Supporting information for Yang *et al.* (2003) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.0137017100

Supporting Methods

Crystallization and Data Collection. Native Rv2002-M3 protein was crystallized in the presence of NAD^+ into trigonal crystals and X-ray data were collected, as described (1). Selenomethionine (SeMet)-substituted Rv2002-M3 protein was crystallized in the presence of NAD^+ into tetragonal crystals, by using the reservoir solution comprising 1.5 M ammonium sulfate, 5.0% (vol/vol) polyethylene glycol 400, and 100 mM Na HEPES (pH 7.5). Each hanging drop of 4 μl volume was prepared by mixing 2 μl each of this reservoir solution and the protein solution (14.4 mg/ml protein in a buffer containing 20 mM Tris·HCl at pH 8.0, 0.1 mM phenylmethylsulfonyl fluoride, and 2 mM NAD^+). The SeMet-substituted crystal was flash-frozen using glycerol as a cryo-protectant, which was added to the reservoir solution to a final concentration of 15% (vol/vol). Multiwavelength anomalous diffraction data were collected at 100 K from a flash-frozen crystal of the SeMet-substituted protein at three different wavelengths using an Area Detector Systems Corporation Quantum 4R charge coupled device detector at the experimental station BL-18B of Photon Factory, Tsukuba, Japan. The raw data were processed and scaled with the programs MOSFLM and SCALA (2). Crystals of the ternary complex with androsterone and NADH were grown using the same method and the same reservoir solution as the SeMet-substituted crystals. Just before preparing the hanging drop, 100 μl of the protein solution (14.7 mg ml^{-1} protein in a buffer containing 20 mM Tris·HCl at pH 8.0 and 0.1 mM phenylmethylsulfonyl fluoride) was mixed with 2 μl of 200 mM androsterone solution and 2 μl of 100 mM NADH solution. Crystals of the ternary complex were very sensitive to cryo-protectants, and thus the data were collected at room temperature with Cu *K*α radiation. The raw data were processed and scaled with the program suite HKL (3). Table 2 summarizes data collection statistics, including unit cell dimensions.

Structure Determination. All of the 20 expected selenium atoms except those of N-terminal methionines in four monomers of the Rv2002-M3 protein in the

crystallographic asymmetric unit were located with the program SOLVE (4). Phases were calculated by SHARP (5) and were improved through 4-fold noncrystallographic symmetry averaging, solvent flattening, and histogram matching with the program DM (2). Model building was done with the program O (6). The model was refined with the program CNS (7) using the data collected at the remote wavelength (Table 2). Subsequently, this model was used to solve the structure of the binary complex of the native Rv2002-M3 protein with NAD^+ by the molecular replacement method with the program AMORE (8). The structure of the ternary complex with androsterone and NADH was determined similarly. Occupancies of androsterone molecules bound to the four crystallographically independent monomers were similar and were set to 0.5. R_{free} (9) was calculated for a randomly chosen set of 10% of the reflections.

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