Supporting Information

Effects of Aromatic Thiols upon Thiol-Disulfide Interchange Reactions that Occur During Protein Folding

Todd V. DeCollo and Watson J. Lees*

Department of Chemistry, Center for Science and Technology, Syracuse University, Syracuse, NY 13244.

wjlees@syr.edu

General Remarks. NMR spectra were recorded at 300 MHz (^{1}H) and at 75 MHz (^{13}C) on a Bruker Spectrophotometer. Chemical shifts were indirectly referenced to TMS via solvent signals ($CDCl_3$, 7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C, and CD_3OD, 49.00 ppm for ^{13}C). Thin-layer chromatography (TLC) was conducted on Aldrich general purpose silica gel on polyester plates with UV indicator. Silica gel chromatography was performed with E.M. Science silica gel (230-400 mesh). Dry THF was obtained by distillation from sodium metal in the presence of benzophenone. Routine drying of organic solutions was carried out with anhydrous magnesium sulfate. All reactions performed under inert atmospheric conditions were carried out under Ar. All reagents purchased were used without purification unless otherwise noted. UV-vis spectra were recorded on a Cary 1 UV-visible spectrophotometer. SDS-PAGE was performed with a Hoefer Mighty Small II apparatus. All proteins, precast plastic gels, and molecular weight marker were purchased from Sigma. Gels were computer scanned using a digital camera and computer program. E & R Microanalytical Laboratory Inc. performed elemental analysis and the mass spectrum was obtained by the Mass Spectroscopy Laboratory at the University of Illinois at Urbana-Champaign.

4-Mercaptobenzoic Acid (1).[1-3] Light yellow crystals: mp 222 ^{0}C (lit. 219 ^{0}C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.4 Hz, 2 H, Ar H), 7.32 (d, *J* = 8.6 Hz, 2 H, Ar H), 3.64 (s, 1 H, SH); ^{13}C NMR (75 MHz, CD_3OD) δ 169.5, 141.0, 131.3, 128.9, 128.3.

Methyl 4-(*O*-Dimethylthiocarbamoyl)phenylacetate.[4,5] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.5 Hz, 2 H, Ar H), 7.03 (d, *J* = 8.5 Hz, 2 H, Ar H), 3.69 (s, 3 H, CO_2CH_3), 3.64 (s, 2 H, *CH_2*-CO_2CH_3), 3.46 (s, 3 H, *Me*-N-Me), 3.34 (s, 3 H, Me-N-*Me*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.6, 171.7, 153.0, 131.5, 130.0, 122.8, 52.0, 43.2, 40.5, 38.9.

Methyl 4-(*S*-Dimethylthiocarbamoyl)phenylacetate.[4,5] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.2 Hz, 2 H, Ar H), 7.30 (d, *J* = 8.2 Hz, 2 H, Ar H), 3.68 (s, 3 H, CH_2-CO_2*Me*), 3.63 (s, 2 H, *CH_2*-CO_2CH_3), 3.05 (br s, 6 H, N-Me_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 166.8, 135.8, 135.0, 129.8, 127.5, 52.1, 40.9, 36.8.

4-Mercaptobenzeneacetic Acid (2).[4,5] Pale yellow crystals: mp 98 ^{0}C (lit. 97-100 ^{0}C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J* = 6.7 Hz, 2 H, Ar H), 7.16 (d, *J* = 6.6 Hz, 2 H, Ar H), 3.61 (s, 2 H, -*CH_2*-CO_2H), 3.43 (s, 1 H, SH); ^{13}C NMR (75 MHz, CD_3OD) δ 176.5, 130.8, 130.1, 129.9, 129.8, 40.2; HRMS (EI) calcd for $C_8H_8O_2S$ 168.0245, found 168.0243.

***O*-4-Cyanophenyl *N,N*-Dimethylthiocarbamate.**[6,7] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.7 Hz, 2 H, Ar H), 7.19 (d, *J* = 8.9 Hz, 2 H, Ar H), 3.46 (s, 3 H, *Me*-N-Me), 3.36 (s, 3 H, Me-N-*Me*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.7, 157.0, 133.3, 124.1, 118.3, 109.8, 43.3, 38.9.

***S*-4-Cyanophenyl *N,N*-Dimethylthiocarbamate**.Krishnamurthy, 1989 #24; Sheley, 1978 #36] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.6 Hz, 2 H, Ar H), 7.61 (d, *J* = 8.6 Hz, 2 H, Ar H), 3.10 (br s, 3 H, *Me*-N-Me), 3.05 (br s, 3 H, Me-N-*Me*).

4-Mercaptobenzonitrile (3).[6,7] mp 48 ^{0}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.6 Hz, 2 H, Ar H), 7.32 (d, *J* = 8.7 Hz, 2 H, Ar H), 3.67 (s, 1 H, SH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.1, 132.4, 128.7, 118.5, 108.7.

***O*-4-Acetylphenyl *N,N*-Dimethylthiocarbamate.**[3] A 500-mL flask equipped with a stirring bar was charged with 4-hydroxyacetophenone (14.2 g, 0.104 mol), DABCO (29.2 g, 0.260 mol), and 120 mL of DMF. Once all of the solid had dissolved, *N,N*-dimethylthiocarbamoylchloride (16.0 g, 0.129 mol) was added. After 1.5 h at 65 ^{0}C, the reaction mixture was poured onto crushed ice and acidified to pH 3 with 6.0 N HCl. The white precipitate that formed was collected and dried. Recrystallization from ethanol provided 20.92 g (91%) of *O*-4-acetylphenyl *N,N*-dimethylthiocarbamate: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.6 Hz, 2 H, Ar H), 7.17 (d, *J* = 8.8 Hz, 2 H, Ar H), 3.46 (s, 3 H, *Me*-N-Me), 3.37 (s, 3 H, Me-N-*Me*), 2.61 (s, 3 H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.7, 186.6, 157.3, 134.4, 129.5, 122.9, 43.1, 38.7, 26.5.

***S*-4-Acetylphenyl *N,N*-Dimethylthiocarbamate.** In a 500-mL flask, *O*-4-acetylphenyl *N,N*-dimethylthiocarbamate (20.9 g, 0.094 mol) was heated over a temperature range of 210-240 ^{0}C under Ar for 2.5 h, yielding a single, clean, rearranged product, *S*-4-acetylphenyl *N,N*-dimethylthiocarbamate. Upon cooling of the reaction mixture, the product crystallized in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.4 Hz, 2 H, Ar H), 7.60 (d, *J* = 8.5 Hz, 2 H, Ar H), 3.09 (br s, 3 H, *Me*-N-

Me), 3.06 (br s, 3 H, Me-N-*Me*), 2.61 (s, 3 H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.5, 165.6, 137.0, 135.2, 134.9, 128.4, 36.9, 26.6.

1-(4-Mercaptophenyl)ethanone (4). *S*-4-acetylphenyl *N,N*-dimethylthiocarbamate (11.0 g, 0.049 mol) was added to a solution of KOH (6.0 g, 0.107 mol) in 25 mL of MeOH and 62.5 mL of THF. This mixture was then stirred for 3 h. Following evaporation of the MeOH, the residue was poured onto crushed ice, acidified with 2.0 N HCl to pH 2 and extracted with EtOAc (3 x 100 mL). The organic layer was washed with brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo to give a brown oil. Recrystallization from ethanol provided a dark yellow solid that melted when warmed to room temperature. This liquid was further purified by distillation (90 ^{0}C, 0.5 mmHg) to afford 5.5 g (73%) of 1-(4-mercaptophenyl)ethanone as a light yellow liquid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 8.5 Hz, 2 H, Ar H), 7.30 (d, J = 8.4 Hz, 2 H, Ar H), 3.62 (s, 1 H, SH), 2.56 (s, 3 H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.6, 138.6, 133.7, 128.7, 127.8, 26.0; Anal. Calcd for C_8H_8OS: C, 63.13; H, 5.30; S, 21.07. Found: C, 63.32; H, 5.01; S, 21.19.

4-Mercaptobenzenemethanol (5).[8,9] A solution of 4-mercaptobenzoic acid (9.07 g, 0.059 mol) in 50 mL of THF was added dropwise over 2 h to a 250-mL flask containing lithium aluminum hydride (11.0 g, 0.290 mol), 50 mL of THF, and a stirring bar. After 14 h, the reaction was quenched by the dropwise addition of 2 mL of H_2O. The mixture was then acidified to pH 1 with 1 N HCl and extracted with diethyl ether (3 x 50 mL). The organic layer was washed with H_2O (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Recrystallization from hexane followed by sublimation yielded 4.20 g (51%) of 4-mercaptobenzenemethanol as a white powder: mp 51.5 ^{0}C (lit. 51- 52

^{0}C)[8]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.4 Hz, 2 H, Ar H), 7.21 (d, *J* = 8.4 Hz, 2 H, Ar H), 4.60 (s, 2 H, CH_2-OH), 3.64 (s, 1 H, SH), 1.94 (s, 1 H, CH_2OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 129.9, 129.6, 127.8, 64.7.

SDS-PAGE of the Reduction of Insulin. Insulin (300 μL of a 10 mg/mL stock solution) was diluted with 900 μL of aqueous buffer (pH 6.5, 0.10 M in potassium phosphate, 2 mM EDTA) to form 1.2 mL of a dilute insulin solution (2.5 mg/mL, 0.418 mM). Four samples containing 100 μL of the dilute insulin solution, 50 μL of a solution of **7** (20.0 mM in buffer) and 50 μL of a DTT solution (20.0 mM in buffer) were prepared. The first three samples were quenched with 100 μL of a iodoacetamide (100.0 mM in buffer) solution at 10, 25, and 60 min respectively. The last sample was not quenched. Four samples containing 100 μL of the dilute insulin solution, 50 μL of buffer, and 50 μL of the DTT solution were prepared and quenched with iodoacetamide as before. All eight samples were then denatured and loaded onto a Tricine-Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Gel.[10] The 10 cm X 10 cm precast gel was run for 1 h at 30 V (constant voltage) followed by 2.5 h at 75 V (constant voltage). The gel was stained using Coomassie Brilliant Blue R 250 and scanned.

Qualitative validation of the light scattering assay was provided by sodium dodecyl sulfate polyacrylamide gel electrophoresis**,** SDS-PAGE. Aromatic thiol **6** was chosen due to its enhanced rate of reduction relative to other aromatic thiols used in the light-scattering assay. The gel indicated that between 10 and 25 min, the reaction containing DTT and **6** had produced as much β-chain as the reaction containing only DTT at 60 min (Figure 4).

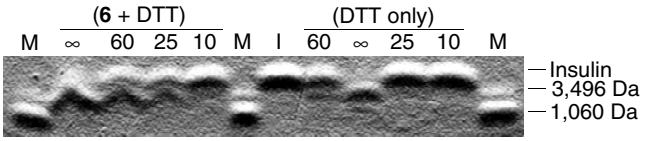

Figure 4. The tricine-SDS-PAGE gel used to follow the reduction of insulin by DTT in the presence or absence of **6**. To achieve greater contrast the gel is shown in relief where the light areas represent protein and the black areas are shadows. Lanes marked M contained molecular weight markers of 1,060 Da and 3,496 Da. The molecular weight marker at 3,496 Da is the β-chain of insulin. The lane marked I contained insulin. Lanes marked with numbers specify the length of time before the reaction mixture was quenched. Lanes marked infinity contained reaction mixtures that were never quenched.

References

(1) Okuno, H. Y.; Uoto, K.; Tomohira, T.; Youinov, M.-T. *J. Chem. Soc. Dalton Trans.* **1990**, 3375.

(2) Chen, K.-Y.; Gorman, C. B. *J. Org. Chem.* **1996**, *61*, 9229-35.

(3) Newman, M. S.; Karnes, H. A. *J. Org. Chem.* **1966**, *31*, 3980-4.

(4) Aultz, D. E.; Helsley, G. C.; Hoffman, D.; McFadden, A. R.; Lassman, H. B.; Wilker, J. C. *J. Med. Chem.* **1977**, *20*, 66-70.

(5) Tagawa, H.; Ueno, K. *Chem. Pharm. Bull.* **1978**, *26*, 1384-93.

(6) Krishnamurthy, S.; Aimino, D. *J. Org. Chem.* **1989**, *54*, 4458-62.

(7) Sheley, C. F. *Mol. Cryst. Liq. Cryst.* **1978**, *44*, 193-5.

(8) Itoh, T.; Gotoh, K.; Ishikawa, N.; Hamaguchi, T.; Kubo, M. *J. Org. Chem.* **1996**, *61*, 1867-9.

(9) Grice, R.; Owen, L. N. *J. Chem. Soc.* **1963**, 1947-54.

(10) Schaegger, H.; Von Jagow, G. *Anal. Biochem.* **1987**, *166*, 368-79.