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Fluorogenic Reaction of Sulfite with *o*-Phthalaldehyde

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o-Phthalaldehyde (OPA) was found to react with sodium sulfite in borate buffer (pH 9.9) to form a fluorescent product (λ_{ex} 360 nm, λ_{em} 424 nm), which was stable only in the solution. The fluorescence of the product in alkaline solution was stable for at least 6 h. Hydrosulfite and pyrosulfite showed the same degree of fluorescence intensity as sulfite. Other sulfur compounds gave little or no fluorescence. The working curve for sodium sulfite was linear in the range of 0.2—7.0 nmol/2 ml. The detection limit of sodium sulfite was 0.2 nmol/2 ml. The presence of metal ions had practically no influence on the fluorescence intensity of the OPA–sodium sulfite system. However, contamination with ammonium ion and amines should be avoided in the determination of sulfite. The fluorescent product seems to be formed through a mechanism different from that of isoindole formation, but its structure has not yet been determined.

Keywords——*o*-phthalaldehyde; sodium sulfite; fluorogenic reaction; fluorescent product characterization; fluorophotometry

o-Phthalaldehyde (OPA) has widely been used as a fluorogenic reagent for the determination of amino acids and amines. Recently, the use of OPA as a derivatization reagent for the determination of thiols in high-performance liquid chromatographic (HPLC) analyses was also reported.[1a,b] The fluorescent isoindole derivatives formed by the reaction of OPA and amines in the presence of organic thiols are generally unstable in aqueous alkaline solution,[2] though some promising results were obtained regarding the stabilization of isoindole fluorophores.[3] In the course of our studies concerning the stabilization of the products obtained from the reaction of OPA and amines, it was occasionally found that OPA reacts with inorganic sulfur compounds, *e.g.*, sodium bisulfite and sodium sulfite, in the absence of amines such as taurine[1a] or 2-aminoethanol[1b] to give intense fluorescence that is quite stable in solution.

Thus, the present paper deals with the fluorogenic reaction of OPA and inorganic sulfur compounds as a means for the determination of the latter. The characteristics of the reaction product are also described.

Experimental

Reagent and Materials——All chemicals used were of reagent grade unless otherwise stated. OPA of analytical reagent grade was purchased from Tokyo Kasei Kogyo Co., Tokyo and purified by sublimation. Inorganic sulfur compounds were purchased from Wako Pure Chemical Industries Co., Osaka. Other chemicals and solvents were obtained from commercial sources. Deionized and double-distilled water was used throughout. Phosphate buffer (pH 6.0 and 7.0) was prepared by mixing 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4 solutions in different ratios. The borate buffer (0.1 M H_3BO_3 and 0.1 M KCl) was adjusted in the pH range of 8.0—12.0 with 0.1 M NaOH.

Apparatus and Methods——A Hitachi 650-60 fluorescence spectrophotometer was used for the fluorescence measurement. Absorption spectra were measured with a Hitachi 150-20 spectrophotometer.

Typical Reaction Procedure: The stock solutions of OPA (800 μM) and sodium sulfite (10 μM) for the test were prepared in borate buffer (pH 9.9). One milliliter portions of the stock solutions were mixed in a reaction tube and

heated at 80 °C for 15 min. The reaction mixture was immediately cooled to room temperature, and the fluorescence intensity was measured at 424 nm, with excitation at 360 nm, against a reagent blank.

Working Curve: The working curve for the determination of sodium sulfite was prepared under the conditions described above. Thus, sodium sulfite (1.26 mg) was dissolved in borate buffer (pH 9.9, 10 ml), and this solution was diluted to prepare the working solutions corresponding to various concentrations. One milliliter of each working solution was mixed with 1 ml of the OPA stock solution in the reaction tube and treated in the manner described above.

Results and Discussion

Reaction Conditions

In order to find suitable conditions for the determination of inorganic sulfur compounds, the reactivity of OPA and a test compound, sodium sulfite, was examined from various point of view.

Effect of pH——The stock solutions of OPA (800 μM) and sodium sulfite (10 μM) were separately prepared in buffer (pH 6.0—12.0). Aliquots of the solutions were mixed in a reaction tube and sealed. The reaction was carried out under heating at 80 °C for 15 min. As shown in Fig. 1, the extent of reaction depended on the pH. The maximum fluorescence intensity at 424 nm was obtained in the range of pH 9.7—10.0 and decreased at higher and lower pH. Thus, pH 9.9 borate buffer was used for the reaction.

Effects of Reaction Temperature and Time——The time courses of the reaction at various temperature (20—80 °C) are shown in Fig. 2. A constant fluorescence intensity was obtained in the range of 15—30 min at 80 °C and the fluorescence was quenched gradually by further heating at the same temperature.

Effect of OPA Concentration——The effect of OPA concentration on the fluorogenic reaction was examined in the range of 100—1000 μM. A constant fluorescence intensity was obtained with OPA concentrations higher than 500 μM OPA. Thus, an 80-fold molar excess of OPA was used in our experiments.

Stability of Fluorescence Intensity——The fluorescence intensity of the reaction product at room temperature was fully maintained for at least 6 h.

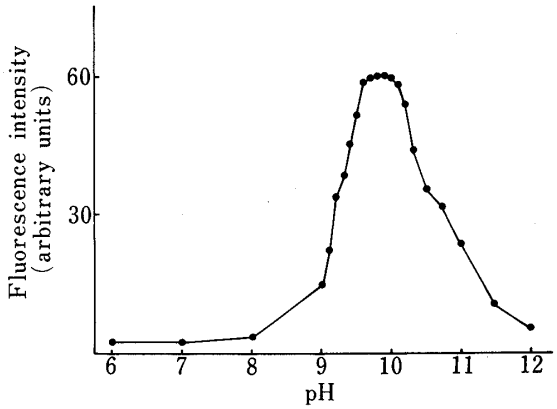

Fig. 1. Dependence of the Fluorogenic Reaction upon pH

One ml of Na_2SO_3 solution (10 μM) was incubated with 1 ml of OPA solution (800 μM) at 80 °C for 15 min. The fluorescence intensity of the cooled reaction solution was measured at 424 nm with excitation at 360 nm.

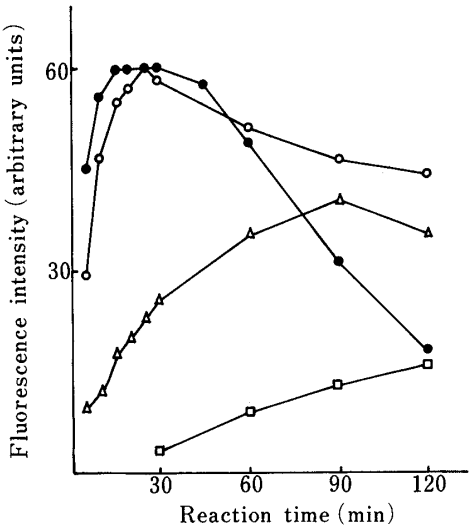

Fig. 2. Time Courses of the Fluorogenic Reaction at Various Temperatures

One ml aliquots of Na_2SO_3 (10 μM) and OPA (800 μM) solutions (pH 9.9) were mixed at various temperatures.

●, 80 °C; ○, 60 °C; △, 40 °C; □, 20 °C.

TABLE I. Relative Fluorescence Intensities of Products Obtained in the Reactions with OPA[a]

Compound	F.I.[b]	Compound	F.I.[b]
Na_2SO_3	100	$Na_2S_2O_6 \cdot 2H_2O$	1
$K_2SO_3 \cdot 2H_2O$	101	$Na_2S_2O_7$	0
$Na_2S_2O_4$	97	$K_2S_2O_7$	0
$Na_2S_2O_5$	99	$Na_2S_2O_8$	1
$K_2S_2O_5$	118	$K_2S_2O_8$	1
$Na_2S \cdot 9H_2O$	4	NaSH	6
Na_2SO_4	1	NaSCN	0
K_2SO_4	1	KSCN	7
$Na_2S_2O_3 \cdot 5H_2O$	0		

a) One ml of sulfur compound solution (pH 9.9, 10 μM) was treated with 1 ml of OPA solution (pH 9.9, 800 μM) at 80 °C for 15 min. Fluorescence intensity was measured at 424 nm with excitation at 360 nm. *b*) The fluorescence intensity (F.I.) of Na_2SO_3 solution was arbitrary taken as 100.

Reaction of Various Inorganic Sulfur Compounds with OPA

Based on the above observations, the standard reaction conditions was chosen as described in Experimental. The reactivities of various inorganic sulfur compounds with OPA were also examined by using the standard procedure. The relative fluorescence intensities of the test compounds are summarized in Table I. As shown in Table I, hyposulfite ($S_2O_4^{2-}$) and pyrosulfite ($S_2O_5^{2-}$) gave approximately equal degrees of fluorescence intensity as compared with sulfite (SO_3^{2-}). However, the former two are not found in nature, and bisulfite (HSO_3^-) can be regarded as being mostly in sulfite form at pH 9.9 on theoretical grounds. On the other hand, analogous sulfur compounds, *e.g.*, SH^-, SCN^-, S_2^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_6^{2-}$, $S_2O_7^{2-}$, and $S_2O_8^{2-}$, gave little or no fluorescence. Thus, this reaction seems to be selective for sulfite.

Interference Study

Effect of Metal Ions——The interference of metal ions was examined. The presence of metal ions (20 nmol) such as Cu^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Sr^{2+}, Cd^{2+}, Ba^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Al^{3+}, Fe^{3+}, and Sn^{4+} had no influence on the fluorescence intensity of the OPA–sodium sulfite system. No addition of ethylenediamine tetraacetic acid (EDTA), therefore, was required for the reaction.

Effect of Ammonium Ion and Amines——OPA is known to react with ammonium ion[4] and aromatic amines[5] giving a color and *N*-phthalimidines, respectively. Thus, the effects of these compounds on the reaction of OPA and sodium sulfite were examined. The addition of ammonium nitrate (20 nmol) to the above system resulted in marked enhancement of the fluorescence intensity, while that of amines (20 nmol) such as 2-aminoethanol, cyclohexylamine, benzylamine, and aniline caused marked quenching. Therefore, contamination with ammonium ion and amines should be avoided in the determination of sulfite.

Working Curve

The working curve of sodium sulfite was linear in the range of 0.2—7.0 nmol/2 ml. The coefficient of variation for 0.5 nmol of sodium sulfite was 4.4% ($n=10$), and the detection limit (at which the fluorescence intensity was twice that of the blank) was 0.2 nmol/2 ml.

Characteristics of the Fluorescent Product

Figure 3 shows the ultraviolet (UV) absorption spectra of OPA treated at 80 °C for 15 min in the absence and presence of sodium sulfite, together with the fluorescence spectrum of the reaction product. The presence of sodium sulfite resulted in a new band having an absorption maximum at 360 nm, indicating the formation of the reaction product from OPA

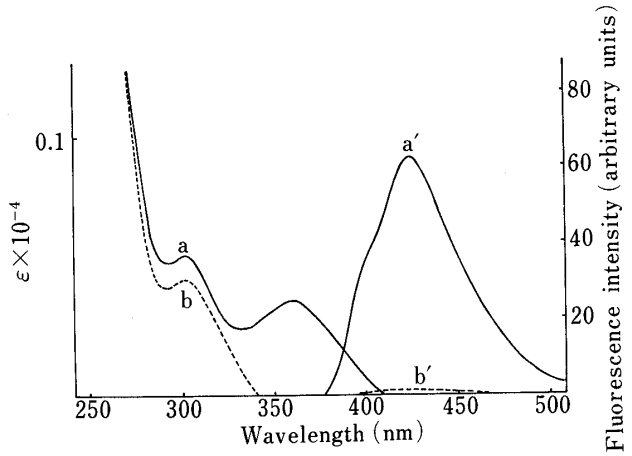

Fig. 3. Absorption and Fluorescence Spectra of OPA in the Absence and Presence of Sodium Sulfite

The solutions used in the measurement were prepared by heating at 80 °C for 15 min. Concentrations for absorption spectra (a and b): OPA, 200 μM; Na_2SO_3, 100 μM. Concentrations for fluorescence spectra (a′ and b′): 800 μM; Na_2SO_3, 10 μM. a and a′, with Na_2SO_3; b and b′, without Na_2SO_3.

and sodium sulfite. Thus, the isolation of the product on a preparative scale was attempted in order to elucidate the structure and the reaction mechanism. The aqueous reaction mixture was extracted with organic solvents, but no product was obtained. Isolation based upon thin layer chromatography (TLC, benzene–ethyl acetate = 4 : 1) gave colorless crystals (mp 143—146 °C), which were practically insoluble in organic solvents. Although this compound was positive to the sodium nitroprusside test for sulfur, the absorption band at 360 nm observed in the reaction mixture was no longer seen. Therefore, this compound can be distinguished from the fluorescent product. It appears from these results that the product is stable only in the reaction solution and changes to some other compound during isolation, and that it is formed through a mechanism quite different from those reported by earlier investigators.[2] Further characterization of the product is under way.

The sensitivity of the present method is somewhat low in comparison with that of the HPLC method presented by Mopper and Delmas,[1b] but it is comparable to those of the indophenol blue method reported by Nakamura and Tamura[6] and another method.[7]

This reaction is simple in operation, and therefore may be convenient for the determination of sulfite, after the removal of interfering ammonium ion and amines by ion-exchange prior to the analyses.

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