM Tris-HCl, pH 7.5 (buffer A), and disrupted by sonication. Cell debris was removed by centrifugation at 12,000 *g* for 30 min. The pellet of an ammonium sulfate precipitation (70% saturation) was resuspended in buffer A and dialyzed against the same buffer before loading onto a DEAE Sepharose column (2.5 x 25 cm, Pharmacia) equilibrated with buffer A. The loaded sample was washed with 70 mL of buffer A and eluted using a gradient of 0-0.3 M NaCl buffer A (1.2 L total volume). Fractions of protein were pooled based on SDS-PAGE analysis and desalted using an Amicon ultrafiltration unit (YM-10). The concentrated sample was further purified using a FPLC Mono Q 10/10 column (Pharmacia) at 3 mL/min using the solvent system of A (20 mM Tris-HCl, pH 7.5) and B (A + 1 M NaCl) with the profile: 0% B from 0-2 min, 22% B from 2-7 min, and a linear gradient of 22% to 35% B from 7-22 min. After concentration with an Amicon unit (YM-10), size-exclusion chromatography using an FPLC Superdex HR200 column (Pharmacia) with buffer A at a flow rate of 1 mL/min was used as the last step in the purification.

Mass Spectrometry. MCRA protein (120 μg) was applied to a PLRP-S narrow bore column (1 mm x 50 mm, 4000 Å pore size, 8 μg particle size) equilibrated with 2%

acetonitrile/0.1% TFA and eluted at 40 μL/mL with a linear acetonitrile/0.1% TFA gradient (2.9%/min) employing a Michrom microbore HPLC system (Michrom BioResources, Inc.). The flow was split 1:10 delivering analyte at 4 μL/min flow rate to an Electrospray VG BioQprobe.[4] MeOH with 0.2% TFA at a flow rate of 2 μL/min was used as a sheath liquid. The nozzle to skimmer bias value was 70 V. Molecular ions were monitored within a 1150-1450 m/z range at a rate of 5.9 sec/scan. Resolution was adjusted so that a width at half height of the 1212 horse heart myoglobin peak was 1.2 Da and myoglobin was used as a mass scale calibrator. The instrument was controlled and data was analyzed using LabBase® software and deconvolution of the ES MS data to a real mass was achieved using a MaxEnt® algorithm.[5] The molecular mass obtained was 49006 Da, which agrees with the calculated mass of methionine deleted MCRA + FAD (49005 Da).

DNA Protection Assay and Analysis. Each reaction was made anaerobic by cycling all reactants except E_3 between vacuum and oxygen-scrubbed argon over a period of 10 min in air-tight glassware. The concentrated E_3 was then added under argon pressure with a Hamilton gas-tight syringe and the reaction was lightly vortexed, incubated for 15 min, and quenched by flushing with air. The reaction sample (~ 1 μg DNA) was denatured by incubation with 0.2 N NaOH for 5-10 min prior to adding the loading buffer and running the agarose gel. The DNA was pure, circular pIJ702 cloning vector isolated (5.8 kb) from *S. lividans*. Densitometric analysis of the gels was performed with NIH Image 1.60. Percent protection was calculated by dividing the area of the bottom band into the total area of the top and bottom bands. MC remaining in each reaction mixture after the incubation period was quantified by removing the protein and DNA from a 25 μL sample using a Microcon 10 concentrator (Amicon). The filtrate was directly loaded onto an Econosil C_{18} column (4.6 mm x 250 mm, 5μ) fitted to a Hewlett-Packard 1090 HPLC and separated using a linear gradient of 6%-18% CH_3CN in 0.02 M NH_4OAc, pH 5.2, over 30 min. Flow rate: 1 mL/min; detection: 217 nm and 363 nm. In-line diode-array scans during the

purification identified one peak as MC with no detectable mitosene derivatives (data not shown). The data in **B** are from multiple trials.

References for Supporting Information

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