Binding and Reactivity of Thiosulfate Dianion in Positively Charged Micelles: A Quantitative Analysis

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Abstract: The combined studies of thiosulfate binding to hexadecyltrimethylammonium bromide (CTAB) micelles and of the effect of hexadecyltrimethylammonium thiosulfate (CTA_2T) micelles on the reaction between thiosulfate and *n*-butyl iodide lead to the prediction of the effect of CTAB on the kinetics of this reaction. These results demonstrate that the reactivity of thiosulfate is identical in CTAB and CTA_2T micelles and that ion-exchange formalisms can be used to analyze quantitatively the effect of micelles on reactions of divalent ions.

Several models proposed for the quantitative analysis of reaction rates in micellar solutions have allowed the evaluation of the factors responsible for micelle-induced rate changes of nucleophilic reactions of hydrophobic and hydrophilic monoanions.[1-7] However, the effect of ionic micelles on reactions of polyvalent anions has received little attention and has not been analyzed quantitatively.[8-11]

The reaction between thiosulfate and alkyl halides is a convenient system for the study of micellar effects on divalent ion–neutral substrate reactions because (a) the mechanism of this reaction has been thoroughly investigated,[12] (b) the distribution of alkyl halides between the aqueous phase and the micellar pseudophase can be easily measured, and (c) thiosulfate binding to the micellar pseudophase can be estimated directly since the absorption spectrum of this dianion depends on the medium.[13]

We report herein a quantitative analysis of the effect of hexadecyltrimethylammonium thiosulfate (CTA_2T) micelles on the rate of reaction of thiosulfate with *n*-butyl iodide (reaction 1).

$$S_2O_3^{2-} + CH_3(CH_2)_3I \rightarrow CH_3(CH_2)_3S_2O_3^- + I^- \quad (1)$$

In addition, we demonstrate that the parameters obtained from the analysis of the effect of CTA_2T on reaction 1 can be used to predict the effect of hexadecyltrimethylammonium bromide (CTAB) micelles on the same reaction.

Experimental Section

Materials and Apparatus. *n*-Butyl iodide, obtained from Aldrich Chemical Co., was distilled and stored at −18 °C. Stock solutions (ca. 0.02 M) in ethanol contained 2×10^{-4} M cyclohexene. Hexadecyltrimethylammonium bromide (CTAB) was obtained from Merck (p.a.) and purified as previously described.[14] All inorganic reagents were analytical grade or superior, and all solutions were prepared in freshly boiled deionized water, doubly distilled in glass, that had been allowed to cool under an argon atmosphere. All measurements were conducted at 30 ± 0.1 °C. Spectrophotometric determinations were performed with a Beckmann M25 or Perkin-Elmer 555 spectrophotometer. Surface tension was measured with an A. Krüss Model 8551 de Noüy tensiometer equipped with a Pt ring. Conductivity measurements were performed with a dipping cell and a Beckman RC 18A conductivity bridge. Elemental analyses were performed by Dr. R. Moscovici from the Instituto de Química da USP, São Paulo, Brazil.

Hexadecyltrimethylammonium Thiosulfate (CTA_2T). Fifty milliliters of a 0.1 M solution of CTAB was passed through a Dowex 21K thiosulfate column (1.25 × 45 cm) that was eluted with water. The detergent-containing fractions were lyophilized, recrystallized from acetone–methanol, and dried under vacuum. Anal. Calcd for $C_{38}H_{84}N_2O_3S_2$: C, 67.00; H, 12.42; N, 4.11. Found: C, 66.50; H, 12.35; N, 4.11. The thiosulfate content of CTA_2T, estimated by I_2 titration,[15] was within 0.1% of the expected value.

Determination of the Critical Micelle Concentration (CMC) of CTA_2T. The CMC of CTA_2T was determined by several methods. Plots of surface tension vs. $[CTA_2T]$ exhibit no minima; a sharp brake corresponding to the cmc is observed at 3.2×10^{-4} M, expressed as concentration of CTA monomer. Conductometric and spectrophotometric measurements and the use of a fluorescent probe yielded the same value for the cmc of CTA_2T. The fluorescence experiments were performed by E. Abuin and E. Lissi (Universidad de Santiago, Santiago, Chile) with CTA_2T prepared by us.

Determination of the Absorptivity of Micelle-Bound Thiosulfate. The value of the observed absorptivity (260 nm) of a CTA_2T solution below the cmc (ϵ_f = 84 M^{-1} cm^{-1}) coincides with published values for the absorptivity of thiosulfate in aqueous $Na_2S_2O_3$.[16] At the cmc, the slope of the linear function relating the observed absorbance per centimeter with $[CTA_2T]$ increases and can be described by eq 2, where C_D, the

$$A = \tfrac{1}{2}[(\text{cmc} + \alpha C_D)\epsilon_f + (1 - \alpha)C_D\epsilon_b] \quad (2)$$

analytical concentration of the micellized detergent, is equal to the total detergent concentration (C_T) minus the cmc (both expressed as molar concentration of CTA monomer). The absorptivity of thiosulfate free in the aqueous phase (ϵ_f) is 84 M^{-1} cm^{-1} (see above) at 260 nm. The degree of counterion dissociation of the CTA_2T micelle (α) was estimated by conductometric measurements[17] and found to be 0.15. From the above data, the absorptivity of micelle-bound thiosulfate (ϵ_b) was calculated to be 120 ± 6 M^{-1} cm^{-1}.

Determination of the Distribution Constant of *n*-Butyl Iodide between the Micelles and the Aqueous Phase (K_S). K_S can be expressed according to eq 3,[1-3] where S_b and S_f are the analytical concentrations of *n*-butyl

$$K_S = S_b/S_fC_D \quad (3)$$

iodide solubilized in the micelles and in the aqueous phase, respectively. The spectral shift caused by addition of CTAB to an aqueous solution

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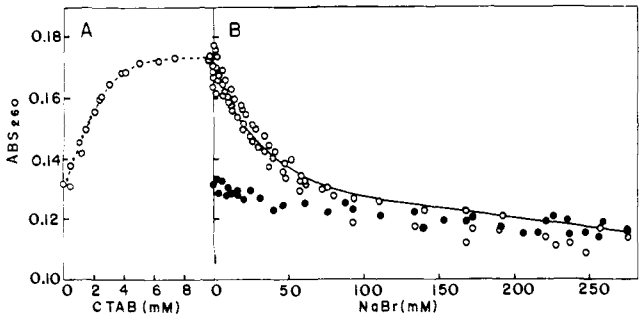

Figure 1. Effect of CTAB and NaBr on the absorbance of an aqueous solution of $Na_2S_2O_3$ (1.57×10^{-3} M). (A) CTAB was added to an aqueous solution of thiosulfate. (B) An NaBr solution containing 0.01 M CTAB was added to a solution of thiosulfate in 0.01 M CTAB (○); the same experiment in the absence of CTAB (●). The solid line was calculated; see text.

of *n*-butyl iodide was used to determine K_S,[14,18] yielding a value of 450 M^{-1} for this constant.

Determination of Rate Constants for Reaction 1. The progress of reaction 1 was followed at 30 ± 0.1 °C by monitoring the decrease in absorbance at 270 nm observed after addition of *n*-butyl iodide to thiosulfate. Reactions were conducted with excess thiosulfate, and pseudo-first-order rate constants (k_ψ) were calculated from ln $(A_\infty - A_t)$ vs. time plots, which were linear for at least four half-lives. k_ψ's obtained in CTAB were corrected for the reaction between bromide and *n*-butyl iodide (I. M. Cuccovia and H. Chaimovich, unpublished results). This correction becomes significant at concentrations of CTAB higher than 0.05 M.

Results and Discussion

The absorbance of an aqueous $Na_2S_2O_3$ solution reaches a plateau upon increasing [CTAB] (Figure 1A), indicating that the dianion binds to the micelle. Using the value of the molar absorptivity of bound thiosulfate ($\epsilon_b = 120 \pm 6$ M^{-1} cm^{-1}) (see Experimental Section), we estimated that ca. 95% of the thiosulfate is bound to CTAB in the plateau region (Figure 1A). The absorbance of the latter system decreases upon addition of NaBr, approaching a value comparable to that presented by $Na_2S_2O_3$ in the absence of CTAB at 0.2 M NaBr (Figure 1B). Thus, bromide can displace thiosulfate from the micellar surface, similarly to what is observed in the case of monovalent ion exchange[4,5,7] according to

$$(S_2O_3^{2-}{}_b) + 2(Br^-_f) \underset{}{\overset{K_{T/Br}}{\rightleftharpoons}} (S_2O_3^{2-}{}_f) + 2(Br^-_b) \qquad (4)$$

where $K_{T/Br}$ is the ion selectivity coefficient[5] for the thiosulfate–bromide exchange.

The decrease of absorbance per centimeter as a function of salt (Figure 1B) was analyzed by making use of eq 5, where T_b and

$$A_{260} = \epsilon_f T_f + \epsilon_b T_b \qquad (5)$$

T_f denote the analytical concentrations of bound and free thiosulfate, respectively. T_b and T_f are related by $K_{T/Br}$ (eq 6), where

$$K_{T/Br} = \frac{(T_b)_l (Br_f)^2}{T_f (Br_b)_l^2} \qquad (6)$$

$(T_b)_l$ and $(Br_b)_l$ are, respectively, the *local* concentrations of thiosulfate and bromide in the micellar pseudophase. The analytical concentrations of bromide free in the intermicellar aqueous phase (Br_f) and bound to the CTAB micelle (Br_b) are given by eqs 7 and 8.[5] The analytical and local concentrations of a given

$$(Br_f) = \alpha C_D + \text{cmc} + 2T_b + (\text{NaBr}) \qquad (7)$$

$$(Br_b) = (1 - \alpha) C_D - 2T_b \qquad (8)$$

species in the micellar pseudophase have been shown to be related according to eq 9,[5] where $\bar{V}$, is the partial molar volume of the

$$(Br_b)_l = (Br_b)/C_D\bar{V} \qquad (9)$$

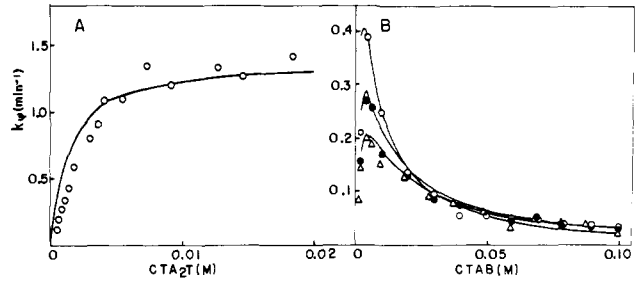

Figure 2. Effect of CTA_2T and CTAB on reaction 1. [*n*-Butyl iodide] = 1×10^{-4} M. Lines were calculated by using $k_2^0 = 0.146$ M^{-1} min^{-1} and $K_S = 450$ M^{-1} and expressing C_T as molar concentration of CTA monomer. (A) Variation of CTA_2T; (B) Variation of CTAB, $Na_2S_2O_3$ = 1.57×10^{-3} M. NaBr concentrations and $K_{T/Br}$ (calculated from Figure 1B) were 0 M, 0.0025 (○); 4.9×10^{-3} M, 0.008 (●); and 9.75×10^{-3} M, 0.011 (△).

micellized detergent. α and $\bar{V}$ for the CTAB micelle were taken as 0.2[4] and 0.37 L/mol,[19] respectively. The data in Figure 1B were fitted by iteration using $K_{T/Br}$ as adjustable parameter, eq 5–9, and the usual assumptions of ion exchange in micellar solutions.[5-7] The best fit values of $K_{T/Br}$ varied between 0.0025 (no NaBr) and 0.03. This latter value can be used to fit all data in Figure 1B for [NaBr] exceeding 0.02 M. As opposed to monovalent–monovalent ion exchange,[5-7,18,20] $K_{T/Br}$ is salt dependent, and therefore, it is an *apparent* selectivity coefficient for the thiosulfate–bromide exchange.

The pseudo-first-order rate constant (k_ψ) for reaction 1 increases with increasing [CTA_2T] above the critical micelle concentration and reaches a plateau (Figure 2A). At 5×10^{-3} M CTA_2T, k_ψ is 1.7×10^3 times larger than that in the absence of micelles at the same total thiosulfate concentration. The CTA_2T dependence of k_ψ (Figure 2A) was fitted to a pseudophase-model equation for a reactive-counterion surfactant:[21]

$$k_\psi = \frac{k_2^0 \alpha C_D + (k_2^m/\bar{V}) K_S (1 - \alpha) C_D}{2(1 + K_S C_D)} \qquad (10)$$

where k_2^m and k_2^0 are the second-order rate constants for reaction 1 in the micelle and in the aqueous phase, respectively. Taking $k_2^0 = 0.146$ M^{-1} min^{-1},[12a] $K_S = 450$ M^{-1}, and $\alpha = 0.15$ (Experimental Section), the best-fit value for the ratio $(k_2^m/\bar{V})$ is 3.3 min^{-1}.

The effect of CTAB upon reaction 1 can be described by eq 11 (which takes into account (i) the ion-exchange equilibria described by eq 6 (ii) the usual assumptions of ion exchange,[5] (iii) independent reactivities in the micellar and aqueous phases,[1-5] and (iv) pseudophase partitioning of a neutral substrate[1-5]), where

$$k_\psi = T_T \frac{k_2^0 + (k_2^m/\bar{V}) K_S K_{T/Br} \dfrac{(Br_b)^2}{(Br_f)^2 C_D \bar{V}}}{(1 + K_S C_D)\left(1 + K_{T/Br} \dfrac{(Br_b)^2}{(Br_f)^2 C_D \bar{V}}\right)} \qquad (11)$$

T_T is the total [$Na_2S_2O_3$]. The lines in Figure 2B were calculated by using eq 11. The values used for $(k_2^m/\bar{V})$, k_2^0, and K_S were 3.3 min^{-1}, 0.146 M^{-1} min^{-1}, and 450 M^{-1}, respectively (see the analysis of the data in Figure 2A). The calculated and the observed k_ψ's are identical within experimental error (Figure 2B).

The value of $(k_2^m/\bar{V}) = 3.3$ min^{-1} was used to calculate the effects of CTA_2T and CTAB on reaction 1. Taking $\bar{V} = 0.37$ L/mol,[19] we find $k_2^m = 1.2$ M^{-1} min^{-1}, a value 8 times larger than that of k_2^0 for the same reaction in water. It has been found that the rate of reaction 1 increases as the dielectric constant of the

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medium decreases.[22] Since the effective dielectric constant of the micellar surface is lower than that of water,[1-3] this could be a contributing factor for the moderate increase in the apparent reactivity of micelle-bound thiosulfate.

In conclusion, we have demonstrated that the combined studies of thiosulfate binding to CTAB micelles and of the effect of CTA_2T micelles on the kinetics of reaction 1 can lead to the prediction of the effect of CTAB on the kinetics of this reaction. This implies that the reactivity of thiosulfate is identical in CTA_2T and CTAB micelles. We also have shown that the ion-exchange formalism is adequate to describe quantitatively the effect of detergents on reactions of divalent ions.

Acknowledgment. Support from the Fundacão de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNP_q), and PNUD/UNESCO (RLA 024/78) is acknowledged. The authors thank P. S. Araujo for careful review of the manuscript and U. Ávila for secretarial assistance.

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Registry No. $S_2O_3^{2-}$, 14383-50-7; CTAB, 57-09-0; CTA_2T, 82209-37-8; butyl iodide, 542-69-8.

Model-Free Approach to the Interpretation of Nuclear Magnetic Resonance Relaxation in Macromolecules. 1. Theory and Range of Validity

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Received November 9, 1981

Abstract: A new approach to the interpretation of nuclear magnetic resonance relaxation experiments on macromolecules in solution is presented. This paper deals with the theoretical foundations and establishes the range of validity of this approach, and the accompanying paper demonstrates how a wide variety of experimental relaxation data can be successfully analyzed by using this approach. For both isotropic and anisotropic overall motion, it is shown that the unique information on fast internal motions contained in relaxation experiments can be completely specified by two model-independent quantities: (1) a generalized order parameter, $\mathcal{S}$, which is a measure of the spatial restriction of the motion, and (2) an effective correlation time, τ_e, which is a measure of the rate of motion. A simple expression for the spectral density involving these two parameters is derived and is shown to be exact when the internal (but not overall) motions are in the extreme narrowing limit. The model-free approach (so called because $\mathcal{S}^2$ and τ_e have model-independent significance) consists of using the above spectral density to least-squares fit relaxation data by treating $\mathcal{S}^2$ and τ_e as adjustable parameters. The range of validity of this approach is illustrated by analyzing error-free relaxation data generated by using sophisticated dynamical models. Empirical rules are presented that allow one to estimate the accuracy of $\mathcal{S}^2$ and τ_e extracted by using the model-free approach by considering their numerical values, the resonance frequencies, and the parameters for the overall motion. For fast internal motions, it is unnecessary to use approaches based on complicated spectral densities derived within the framework of a model because all models that can give the correct value of $\mathcal{S}^2$ work equally well. The unique dynamic information ($\mathcal{S}$ and τ_e) can be easily extracted by using the model-free approach. Moreover, if one desires a physical picture of the motion, the numerical values of $\mathcal{S}^2$ and τ_e can be readily interpreted within a physically reasonable model.

I. Introduction

Nuclear magnetic relaxation data on macromolecules in solution contain information concerning the nature of internal motions that occur in these systems. The usual approach[1] to extracting such information involves the use of dynamical models that are based on physical intuition and/or the ease of formulation. While such analyses can be useful, there is the danger of overinterpretation of limited data and the possibility that the resulting physical picture is not unique. Models cannot be proven; they can only be eliminated.

In this paper we seek to answer the questions: (1) what is the unique information content of a given set of relaxation data and (2) how can one extract that information? In order to clarify the nature of the problem, let us consider a hypothetical example of a ^{13}C NMR relaxation study of a lysine side chain in an isotropically reorienting protein. The relaxation of each carbon nucleus is determined by the fluctuations of the $^{13}C-H$ vectors with respect to the external magnetic field. The observed quantities are determined by the Fourier transform (the spectral density) of an appropriate time-correlation function evaluated at certain frequencies whose values depend on the external field strength. To obtain the time dependence of the correlation function, which contains all the potentially available dynamic information, one would, in principle, need to perform experiments at an infinite number of magnetic field strengths. Even then the dynamic information would be limited because of the nature of the correlation function. With current NMR technology, a typical data set consists of a few numbers (say, T_1's and NOE's at two magnetic fields). As a result of steric constraints and concerted motions, the dynamics of a side chain are extremely complicated, and one cannot expect to construct a detailed picture of the dynamics from a few experimentally accessible numbers that, as we shall see, may contain redundant information.

The simplest possible description of the internal dynamics of the side chain involves specifying (1) the rate (time scale) and (2) the spatial restriction of the motion of each carbon in the chain. Suppose we sit in a frame rigidly attached to the macromolecule

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