Anal. Calcd for $[C_{42}H_{24}N_{10}Ru]_n$ (M_r = 769.8): C, 65.53; H, 3.14; N, 18.20. Found: C, 65.17; H, 3.21; N, 18.07. UV/vis (C_6H_5Cl) λ_{max} = 693 nm.

Bis(1,4-diisocyanobenzene)(phthalocyaninato)ruthenium(II) (12). A 0.2-g (0.33-mmol) sample of PcRu (**4**) and 2 g (15.6 mmol) of 1,4-diisocyanobenzene were heated in 100 mL of chloroform under reflux for 1 h. After cooling to room temperature, the reaction mixture was filtered; the deep blue filtrate was concentrated to one-third of its parent volume and subsequently treated with 50 mL of *n*-hexane. The precipitate was suction-filtered, washed with methanol, and dried in vacuo at 70 °C. Yield: 0.24 g (85%), violet powder.

^{1}H NMR: δ 9.32 m (8 Pc H^2); 8.01 m (8 Pc H^1); 5.21 m (4 H^a), 6.45 m (4 H^b) (for nomenclature see Table I). UV/vis (C_6H_5Cl) λ_{max}, nm: 644, 619 sh, 583, 357, 310.

(μ-1,4-Diisocyanobenzene)(phthalocyaninato)ruthenium(II) (13). A 0.61-g (1-mmol) sample of PcRu (**4**) and 0.14 g (1.1 mmol) of 1,4-diisocyanobenzene were heated in 70 mL of acetone under reflux for 24 h. After cooling to room temperature, the reaction mixture was centrifuged; the residue was washed with 200 mL of acetone and then dried in vacuo at 70 °C. Yield: 95%, blue-black powder.

Anal. Calcd for $[C_{40}H_{20}N_{10}Ru]_n$ (M_r = 741.7): C, 64.77; H, 2.72; N, 18.89. Found: C, 63.60; H, 2.80; N, 17.95. UV/vis (C_6H_5Cl) λ_{max}, nm: 639, 619 sh, 582, 395, 360, 309.

Doping of [PcRu(pyz)]$_n$ (10) and [PcRu(dib)]$_n$ (13) with Iodine. Weighed quantities of polymers **10** and **13** and appropriate amounts of iodine were rigorously ground together (10–15 min) in a mortar in the presence of a few drops of benzene. After evaporation of the solvent at elevated temperature (50–70 °C) in an inert-gas stream, finely divided blue-black solids $[PcRuLI_x]_n$ (L = pyz, dib; x = 1.5, 2) were obtained. Stoichiometries of the iodine-doped polymers were confirmed by elemental analysis.

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Registry No. **3**, 99547-51-0; **4**, 27636-56-2; **5**, 87195-51-5; **6a**, 99547-52-1; **6b**, 99547-53-2; **6c**, 99547-54-3; **6d**, 99547-55-4; **6e**, 99547-56-5; **7**, 88344-21-2; **8**, 99547-57-6; **9**, 99547-58-7; **10**, 87189-21-7; **11**, 99547-60-1; **12**, 99559-72-5; **13**, 90654-28-7; iodine, 7553-56-2.

Contribution from the Department of Biology, University of California at San Diego, La Jolla, California 92093

Interaction of Copper(II) with *N*-(2-Hydroxyethyl)piperazine-*N*′-ethanesulfonic Acid (HEPES)

Kaspar Hegetschweiler and Paul Saltman*

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The widespread use of HEPES as the biochemical buffer of choice at neutral pH is predicted on the assumption of its lack of binding affinity and reactivity with metal ions. In fact, under conditions encountered in studying Cu(II) reactions, complexation with and oxidation of HEPES by the metal was observed. The redox reaction only occurs in presence of ligands that stabilize Cu(I). The reduction of Cu(II) is first order in HEPES and second order in Cu(II). The pH profile suggests a direct interaction between HEPES and Cu(II). Several alcohols were tested for their ability to reduce Cu(II). In the absence of a N ligand the reaction is very slow or zero. Caution in the use of HEPES to study Cu reactions is advised.

Introduction

The selection of proper buffers for investigating the chemistry and biochemistry of trace metals in a variety of systems is a significant problem. Every buffer provides a potential ligand for cations. The use of hydrogen phosphate or hydrogen carbonate, e.g., is severely restricted because of the insolubility of many trace metal phosphates and carbonates. The application of amine buffers such as tris(hydroxymethyl)aminomethane (Tris) seemed to obviate these problems. However, it was soon recognized that Tris could form complexes with some of the trace metals.[1] The search for a more "innocent" buffer led to *N*-(2-hydroxyethyl)-piperazine-*N*′-ethanesulfonic acid (HEPES) (Figure 1). This buffer has become widely accepted and applied.[2-5] In the course of experiments concerning the reduction of Cu(II) by heme proteins,[6] we found a weak but significant interaction between HEPES and Cu(II). The nature of the Cu(II) reaction with HEPES and other related compounds, in particular the structurally related compound dimethylethanolamine (DMEA) (Figure 1), is reported here.

It has been long known that Cu(II) is able to oxidize certain organic compounds, if the resultant Cu(I) can be held in a stabilized form. For example, acetonitrile binds Cu(I) and enhances oxidation reactions. In the Fehling reaction, aldehydes are oxidized and the Cu(I) is stabilized in form of the insoluble copper(I) oxide.[7] Bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) and its disulfonated derivative (batho) stabilize Cu(I) because of their peculiar steric properties. The redox potential $E°$(Cu(II)/Cu(I)) in the presence of batho is 0.62 V compared to 0.167 V for the aqua ion.[8,9] We have carried out kinetic studies of the oxidation reaction of HEPES and other alcohols by Cu(II) in the presence of batho, and have characterized the significant interaction of the Cu^{II}-$(batho)_2$ complex with HEPES.

Experimental Section

Copper(II) sulfate pentahydrate (Mallinckrodt, analytical reagent), HEPES (Sigma), and disodium bathocuproinedisulfonate (Sigma) were used. All solutions with batho were kept in the dark prior to use. All other chemicals were of reagent grade quality. The copper complexes were prepared by mixing aqueous solutions of copper sulfate and the ligands at the concentrations indicated. All reactions were carried out at an ionic strength of 1.0 M NaCl. The reduction of Cu(II) was measured spectrophotometrically. The alcohol was present in 3.0 mL of 1.0 M NaCl. Following equilibration to the desired temperature, 30 μL of 0.1 mM Cu(II) aqua complex or Cu(II) complex solution was added and rapidly mixed. In the case of DMEA, 20 mM phosphate buffer was used

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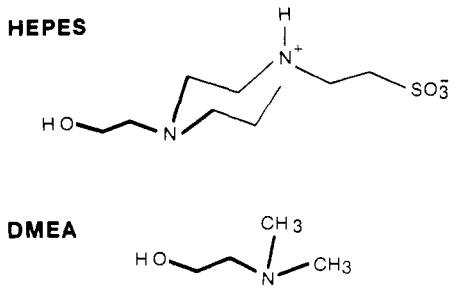

Figure 1. Structure of *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES) and *N,N*-dimethylethanolamine (DMEA).

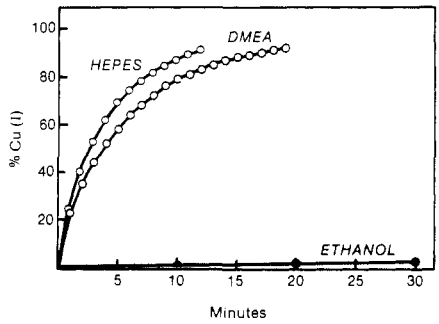

Figure 2. Reduction of Cu(II) in the presence of batho by different alcohols. Initial reaction conditions: [Cu] = 0.1 mM, [batho] = 2 mM, [NaCl] = 1.0 M, 25 ± 1 °C. [HEPES] = 0.2 M, pH 7.0; [DMEA] = 0.2 M, pH 8.2; [ethanol] = 0.2 M, pH 7.0.

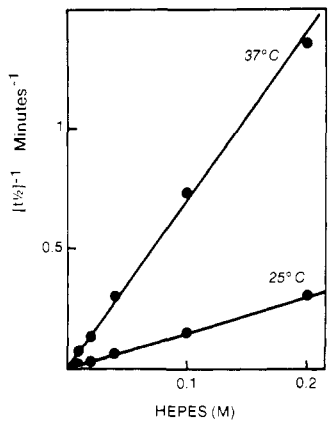

Figure 3. Reduction of Cu(II) by HEPES in the presence of batho. Data are presented as a plot of the reciprocal half-life time, $(t_{1/2})^{-1}$ vs. HEPES concentration. First-order dependence in [HEPES] is demonstrated. Initial conditions: [Cu] = 0.1 mM, [batho] = 2 mM, [NaCl] = 1.0 M, pH 6.8.

to adjust the pH to either 5.9 or 6.8. For all other alcohol solutions no buffering was required. Immediately after mixing, the formation of $Cu^{I}(batho)_2$ was measured with a Hitachi UV–vis double-beam spectrophotometer, Model 110A, by recording A at 483 nm (ϵ_{483} = 12 250 M^{-1} cm^{-1}).[8,9] To complete the reduction of copper and to determine the final value of absorbance, 5 μL of 10% sodium dithionite solution was added. The number of equivalents of Cu(II) reduced by 1 equiv of HEPES was determined by dissolving 21.1 mg (88.6 μmol) of HEPES with respectively 1, 2, 4, and 8 equiv of $Cu^{II}(batho)_2$ in water, adding 1 mL of 1 M phosphate buffer pH 7, and diluting to a final volume of 10 mL. Aliquots of 1 mL were taken after 1, 2, 4, and 7 days. The samples were diluted to a volume of 3 L, and the absorbance was measured at 483 nm.

Results

The redox properties of the alcohols tested were quite different for the Cu(II)–batho system. 2-Propanol, Tris, and methanol did not show any reduction reaction even after several days. Ethanol and glycerol reacted very slowly. HEPES and DMEA reacted about 1000 times faster than ethanol (Figure 2). No change in rate was observed under anaerobic conditions.

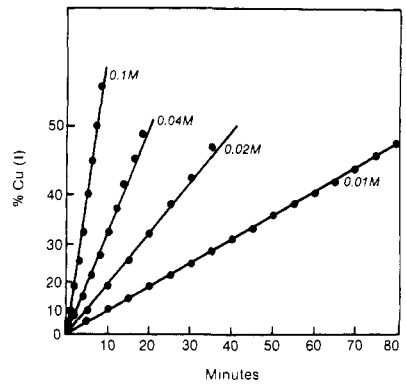

Figure 4. Reduction of Cu(II) by various concentrations of HEPES in the presence of batho presented as a plot of reciprocal Cu(I) concentration, $[100\% - \%\ Cu(I)]^{-1}$ vs. time. Second-order dependence in [Cu(II)] is demonstrated. Initial conditions: [Cu] = 0.1 mM, [batho] = 2 mM, [NaCl] = 1.0 M, pH 6.8, 25 ± 1 °C, [HEPES] as indicated on figure.

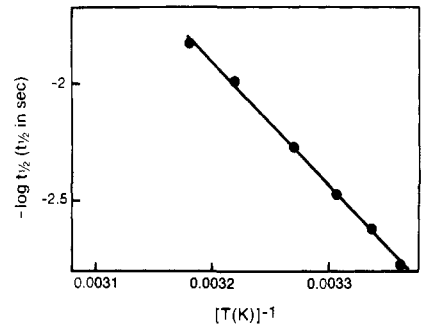

Figure 5. Temperature dependency of the reduction of Cu(II) by HEPES in the presence of batho presented as log of half-life time vs. reciprocal absolute temperature. Initial conditions: [Cu] = 0.1 mM, [batho] = 2 mM, [NaCl] = 1.0 M, [HEPES] = 0.2 M, pH 6.5.

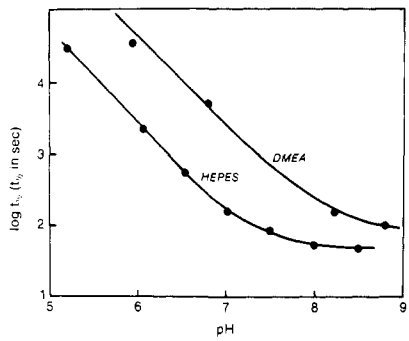

Figure 6. pH dependency of the reduction of Cu(II) by HEPES or DMEA in the presence of batho presented as log of half-life vs. pH. Initial conditions: [Cu] = 0.1 mM, [batho] = 2 mM, [NaCl] = 1.0 M, [HEPES] = 0.2 M, [DMEA] = 0.2 M, 25 ± 1 °C.

The kinetics of the reduction of Cu(II) by HEPES were studied in detail. The reactions were carried out in the presence of a large excess of reducing agent. The change in concentration of reducing agent was negligible. Under these conditions, the rate of reduction was linearly dependent upon the HEPES concentration (Figure 3). Figure 4 presents Cu(I) formation as a function of time. The initial phase can be reasonably expressed with the rate law

$$\frac{d[Cu^+]}{dt} = k[Cu^{2+}]^2[HEPES]$$

with $k_{25°C}$ = 2.4 × 10^2 s^{-1} M^{-2} and $k_{37°C}$ = 1.16 × 10^3 s^{-1} M^{-2}. The kinetics of the complete reaction was complex and could not be approximated with this expression.

For determination of the energy of activation, reaction rates were measured at various temperatures between 24 and 42 °C, and the data analyzed by an Arrhenius plot (Figure 5). The

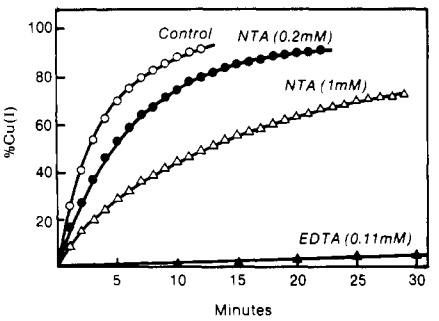

Figure 7. Reduction of Cu(II) by HEPES in the presence of batho as influenced by additional ligands stabilizing Cu(II). Initial conditions: [Cu] = 0.1 mM, [batho] = 2 mM, [NaCl] = 1.0 M, [HEPES] = 0.2 M, pH 7.0, 25 ± 1 °C. Stabilizing ligands are at the concentrations indicated.

energy of activation was found to be 24.3 kcal/mol.

For both HEPES and DMEA, the pH profile was measured (Figure 6). For low pH values (pH < pK_a) the rate decreased linearly with decreasing pH. For high pH values (pH > pK_a), there was only weak, if any, pH dependency (pK of HEPES = 7.48;[2] pK of DMEA = 9.29[10]).

The influence of ligands, which stabilize the Cu(II), is shown in Figure 7. Nitrilotriacetate (NTA) and ethylenediaminetetracetate (EDTA) were investigated. Increasing ligand concentration and incresing stability for Cu(II) inhibit the reduction (stability constants for Cu(II) with NTA, log K_1 = 12.4 and log K_2 = 4.5, and with EDTA, log K = 18.7[10]).

Discussion

The results demonstrate that HEPES is oxidized by Cu(II) in the presence of ligands that stabilize Cu(I). The similar behavior of HEPES and DMEA suggests the oxidation of the alcoholic groups of both components with participation of nitrogen in ligand formation (Figure 1). The enhancement of the rate in the case of HEPES and DMEA compared with ethanol or glycerol can be explained on the basis of the formation of a weak ternary complex with the Cu(II)-batho system. Similar ternary complexes with the Cu(II)-2,9 dimethyl-1,10-phenanthroline system are known.[11,12] The direct interactions of Cu(II) with several N,N-disubstituted ethanolamines have been characterized. The first stability constant for complex formation with DMEA is $K_1 = 5 \times 10^4$.[13] The assumption that the Cu(II)-batho system forms a ternary complex with HEPES or DMEA as an intermediate in the reaction is strongly supported by the pH dependency of the reaction (Figure 6). Only the deprotonated species are able to reduce the Cu(II). This model is supported by the finding that the reaction is inhibited with NTA or EDTA.

Because the reaction is second order with respect to Cu(II), it appears that a pair of electrons must be transferred from the HEPES to two molecules of $Cu^{II}(batho)_2$. We therefore propose the following mechanism:

$$2Cu^{II}(batho)_2 + HEPES \overset{K}{\rightleftharpoons} [Cu^{II}(batho)_x]_2\text{-}HEPES \rightarrow 2Cu^{I}(batho)_2 + \text{oxidation products}$$

An estimated value for these interactions from kinetic data is $10^2 < K < 10^4$ mol^{-1}. Direct data for the complexation of Cu(II) by 2,9-dimethyl-1,10-phenanthroline are available:[10] log K_1 = 6.1, log K_2 = 4.9. It has been suggested that similar constants are obtained for the Cu(II)-batho system. Under conditions chosen in this work, Cu(II) is present as a bis complex.[8,9]

In agreement with the proposed mechanism, tris(phenanthroline)iron(III) does not oxidize HEPES, even though it is a stronger oxidizing agent than the Cu(II)-batho complex ($E°$ = 1.1 V[14]). As is well-known, Fe(III), unlike Cu(II), has a very low affinity for saturated amines.

We found that 1 equiv of HEPES is able to reduce 6 equiv of copper. This might be an explanation for the complex kinetics of this reaction in the later stages. Intermediates formed during the reaction could reduce the Cu(II) at a faster rate than HEPES itself. The fact that methanol, 2-propanol and Tris do not undergo a redox reaction may have to do with their lack of a specific structure or intermediate required for the reactivity.

In studies involving trace metals, especially Cu(II), HEPES should be used with caution as a buffer. There are weak but significant interactions between the metal and HEPES which may introduce experimental artifacts. If Cu(II) is present together with ligands that stabilize Cu(I), HEPES should never be used due to the reduction of Cu(II).

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Notes

Contribution No. 3518 from the Central Research & Development Department, E. I. du Pont de Nemours & Company, Wilmington, Delaware 19898

Hyper-Closo Metallaboranes

R. T. Baker

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Greenwood, Kennedy, and co-workers have recently isolated and structurally characterized a wide variety of new phosphinometallaborane complexes.[1,2] Two complexes prepared via the cage-closure reactions (1) and (2) have been termed iso-closo

$$nido\text{-}(PMe_3)_2(CO)Ir(B_8H_{10}Cl) \xrightarrow[-H_2,\,CO]{135\ °C} H(PMe_3)_2Ir(B_8H_7Cl) \quad (1)$$

$$nido\text{-}H(PPh_3)(Ph_2PC_6H_4)Ir(B_9H_{12}) \xrightarrow[-2H_2]{85\ °C} H(PPh_3)(Ph_2PC_6H_4)Ir(B_9H_8) \quad (2)$$

metallaboranes, as their observed closed, polyhedral structures are not those normally found for nine- and ten-vertex closo metallaboranes. Greenwood et al. thus regard these complexes as simply different closo isomers. We suggest, on the basis of electron-counting arguments and by reference to known structural analogues, that these complexes contain two skeletal electrons

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