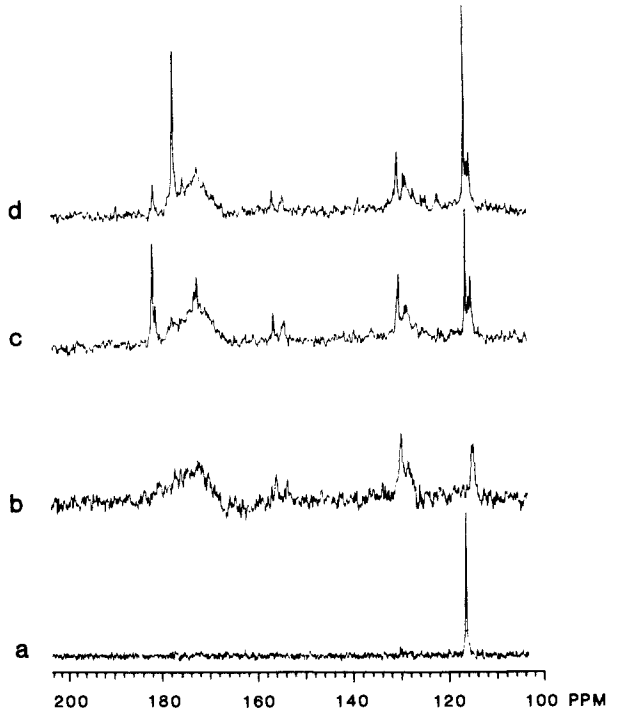

Figure 1. ^{13}C NMR spectra. Samples were prepared in 10-mm NMR tubes in 50 mM phosphate buffer, pH 6.20, containing 1% MeOH and 50% D_2O. The pH of all samples was checked before and after spectral acquisition and did not change. All spectra were recorded at 15 °C by using a Varian XL-300 spectrometer. Broad-band decoupling was performed in all experiments using the Waltz-16 program provided by Varian (1.0-W power). A pulse width of 5.0 μs and a pulse delay of 0.0 s were used with an acquisition time of 0.750 s. Methanol was used as the internal reference (49.00 ppm). The concentrations of papain given are based on active site titration.[19,20] (a) 1.0 mM Ac-L-PheGly^{13}CN (800 transients). (b) 1.0 mM papain (43 000 transients). After recording this spectrum Ac-L-PheGly^{13}CN was added (1.5 mM) and the spectrum shown in (c) was recorded (50 000 transients). The sample was next acidified with 1% v/v glacial acetic acid (pH 4.05), 2,2'-dipyridyl disulfide (6 mM) was added, and the spectrum shown in (d) was recorded (39 800 transients; 179 ppm = HOAc).

Scheme I

derives from the *reversible* addition of the active site sulfhydryl of papain to the ^{13}C nitrile carbon, the sample used to record spectrum (c) was acidified and treated with the thiol reagent 2,2'-dipyridyl disulfide to trap free E-SH covalently.[8,19] This resulted in the rapid (i.e., within 10 min) and complete disappearance of the 182.08 ppm peak with concomitant growth in intensity of the peak due to free inhibitor (spectrum d).

The above experiments clearly indicate that **1b** and papain interact via reversible formation of a covalent adduct involving the nitrile carbon. Since the nitrile carbon in **1b** corresponds directly with the carbonyl carbon of typical papain substrates (e.g. **1c**), the adduct formed is most likely the thioimidate depicted in Scheme I. This is completely consistent with the known chemical reactivity of nitriles and thioimidate esters. It is surprising, however, that the thioimidate adduct fails to yield hydrolysis products, since it is a close analogue of the acyl enzyme intermediate formed during turnover of normal substrates by papain.

Acknowledgment. Financial support for this research was provided by the University of Kansas, NIH Predoctoral Training Grant GM-07775, and a grant from the NIH Shared Instrumentation Program (RR-413). We also appreciate helpful suggestions from Dr. Grover Everett and the assistance of Dr. Charles Decedue in the purification of papain.

Characterization of [Dimethyl *N,N'*-ethylenebis(L-cysteinato)(2-)-*S,S'*]copper(II), $Cu(SCH_2CH(CO_2CH_3)NHCH_2-)_2$, a Stable Cu(II)-Aliphatic Dithiolate

Parimal K. Bharadwaj, Joseph A. Potenza,* and Harvey J. Schugar*

Department of Chemistry, Rutgers The State University of New Jersey New Brunswick, New Jersey 08903

Received October 8, 1985

We describe here the first stable Cu(II) complex (**1**) that incorporates "biological" S(cys) ligation. Except for Cu(tet *b*)-$SCH_2CH_2CO_2$ (**2**),[1] stable Cu(II) aliphatic thiolates have been limited to type 1 proteins[2] and possibly the Cu_A site in cytochrome *c* oxidase.[3-7] From X-ray absorption edge,[8,9] EXAFS,[10] EPR, and ENDOR studies, Chan et al.[3] suggested that the Cu_A site is a pseudotetrahedral $CuN_2(his)S_2(cys)$ unit with considerable Cu(I)-thiyl radical[11] or unusually covalent Cu(II)-thiolate character.[5] The large covalency of Cu(II)-thiolate bonding recently has been evaluated.[12]

A solution of *N,N'*-ethylenebis(L-cysteine)[13] (3 g) in 100 mL of dry methanol (saturated with HCl(g) at 268 K) was heated to 318 K for 10 h. The resulting diester dihydrochloride was isolated (2.9 g) after the solution was reduced to 50 mL and cooled to 298 K. A suspension of the dihydrochloride in dry ether was treated with NH_3(g) for 0.5 h, NH_4Cl was removed, and the solvent evaporated to yield the free ester. The title complex deposited as aggregated red-brown plates (≈90% yield) from an argon-purged solution of 0.2 mM of ester and 0.2 mM of Cu(tet *a*)·$2ClO_4$[14] in 10 mL of DMF/MeOH/H_2O (5:1:1). This ligand-exchange reaction depends on the Cu(II) starting material.[16]

Due to the importance of the structure, a data set was collected on the only apparently single plate even though twinning was

(1) John, E.; Bharadwaj, P. K.; Potenza, J. A.; Schugar, H. J., unpublished results.
(2) (a) Colman, P. M.; Freeman, H. C.; Guss, J. M.; Murata, M.; Norris, V. A.; Ramshaw, J. A. M.; Venkatappa, M. P. *Nature (London)* **1978**, *272*, 319. (b) Adman, E. T.; Steinkamp, R. E.; Sieker, L. C.; Jensen, L. H. *J. Mol. Biol.* **1978**, *123*, 35. (c) Maret, W.; Dietrich, H.; Ruf, H.-H.; Zeppezauer, M. *J. Inorg. Biochem.* **1980**, *12*, 241. (d) Solomon, E. I.; Hare, J. W.; Dooley, D. M.; Dawson, J. H.; Stephens, P. J.; Gray, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 168. (e) Dooley, D. M.; Rawlings, J.; Dawson, J. H.; Stephens, P. J.; Andreasson, L.-E.; Malmstrom, B. G.; Gray, H. B. *J. Am. Chem. Soc.* **1979**, *101*, 5038. (f) Dawson, J. H.; Dooley, D. M.; Clark, R.; Stephens, P. J.; Gray, H. B.; *J. Am. Chem. Soc.* **1979**, *101*, 5046.
(3) Stevens, T. H.; Martin, C. T.; Wang, H.; Brudvig, G. W.; Scholes, C. P.; Chan, S. I. *J. Biol. Chem.* **1982**, *257*, 12106 and references cited therein. Brudvig, G. W.; Blair, D. F.; Chan, S. I. *J. Biol. Chem.* **1984**, *259*, 11001.
(4) Froncisz, W.; Scholes, C. P.; Hyde, J. S.; Wei, Y.-H.; King, T. E.; Shaw, R. W.; Beinert, H. *J. Biol. Chem.* **1979**, *254*, 7482.
(5) Hoffman, B. N.; Roberts, J. E.; Swanson, M.; Speck, S. H.; Margoliash, E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1452.
(6) Aasa, R.; Albracht, S. P. J.; Falk, K.-E.; Lanne, B.; Vanngard, T. *Biochim. Biophys. Acta* **1976**, *422*, 260.
(7) Greenaway, F. T.; Chan, S. H. P.; Vincow, G. *Biochim. Biophys. Acta* **1977**, *490*, 62.
(8) Hu, V. W.; Chan, S. I.; Brown, G. S. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3821.
(9) Powers, L.; Blumberg, W. E.; Chance, B.; Barlow, C. H.; Leigh, J. S.; Smith, J.; Yonetani, T.; Vik, S.; Peisach, J. *Biochim. Biophys. Acta* **1979**, *546*, 520.
(10) Scott, R. A.; Cramer, S. P.; Shaw, R. W.; Beinert, H.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 664.
(11) Peisach, J.; Blumberg, W. E. *Arch. Biochem. Biophys.* **1974**, *165*, 691.
(12) Penfield, K. W.; Gewirth, A. A.; Solomon, E. I. *J. Am. Chem. Soc.* **1985**, *107*, 4519.
(13) Blondeau, P.; Berse, C.; Gravel, D. *Can. J. Chem.* **1967**, *45*, 49.
(14) Prepared in a manner similar to that used for Cu(tet *b*)·$2ClO_4$; see ref 15.
(15) Hughey, J. L.; Fawcett, T. G.; Rudich, S. M.; Lalancette, R. A.; Potenza, J. A.; Schugar, H. J. *J. Am. Chem. Soc.* **1979**, *101*, 2617.
(16) Substitution of Cu(en)$_2$·$2ClO_4$ gives redox decomposition; a mixed-valence pentamer containing three of the title species and two $CuClO_4$ units has been isolated: Bharadwaj, P. K.; John, E.; Zhang, D.; Xie, M.; Hendrickson, D. N.; Potenza, J. A.; Schugar, H. J. unpublished results.

0002-7863/86/1508-1351$01.50/0

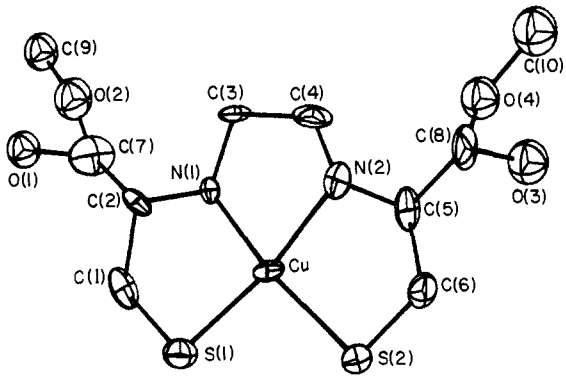

Figure 1. Structure of the title compound (**1**) showing the atom numbering scheme. For clarity, H atoms have been omitted and only one orientation of the ester groups is shown. Selected interatomic distances: Cu-S(1), 2.230 (5); Cu-S(2), 2.262 (4); Cu-N(1), 2.002 (11); Cu-N(2), 2.059 (13); S(1)···S(2), 3.406 (6) Å. Selected bond angles: S(1)-Cu-S(2), 98.6 (2)°; S(1)-Cu-N(1), 89.7 (4)°; S(1)-Cu-N(2), 165.1 (3)°; S(2)-Cu-N(1), 162.6 (4)°; S(2)-Cu-N(2), 88.4 (4)°; Cu-S(1)-C(1), 97.0 (5)°; Cu-S(2)-C(6), 96.8 (6)°; N(1)-Cu-N(2), 87.2 (5)°.

present.[17] The structure of this neutral monomer is shown in Figure 1. The Cu-S(1)-S(2)/Cu-N(1)-N(2) angle (21.0°) defines the small pseudotetrahedral twist of the approximately planar cis CuN_2S_2 unit; the Ni(II) analogue is structurally similar.[19] Observed Cu-S distances are longer than that in plastocyanin (X-ray, 2.13 Å; EXAFS, 2.08-2.10 Å),[20] shorter than those in Cu(tet *b*)-2-mercaptobenzoate[15] (2.359 (4) Å) (**3**) and the mercaptopropionate analogue **2**[1] (2.314 (2) Å), *and agree well with the average Cu-S distance determined by EXAFS (2.27 (2) Å) for cytochrome c oxidase.*[10] The Cu-S-C bond angles are smaller than those reported for **3**[15] (108.4 (4)°) plastocyanin[21] (107°), and **2**[1] (115.9(2)°). The intramolecular S(1)···S(2) distance is shorter than the van der Waals contact of 3.7 Å. Shorter S···S contacts (≈2.8 Å) are present in Mo(VI) complexes with $O_2N_2S_2$ donor sets.[22] The bulky ester groups are equatorially oriented on the λ conformation NS chelate rings.[23] Stable analogues of **1** can be prepared from ester-free, linear tetradentate amino thiol ligands.[24]

(17) Crystallography: $CuS_2O_4N_2C_{10}H_{18}$, $P\bar{1}$, a = 5.508 (1) Å, b = 12.402 (2) Å, c = 5.500 (6) Å, α = 102.23 (2)°, β = 90.14 (2)°, γ = 77.75 (2)°, V = 358.4 (6) Å^3, Z = 1; d_{obsd} = 1.64 (1), d_{calcd} = 1.66 g/cm^3. The structure was solved by using 884 unique reflections ($I > 3\sigma(I)$) collected with Mo Kα radiation (λ = 0.71073 Å) to 2θ = 50° using an Enraf-Nonius CAD-4 diffractometer. All atoms were located on completely occupied sites except for certain ester group atoms (O(1)-O(4), C(9), C(10)), which were located on half-occupied sites. Refinement with all atoms anisotropic except those of the ester groups yielded R_F = 0.057, R_{wF} = 0.065, and GOF = 1.80. The crystal is multiply twinned with [101] as the twin diad axis which interchanges a and c and converts b to $-b$. Except for the disordered atoms, it generates a pseudo-two-fold axis in the molecule with equivalent atoms at x,y,z and $z,-y,x$. The structure factors reflect this equivalence: $|F_{hkl}| = |F_{lkh}|$. Refinement of the multipliers of the disordered C and O atoms showed that these sites were indeed half-occupied and that the twin volumes were equal. Thus, it is not possible to refine the twins individually.[18] Finally, the twinning causes unusual orientations of some thermal ellipsoids and larger than expected esd's of coordinates and derived parameters for a structure at this level of refinement.

(18) Sabelli, C.; Tangocci, P.; Zanazzi, P. F. *Acta Crystallogr., Sect B* **1969**, *B25*, 2231.

(19) Both the Ni(II) analogue of **1** and $H_3NCH_2CH_2NH_3[Ni(SCH_2CH(CO_2)NHCH_2-)_2]$, the diacidate form of the N_2S_2 ligand, have been characterized: Bharadwaj, P. K.; Potenza, J. A.; Schugar, H. J., manuscript in preparation.

(20) Scott, R. A.; Hahn, J. E.; Doniach, S.; Freeman, H. C.; Hodgson, K. O. *J. Am. Chem. Soc.* **1982**, *104*, 5364 and references cited therein.

(21) Penfield, K. W.; Gay, R. R; Himmelwright, R. S.; Eickman, N. C.; Norris, V. A.; Freeman, H. C.; Solomon, E. I. *J. Am. Chem. Soc.* **1981**, *103*, 4382.

(22) Berg, J. M.; Spira, D. J.; Hodgson, K. O.; Bruce, A. E.; Miller, K. F.; Corbin, J. L.; Stiefel, E. I. *Inorg. Chem.* **1984**, *23*, 3412 and references cited therein.

(23) Buchanan, I.; Minelli, M.; Ashby, M. T.; King, T. J.; Enemark, J. H.; Garner, C. D. *Inorg. Chem.* **1984**, *23*, 495.

(24) Bharadwaj, P. K.; Firkar, R.; Potenza, J. A.; Schugar, H. J., unpublished results.

The magnetic moment (1.70 (4) μ_B)[25] and spectroscopic features of **1** are those of Cu(II). The isotropic EPR parameters (DMF, 298 K) are g_{iso} = 2.066 and A_{iso}^{Cu} = 88 × 10^{-4} cm^{-1}; at 80 K (in DMF or the Ni(II) analogue), the spectra are those of a tetragonal Cu(II) species with $g_\parallel$ = 2.126, $g_\perp$ = 2.039, $A_\parallel^{Cu}$ = 182 × 10^{-4}, and $A_\perp^{Cu}$ = 49 × 10^{-4} cm^{-1}. An absorption at 545 nm (ε ≈1000) is assigned as a LF band, possibly enhanced by "intensity stealing" from the nearby LMCT bands.[26] The near-UV bands at ≈400 nm (sh, ε >1000) and 345 nm (ε ≈5800) are those of thiolate → Cu(II) LMCT for complexes having typical (i.e., nontetrahedral) geometries.[1,15,27] The EPR parameters and absorption maxima are remarkably similar to those of the unstable bis Cu(II) complexes of L-cysteine and other NS donor ligands.[28-30] Finally, the presence of two cysteine ligands does not in itself generate either the peculiar EPR or intense 600-nm absorption of the Cu_A protein site.[3] The additional effects of imidazole ligation and tetrahedral distortion are being explored.

Acknowledgment. This research was supported by the National Science Foundation (Grant CHE-8417548), the David and Joanna Busch Foundation, and the National Institutes of Health (Instrumentation Grant 1510 RRO 1486 O1A1).

Supplementary Material Available: Tables of atomic coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors (13 pages). Ordering information is given on any current masthead page.

(25) The magnetic moment at 298 K includes a diamagnetic correction of −181 × 10^{-6} cgs from Pascal's constants.

(26) Miskowski, V. M.; Thich, J. A.; Solomon, R.; Schugar, H. J. *J. Am. Chem. Soc.* **1976**, *98*, 8344.

(27) Anderson, O. P.; Perkins, C. M.; Brito, K. K. *Inorg. Chem.* **1983**, *22*, 1267.

(28) Cavallini, D.; DeMarco, C.; Dupre, S.; Rotilio, G. *Arch. Biochem. Biophys.* **1969**, *130*, 354.

(29) Hanaki, A.; Kamide, H. *Chem. Pharm. Bull.* **1975**, *23*, 1671.

(30) Davis, F. J.; Gilbert, B. C.; Norman, R. O. C. *J. Chem. Soc., Perkins Trans. 2* **1983**, 1763 and references therein.

Models of Heme d_1. Structure and Redox Chemistry of Dioxoisobacteriochlorins

C. K. Chang*

Department of Chemistry, Michigan State University East Lansing, Michigan 48824

K. M. Barkigia,[1] L. K. Hanson, and J. Fajer*

Department of Applied Science Brookhaven National Laboratory Upton, New York 11973

Received November 22, 1985

Unlike the ubiquitous iron porphyrin containing cytochromes found in animals and plants, *d*-type cytochromes present in many microbial nitrite reductases–cytochrome oxidases contain a green heme prosthetic group whose structure has been assumed for the last 2 decades to be a chlorin (dihydroporphyrin).[2] On the basis of spectroscopic data collected by Timkovich et al.,[3] one of us (C.K.C.) has recently proposed that heme d_1 isolated from *Pseudomonas aeruginosa* and *Parracoccus denitrificans* is not a chlorin but a dioxoisobacteriochlorin.[4] Crucial to this as-

(1) Address inquiries regarding the crystallographic data to this author.

(2) Yamanaka, T.; Okunuki, K. *Biochim. Biophys. Acta* **1963**, *67*, 407-416.

(3) Timkovich, R.; Cork, M. S.; Taylor, P. V. *J. Biol. Chem.* **1984**, *259*, 1577-1585.

(4) Chang, C. K. *J. Biol. Chem.* **1985**, *260*, 9520-9522.

0002-7863/86/1508-1352$01.50/0