

Electronic supplementary information

Enzyme-mediated protein refolding

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Experimental

The urease-immobilized resin was prepared as follows. A slurry of an NHS-activated Sepharose resin (1 ml) was added to 10 ml of a urease (from Jack bean) solution (1 mg/ml) in 0.05 M sodium phosphate buffer (pH 7.5), followed by stirring for 5 h at 25°C. The suspension was filtered and washed with water to remove unreacted urease. The concentration of unreacted urease in the eluent was measured by the Bradford method to determine the amount of immobilized urease. To inactivate unreacted NHS ester on the resin, 5 ml Tris(HCl) buffer (50 mM, pH 7.5) was added to the resin and mixed for 3 h at 25°C.

The hydrolysis of urea was carried out in Tris(HCl) buffer (50 mM, pH 7.5) using the immobilized urease at 25°C. Urea concentrations were determined by the diacetyl monoxime method.¹

Native carbonic anhydrase B was dissolved in a 0.05 M Tris(HCl) buffer solution (pH 7.5) containing 8 M urea, and incubated overnight at 25°C to prepare denatured CAB (10 mg/ml). The denaturation of CAB was confirmed by comparing the circular dichroism (CD) spectra of native and denatured CAB. The denatured CAB solution was diluted by the equivalent volume of a 50 mM Tris(HCl) buffer solution (pH 7.5) containing 0.4 M arginine.^{2,3} Ribonuclease A (RNaseA) and hen egg white lysozyme (10 mg) were denatured in a 0.05 M Tris(HCl) buffer solution (pH 7.5 1 ml) containing 8 M urea and 30 mM mercaptoethanol, and incubated for 5 h at 25°C. The denatured proteins were precipitated with 5 ml ice-cold acetone, harvested by centrifugation and lyophilized.⁴ The denatured protein was solubilized in an 8 M urea solution. The denatured RNaseA solution was diluted by the equivalent volume of a 50 mM Tris(HCl) solution (pH 7.5) containing 0.4 M arginine, reduced and oxidized forms of glutathione (2.5 mM and 7.5 mM, respectively). The denatured lysozyme solution was diluted by the equivalent volume

of a 0.1 M acetate buffer solution (pH 5.0) containing 8 M urea, 0.1 M arginine, reduced and oxidized forms of glutathione (10 mM and 1 mM, respectively).

The equivalent volumes of a denatured protein solution and immobilized urease suspension were mixed to yield a certain protein concentration. After gently stirring the mixture for 24 h at 25°C, ammonia, which was produced by the urea hydrolysis, was removed from the reaction mixture using a desalination column (PD-10, GE-healthcare). The protein concentration was determined by the Bradford method and the enzymatic activity of the renatured protein was measured. The refolding yields of proteins were determined by comparing the catalytic activities between native and renatured proteins under the same conditions.

The CAB activity in Tris(HCl) buffer (pH 7.5) was assayed using 1 mM *p*-nitrophenylacetate (2.9 ml) and a CAB solution (0.1 ml).⁵ The initial rate of hydrolysis of *p*-nitrophenylacetate (1 mM) was followed by monitoring the increase in absorbance at 348 nm at 25°C. The RNaseA activity assay was carried out using cytidine 2',3'-cyclic monophosphate (0.2 mg/ml) as a substrate in Tris(HCl) buffer (pH 7.5) at 25°C.⁶ The RNase A solution (0.3 ml) prepared was added to the substrate solution (2.7 ml) to start the hydrolysis reaction, followed by the measurements of the absorbance at 286 nm.

The lysozyme activity was assayed by mixing a 0.05 ml lysozyme solution with 2.95 ml *Micrococcus lysodeikticus* cell wall suspension (0.25 g/l) in 50 mM Tris(HCl) buffer at pH 7.0, and then by monitoring the decrease in absorbance at 450 nm at 25°C.

The 50-fold dilution was carried out by an diluting unfolded protein solution containing 8 M urea using a 49-fold volume of a refolding buffer at 25°C. Refolding buffer was a 50 mM Tris(HCl) buffer solution (pH 7.5) containing 0.4 M arginine for CAB, a 50 mM Tris(HCl) solution (pH 7.5) containing 0.4 M arginine, reduced and oxidized forms of glutathione (2.5 mM and 7.5 mM, respectively) for RNaseA and 50 mM Tris(HCl) solution (pH 7.5) containing 8 M urea, 0.1 M arginine, reduced and oxidized forms of glutathione (10 mM and 1 mM, respectively) for lysozyme.

CD spectra of CAB in Tris(HCl) buffer at pH 7.5 were measured using a JASCO J-725 spectropolarimeter at 25°C. The CD spectra bandwidth was set at 2 nm and the scanning speed was set to

be 10 nm/min. Cuvettes with a 10 mm path length were used. All spectra shown are the averages of 8 scans.

References for ESI

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