Role of the His57-Glu214 Ionic Couple Located in the Active-Site of *Mycobacterium tuberculosis* FprA

Andrea Pennati[‡], Adelia Razeto[§], Matteo de Rosa[‡], Vittorio Pandini[‡], Maria Antonietta Vanoni[‡], Andrea Mattevi[§], Alessandro Coda[§], Alessandro Aliverti[‡] and Giuliana Zanetti[‡]

[‡]Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, via Celoria 26, 20133 Milano, Italy, and [§]Dipartimento di Genetica e Microbiologia, Università degli Studi di Pavia, Via Ferrata 1, 27100 Pavia, Italy.

SUPPORTING INFORMATION

Five pages total.

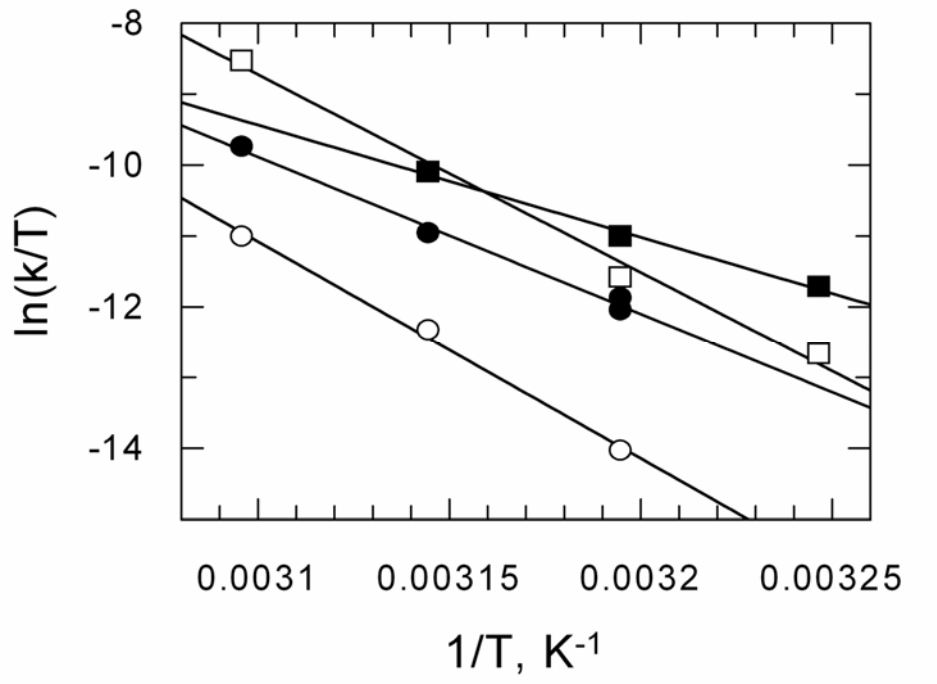

Figure 1. Thermal irreversible unfolding of FprA forms. Eyring plots of the rate constants (expressed in s^{-1}) for the inactivation of the wild-type and mutant FprA forms at different temperatures as a function of incubation temperature. Proteins (ca. 10 μM) were incubated in 10 mM HEPES-NaOH, pH 7.0, containing 100 mM NaCl and 10 % glycerol. Rate constants were determined by fitting time courses of residual enzyme activity with exponential decay equations. Open circles, FprA; open squares, FprA-E214A; filled squares, FprA-H57A; filled circles, FprA-H57Q.

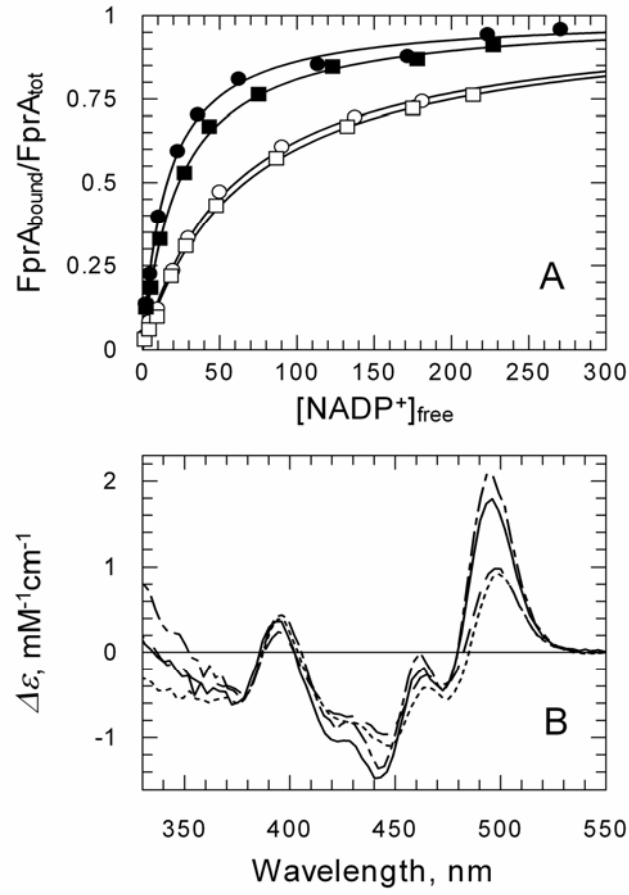

Figure 2. Titration of FprA forms with NADP$^+$. A) Titration curves obtained by successive additions of NADP$^+$ to solutions of wild-type FprA (open circles), Glu214Ala (open squares), His57Ala (filled squares), His57Gln (filled circles) FprA forms in 20 mM HEPES-NaOH, pH 7.0, containing 300 mM NaCl and 10 % glycerol. Curves represent the theoretical equations for a 1:1 protein-ligand binding model drawn using K_d and Δε parameters obtained by nonlinear fitting of experimental data as described in the Materials and Methods section. **B**) Difference spectra of FprA-NADP$^+$ complexes extrapolated at infinite NADP$^+$ concentration. Solid line, His57Gln mutant; dash-dot line, His57Ala mutant; dashed line, Glu214Ala mutant; dotted line, wild-type FprA.

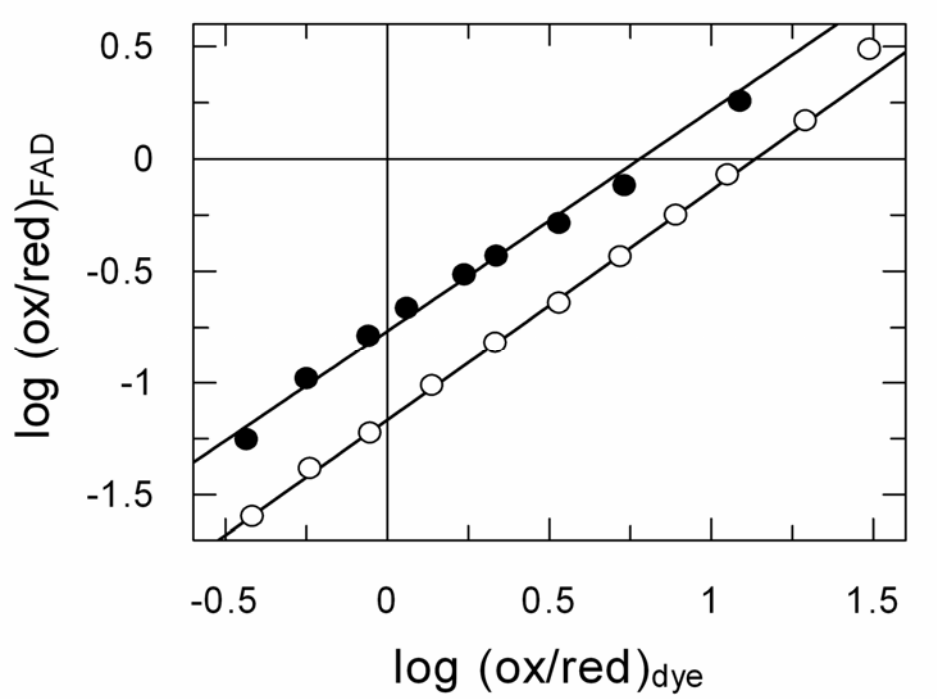

Figure 3. Determination of the redox potential of the enzyme-bound FAD of FprA and FprA-H57Q. Nernst plots of wild-type (open circles) and His57Gln (filled circles) FprA forms obtained by spectrophotometrically monitoring the degree of reduction of FAD and antraquinone-2-sulfonate during stepwise photoreductions under anaerobiosis. After each illumination period, absorbance values were registered when equilibrium conditions were reached. The slopes of the interpolating straight lines are 1.03 and 0.98 for the wild-type and the mutant enzyme, respectively.

Figure 4. Overall structure of the FprA-H57Q monomer. The FAD-binding domain is shown in red and the NADP-binding domain in green. The Gln57 side chain, the FAD and the modified $NADP^+$ (NADPO) are shown in black, yellow and magenta ball-and-stick representation, respectively. Generated with MOLSCRIPT (Kraulis, P. J. J. (1991) *Appl. Crystallogr*. *24*, 946–950).