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(15) This conclusion based on our experimental data and the two-center-only epimerization model is in conflict with intuitive judgments on the relative magnitudes of these rate constants expressed by two referees, but we know of no conflicting evidence; indeed, k_{23} in 1-phenyl-2-deuteriocyclopropane may, according to our calculations, be only some one-fifth smaller than k_{12}.

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Temperature and pH Dependence of the Tetramer-Dimer Equilibrium of Carbomonoxyhemoglobin A$_0$

Sir:

We wish to report: (1) a new method for measuring the equilibrium constant for the tetramer-dimer dissociation of human hemoglobin, and (2) the temperature and pH dependence of that constant.

We have determined the tetramer-dimer dissociation constant ($K_{4,2}$) of carbomonoxyhemoglobin A$_0$ (HbCO) by the difference in tritium outexchange rates of dimer and tetramer. Differential reactivity of monomers and polymers has been well established as a means for estimating macromolecular subunit dissociation constants.[1] The general principles of the method as applied to the tritium outexchange rates of tetrameric and dimeric hemoglobin can be illustrated as follows. Let each chain of hemoglobin contain the same number of tritium labeled sites and let each state (dimer and tetramer) have a unique set of outexchange rate parameters, as is common in protein systems.[2] Then, at some time after initiation of outexchange, the fraction dissociation, α, is given by:

$$\alpha = [(\text{Hrem/Fe})_{\text{C},4} - (\text{Hrem/Fe})_{\text{C},i}] / [(\text{Hrem/Fe})_{\text{C},4} - (\text{Hrem/Fe})_{\text{C},2}] \quad (1)$$

where Hrem/Fe = the amount of tritium left per chain, C,4 and C,2 indicate concentrations of protein where only tetramer or dimer exist, and C,i represents some intermediate concentration at which the two states are both present.

In this manner, fractional hydrogen exchange[3] gives values of the fraction dissociation as a function of protein concentration in a manner strictly analogous to the results obtained from any other physical measurement,[4] provided the protein concentration is well known.

In order to accurately know the concentration of the protein (which must span four or so orders of magnitude) we have labeled the HbCO with trace amounts of ^{14}C which remains affixed to the chains. The ^{14}C counts thereby afford a measure of [HbCO] at concentrations well above or well below those suitable for spectrophotometric determination. The relationship between ^{14}C radioactivity and [HbCO] is established at concentrations where spectral work is feasible.

$K_{4,2}$ at a given set of conditions is determined in a single experiment. A concentrated batch of double-labeled HbCO is apportioned and diluted to yield approximately 18 samples ranging over four orders of magnitude in [HbCO]. Outexchange kinetics in each sample are followed by the rapid dialysis technique.[5] At several times after initiation of outexchange an aliquot is removed from each sample for scintillation counting to simultaneously determine [HbCO] and Hrem/Fe.

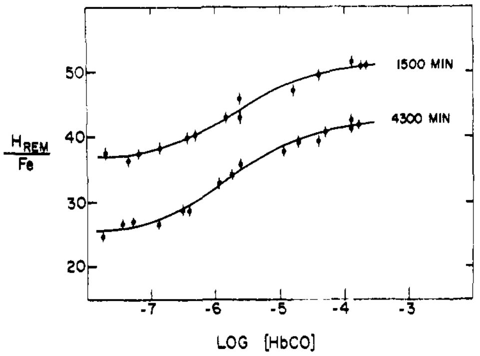

Figure 1. Results of a typical experiment: conditions, pH 6.0, 0.1 M phosphate buffer, 15°; vertical axis, amount tritium remaining per chain; horizontal axis, logarithm of the total concentraton of HbCO in moles of tetramer per liter; symbols, Hrem/Fe vs. log [HbCO] at 1500 min (●) and 4300 min (○) after initiation of tritium outexchange; error bars, ±1 SD of Hrem/Fe as calculated from errors in dilutions, scintillation counts, and the ^{14}C to [HbCO] relationship; solid lines, best fit of data to eq 1 by iterative weighted least-squares analysis; results, at 1500 min, $K_{4,2}$ = (4.4 ± 1.2) × 10^{-6}, at 4300 min, $K_{4,2}$ = (3.6 ± 0.7) × 10^{-6}.

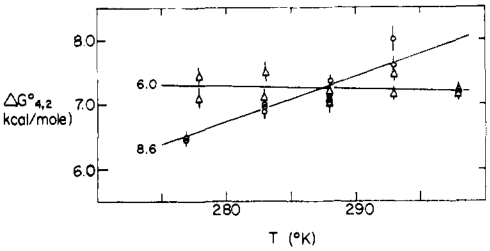

Figure 2. Free energy-temperature profile of $K_{4,2}$ as a function of pH: symbols, $\Delta G°_{4,2}$ at pH 6 (△) and 8.6 (○); error bars, ±1 SD of $\Delta G°_{4,2}$; solid lines, weighted least-squares fit to determine enthalpy and entropy of dissociation as $\Delta G°_{4,2} = \Delta H°_{4,2} - T\Delta S°_{4,2}$.

The results of a typical experiment are shown in Figure 1. Experiments at other sets of conditions gave results of equally high quality. The precision of our values of $K_{4,2}$ is about the same as that most recently reported by others,[6] and betters the precision in earlier.[7,8] The accuracy of these values, moreover, is independent of the assumptions needed for data interpretation in gel filtration[9] or in ultracentrifugation studies.[10] In short, measurement of differential tritium exchange, together with the ^{14}C concentration determination, yields $K_{4,2}$ simply, directly, and precisely.

We have studied the temperature and pH dependence of $K_{4,2}$ for HbCO. The results, displayed as a free energy profile, appear in Figure 2. Weighted least-squares analysis[11] of the data in Figure 2 yields the following thermodynamic quantities: at pH 6, $\Delta H°_{4,2}$ = 8.2 ± 1.8 kcal/mol and $\Delta S°_{4,2}$ = 4.4 ± 6.3 eu; at pH 8.6, $\Delta H°_{4,2}$ = −13.2 ± 2.0 kcal/mol and $\Delta S°_{4,2}$ = −71.0 ± 7.1 eu. These results more than suggest that the liganded or R form of hemoglobin has its own measure of conformational lability in response to pH changes. That such should be the case was, in fact, our motivation for undertaking this work. (There had been a report[8] that $\Delta G°_{4,2}$ for HbCO changes substantially with ionic strength but little with pH, findings which to us seemed inconsistent.) The free energy profiles in Figure 2 intersect at about 15° which may indicate that enthalpy-entropy compensation[12] is operative in this system. Regardless, the free energies of dissociation at 20° are not very different which, given the precision of earlier measurements,[8] would account for the apparent pH independence of $\Delta G^\circ_{4,2}$ at that temperature.

A subsequent manuscript will describe in detail the double-label method for determining $K_{4,2}$ and will correlate the results given here with those of further experiments.

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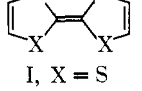
Electrochemical Preparation and Control of Stoichiometry for Donor-Halide Salts: TTFX$_n$ and TSeFX$_n$

Sir:

Considerable interest has been shown recently in the charge transfer salts of tetrathiafulvalene[1-3] (I, TTF) and its derivatives.[4] Depending upon the identity of the anion[5] and the stoichiometry[5,6] in these compounds, $(TTF)_xA_y$, a wide range of unique electric,[1b,2,4,7] magnetic,[8] and optical[6a,9] properties have been observed. These salts were previously prepared by direct chemical oxidation of the donor with added acceptor or by metathetical[5] reactions of $TTF_3(BF_4)_2$ with an anion, but these methods do not offer the desired selectivity or control over the multiple product phases often produced for reactions with anions such as halides.[5,6] It appeared to us that reaction of donor cations generated *electrochemically* would afford the most controlled conditions for preparing donor salts of predictable stoichiometry with a wide variety of inorganic and organic acceptors. Therefore, we have studied the electrochemical preparation of some of these salts and wish to report our results here.



I, X = S
II, X = Se

To test the generality of this method, separate CH_3CN solutions containing 100% TTF^+ or $TSeF^+$,[4b] oxidized electrochemically, were treated with Br^-, and the resulting bromide salts were collected.[10] For the reaction of TTF^+, the electrically *insulating* 1:1 bromide salt[6] (III, TTF-Br) was formed. However, in the identical reaction with $TSeF^+$ (Table I), a *conducting*, mixed valence bromide salt (IV, $TSeF\text{-}Br_x$) containing formally neutral TSeF is produced. To understand the reasons for the striking difference in products formed, cyclic voltamograms of I or II in the presence of Br^- were obtained.

Table I. Properties of Electrochemically Prepared Donor Halide Salts

Reactants[a]	Product	$\rho(\Omega\ \mathrm{cm})$[d]	ΔE^0, eV[e]
$TTF^+ + Br^-$	(**III**) $TTF\text{-}Br$[b]	$>10^6$	-0.147
$TSeF^+ + Br^-$	(**IV**) $TSeF\text{-}Br_{0.8}$[c]	10^{-1}	$+0.017$
$TSeF^+ + Cl^-$	(**V**) $TSeF\text{-}Cl$[b]	$>10^6$	-0.246

[a] For experimental conditions cf. ref 10. [b] Correct elemental analysis was obtained. [c] Average stoichiometry determined by elemental analysis. [d] Resistivity determined on compacted samples at room temperature. [e] Formal emf (CH_3CN, 0.1 M Et_4NClO_4) of the reactions, $2TXF^+ + 3Br^- \rightleftarrows 2TXF + Br_3^-$ (X = S or Se) or $2TSeF^+ + 2Cl^- \rightleftarrows 2TSeF + Cl_2$.

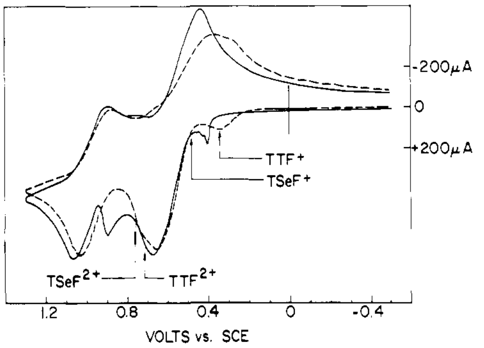


Figure 1. Cyclic voltammograms of donors (6.5×10^{-4} M) I, TTF (- - -), and II, TSeF (—), in CH_3CN (0.1 M Et_4NClO_4) with Br^- added (7.8×10^{-3} M Bu_4NBr) at 340 mV/s on platinum electrode. Arrows indicate E_p for donor cations (+) and dications (2+) in absence of Br^- (cf. ref 11).

Previously we had found[11] that the electrochemistry of I and II was characterized by two simple one-electron couples corresponding to formation of the radical cation and dication. No evidence of any distortion of the current-potential ($I\text{-}\mathcal{E}$) curves was observed with potential cycling, indicating these donor redox systems were uncomplicated by any slow coupled chemical reactions, adsorption processes, or electron transfer steps. For TTF there is no change (at these concentrations) in the voltammograms in the presence of Br^- (Figure 1, dotted line) except for the appearance of two reversible bromide[12] couples. However, in the case of TSeF (Figure 1, solid line) addition of Br^- causes sharpening of the oxidation waves, decrease in the peak width, and a shift towards negative potential. Such voltammetric behavior is typical[13] of electrode processes in which species are formed coupled to a follow-up chemical reaction. Formation of IV is the suspected follow-up reaction since this species is formed in bulk reactions of the electrolysis solutions with bromide ion.

These results and the known proximity[11,12] of the Br^-/Br_3^- and $TSeF^+/TSeF$ couples in the cyclic voltammograms suggest that homogeneous electron transfer reactions may occur in the reaction of $TSeF^+$ and Br^-. Specifically, reaction 1

$$2TSeF^+ + 3Br^- \rightleftarrows 2TSeF + Br_3^- \qquad (1)$$

has a positive ΔE^0 (Table I, negative ΔG under standard conditions) and could represent the means by which neutral TSeF is produced and thereby made available for incorpo-