A Decolorized Analog of Coomassie Brilliant Blue for Electrophoreses

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Background

Polyacrylamide gel electrophoresis (PAGE) is commonly used to analyze complex mixtures of proteins and effects separations of proteins primarily according to their size, but also based on their charge. Clear-native PAGE[1] relies on a protein's intrinsic charge, while blue-native PAGE (BN-PAGE) uses a dye to improve the charge characteristics of proteins such that they run on gels in narrower, more defined bands.[2] This is because the dyes generally used, of the brilliant blue or Coomassie family, form complexes with proteins and improve their charge characteristics.[3] These dyes irreversibly color the gel-bound proteins a deep blue, which is excellent for ready eye detection, but renders further analyses of the proteins in the gel, such as those using advanced fluorescence techniques,[4] very difficult. A useful novel reagent might be one that favorably alters protein charge characteristics for gel studies but does not impart the deep blue color to the gel-bound proteins. Since the Coomassie dyes are used so extensively and their protein binding properties are understood more than most other dyes, it seemed reasonable to try to generate a minimally modified colorless Coomassie analog.

Chemistry

The structures of Coomassie Brilliant Blue G-250 and R-250 dye analogs are shown in Figure 1. It was decided to chemically reduce the quinone-like structure (shown in red circles) in one of the triphenylmethyl rings such that generation of the third aromatic phenyl group would break the hyper-conjugated unsaturated chain that gives rise to the intense blue colors of the dyes. Such an analog would fulfill the need for a colorless dye, minimally altered, which might have the best possibility of retaining the original and advantageous protein binding characteristics of the blue Coomassie dye.

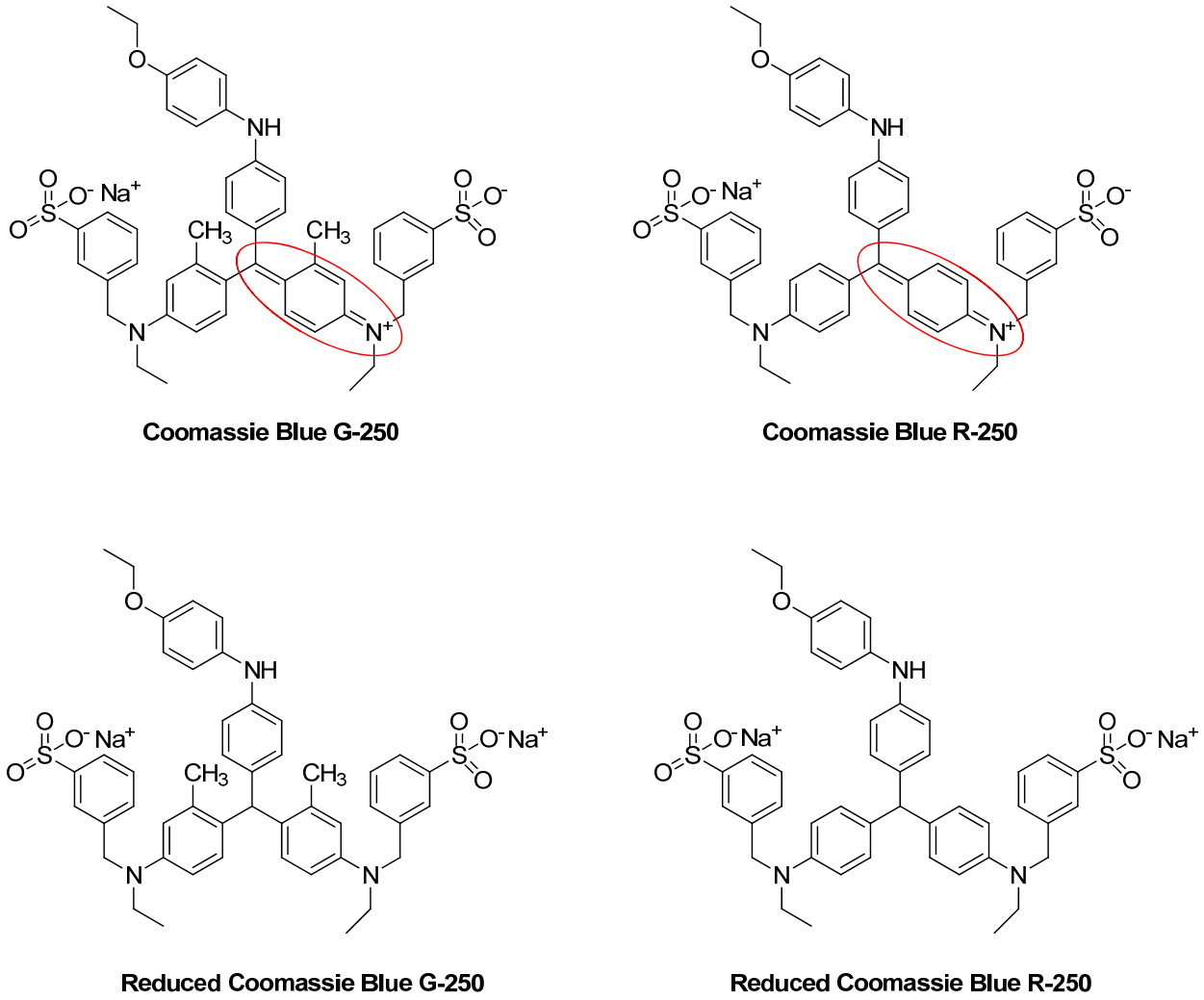

Figure 1. The structures of Coomassie G-250, R-250 and their reduced congeners.

Experimental

General. Solvents and reagents were obtained from commercial sources and used as received. Coomassie Briliant Blue G-250 was purchased from Pierce and Coomassie Brilliant Blue R-250 was purchased from Bio-Rad. Flash chromatography was performed using an Analogix Intelliflash Workstation and SuperFlash Sepra Si 50 SF40-80NP columns. The samples were each evaporated on 9 grams of silica and dry-loaded with an Analogix DASI (Dissolve/Absorb/Sample/Injection). ^{1}H NMR spectra were acquired using a Varian spectrometer operating at 400 mhz. Chemical shifts are reported in parts per million (δ) and referenced to tetramethylsilane (TMS). APCI mass spectrometry (APCI-MS) was performed on a LC/MSD Trap XCT by Agilent Technologies.

Reduced Coomassie Brilliant Blue G-250. Coomassie Brilliant Blue G-250 (2.44 g, electrophoresis purity) was dissolved in MeOH (80 mL) and held under an argon atmosphere. To this solution was added sodium cyanoborohydride (185 mg, 2.94 mmol) and the reaction was stirred for 1.5 h. During this time the color changed from a thick dark blue solution to a thin light green. TLC indicated a completed reaction. Reduced G-250 purification: 0% MeOH (100% CH_2Cl_2) 2 min; to 20% MeOH over 10 min; 20% for 10 min; to 33% MeOH over 10 min; to 50% MeOH over 20 min on a SuperFlash Sepra Si 50 SF40-80NP column, with a flow rate of 85 mL/min. The fraction eluting at 25-33 min was collected and combined, and shown to have an R_F of 0.29 on Silica Gel-60 TLC plates run in 3:1 dichloromethane:methanol. After evaporation of solvent and subsequent lyophilization, the product was a light green solid (1.05 grams, 43% yield). The product was stored under nitrogen. ^{1}H NMR (CD_3OD, 360 MHz) δ H NMR (CD_3OD, 360 MHz) δ 7.80-7.69 (m, 4H), 7.37-7.30 (m, 4H), 7.02-6.99 (m, 2H), 6.84-6.76 (m, 6H), 6.54-6.42 (m, 6H), 5.36 (s, 1H), 4.50 (s, 4H), 3.98 (q, 2H, *J* = 6.8 Hz), 3.45 (m, 4H), 2.04 (s, 6H), 1.34 (t, 3H, *J* = 7.0 Hz), 1.16 (t, 6H, *J* = 6.8 Hz). MS (m/z): 832.2 $[M-2Na+H]^-$; 854.3 $[M-Na]^-$.

Reduced Coomassie Brilliant Blue R-250. Coomassie Brilliant Blue R-250 (2.68 g, electrophoresis purity) was dissolved in MeOH (60 mL) and held under an argon atmosphere. To this solution was added sodium cyanoborohydride (185 mg, 2.94 mmol) and the reaction was stirred for 2.5 h. During this time the color changed from a thick dark blue to a thin reddish brown. TLC indicated a completed reaction. Reduced R-250 dye purification: 0% MeOH (100% CH_2Cl_2) 2 min; to 25% MeOH over 5 min; 25% for 5 min; to 35% MeOH over 5 min; 35% MeOH for 15 min on a SuperFlash Sepra Si 50 SF40-80NP column, with a flow rate of 85 mL/min. The fraction eluting at 17-21 min was collected and combined, and shown to have an R_F of 0.23 on Silica Gel-60 TLC plates run in 3:1 dichloromethane:methanol. After evaporation of solvent and subsequent lyophilization, the product was a light gray solid (1.76 grams, 64% yield). ^{1}H NMR (CD_3OD, 360 MHz) δ 7.81-7.66 (m, 4H), 7.38-7.29 (m, 4H), 7.02-6.57 (m, 16H), 5.14 (s, 1H), 4.50 (s, 4H), 3.96 (q, 2H, *J* = 6.9 Hz), 3.45 (q, 4H, *J* = 6.8 Hz), 1.34 (t, 3H, *J* = 6.8 Hz), 1.16 (t, 6H, *J* = 6.9 Hz). MS (m/z): 804.3 $[M+H-2Na]^-$.

Stability Note. In preliminary studies in gels, reduced R-250 appeared to auto-reoxidize. Based on repeated mass spectroscopic, proton NMR and gel performance studies, reduced G-250 was found to be stable in gels and during storage (12 months at -80°C under nitrogen). Some apparent re-oxidation was observed when reduced G-250 was stored at ambient conditions as a powder.

Spectral Data for Coomassie G-250. ^{1}H NMR (CD_3OD, 360 MHz) δ 7.81-7.73 (m, 3H), 7.44-7.21 (m, 8H), 7.01-6.96 (m, 5H), 6.80-6.75 (m, 4H), 4.86-4.82 (m, 4H), 4.04 (q, 2H, *J* = 7.0 Hz), 3.74-3.67 (m, 4H), 3.34 (m, 2H), 1.83 (s, 6H), 1.39 (t, 3H, *J* = 7.0 Hz), 1.30 (t, 6H, *J* = 6.8 Hz). MS (m/z): 830.2 $[M-Na]^-$; 876.0 $[M+Na]^+$.

Spectral Data for Coomassie R-250. ^{1}H NMR (CD_3OD, 360 MHz) δ 7.95-6.58 (m, 22H), 4.85 (m, 4H), 4.03 (m, 2H), 3.78-3.70 (m, 4H), 3.30 (m, 2H), 1.38 (t, 3H, *J* = 6.4 Hz), 1.36-1.27 (m, 6H). MS (m/z): 802.5 $[M-Na]^-$.

References

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