

FIGURE 3: EPR spectra at 20 K of photoreduced SiR-HP. Anaerobic solutions containing 210  $\mu$ M SiR-HP, 40  $\mu$ M Dfl, and 10 mM EDTA in standard buffer were illuminated in anaerobic EPR tubes for times between 0 and 100 min. The extent of reduction in each tube was determined from the optical spectrum as described in the text. EPR spectra were recorded at microwave frequency 9.12 GHz, 50-mW microwave power, and at a temperature of 20 K. The high-field portion of the spectra was recorded by using an instrument gain 3.15 times that used for the low-field portion. The high-field portion of the spectra for the 1.51-, 1.69-, and 2.00-electron-reduced samples has been displaced slightly upward for the sake of clarity. The bottom magnified spectrum of the low-field region in the 2.00 electron reduced enzyme sample was run at 200-mW power at a relative gain of 32.