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The Color Reaction of Purine Bases by Ternary-complex Formation with Eosin and Silver(I)†

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A color reaction of purine bases utilizing a ternary-complex system among a purine base, a metal ion, and a xanthene dye was studied, and the fundamental conditions for the spectrophotometric determination of purine bases(as adenine) with eosin and silver(I) were established. The absorption maxima of the ternary-complexes lie at 550—560 nm against water. The optimum pH ranges for the color formation of the complexes are pH 4.8—5.4. Beer's law is obeyed over the range of 1—10 μg/10 cm³ of adenine, and the apparent molar absorptivity is 1.1×10^5 dm³ mol⁻¹ cm⁻¹ at 560 nm. In the determination of adenine, the coexistence of pyrimidine bases, such as uracil and cytosine, and nucleotides containing adenine, such as 5′-adenosine triphosphate(ATP) and β-nicotinamide-adenine dinucleotide(NAD), scarcely interfere.

Various photometries and fluorometries of ions and substances utilizing the ternary-complex system (metal ion-electronegative ligand-organic base system) have been reported[1-8]; for example, the ionic-association complex system between a metal ion-1,10-phenanthroline(phen) cation and the anion of a dyestuff such as a xanthene derivative have been studied.

Eosin(2,4,5,7-tetrabromofluorescein) as a xanthene derivative is already used for the determination of such metal ions as silver(I), cadmium(II), iron(III), and cobalt(II).[9-15] However, the method for the determination of organic compounds by using eosin and a metal ion have not been reported entirely.

On the other hand, we recognized that the addition of a purine base to an eosin-silver(I) solution (Solution C) showed a red shift compared with that of Solution C or eosin solution (Solution B), and that the magnitude of the increase in the absorbance of this mixed solution at around 550—560 nm was proportional to the concentration of a purine base.

In this paper, therefore, the fundamental conditions for a new color reaction of purine bases by ternary-complex formation with eosin and silver(I) were studied, and the spectrophotometric determination of purine bases(as adenine) using this color reaction has been established.

Experimental

Reagents and Materials. Stock solutions (1.0×10^{-2} mol dm⁻³) of purine bases were prepared by dissolving proper quantities of purine bases in 2 cm³ of 10% sulfuric acid and diluting the mixtures to 100 cm³ with water. The working solution was made up to the suitable dilution by the use of this stock solution as required. A stock solution (1.0×10^{-1} mol dm⁻³) of silver(I) was prepared by dissolving silver nitrate in water, and then corrected by potentiometry. The working solution (1.0×10^{-3} mol dm⁻³) was made by the suitable dilution of this stock solution. A solution (1.0×10^{-3} mol dm⁻³) of eosin was prepared by dissolving eosin ($C_{20}H_6O_5Br_4Na_2$, Tokyo Kasei Kogyo Co., Ltd., Tokyo) in water. A 2.0% poly(*N*-vinylpyrrolidone)(PVP) solution was prepared by dissolving PVP (K-15, Tokyo Kasei Kogyo, Co., Ltd.) in water. A buffer solution (pH 5.0) was prepared by mixing 0.2 mol dm⁻³ acetic acid and 0.2 mol dm⁻³ sodium acetate solutions. All the other reagents and materials were of an analytical grade and were used without further purification. Doubly distilled water was used.

Apparatus. A Shimadzu Model UV-240 recording spectrophotometer with 1.0-cm quartz cells was used for the absorption spectra and absorbance measurements. A Hitachi-Horiba Model F-7 AD glass electrode pH meter was used for the pH measurements. A Metrohm Model E336A potentiograph was employed for the potentiometric titrations.

Standard Procedure for the Determination of Adenine. A solution containing 1—10 μg adenine was transferred to a 10 cm³ volumetric flask; to this solution 1.0 cm³ of a 2.0% PVP solution, 3.0 cm³ of the buffer (pH 5.0) solution, 0.5 cm³ of a 1.0×10^{-3} mol dm⁻³ eosin solution and 1.0 cm³ of a 1.0×10^{-3} mol dm⁻³ silver(I) solution were added. The mixture was diluted to 10 cm³ with water, kept at 45 °C for 20 min, and then cooled for 10 min in water. The absorbance of the eosin-silver(I)-adenine solution (Solution A) was measured at 560 nm against a eosin solution (Solution B).

Results and Discussion

Color Reaction and Absorption Spectra. On the addition of adenine, a purine base, to the eosin-silver(I) solution (Solution C), the maximum absorption of the eosin-silver(I)-adenine ternary complex solution (Solution A) at around 560 nm was distinctly observed; its absorbance was proportional to the concentration of adenine. Solution A without a surfactant was unstable, and it gradually precipitated on standing. The use of a nonionic surfactant was effective as a dispersion agent. By the way, no difference in absorption spectra between Solution C and the eosin solution (Solution B) in the absence of adenine was observed. The absorption spectra of Solutions A, B, and C in the presence of PVP at pH 5.0 are shown in Fig. 1.

On the other hand, other purine bases, such as guanine or phen, also formed the colored complexes, but the difference in absorbance between Solution B and its ternary complex solution was small as compared with that of the adenine complex solution. The absorption spectrum of the eosin-silver(I)-guanine solution or the eosin-silver(I)-phen solution under the same condi-

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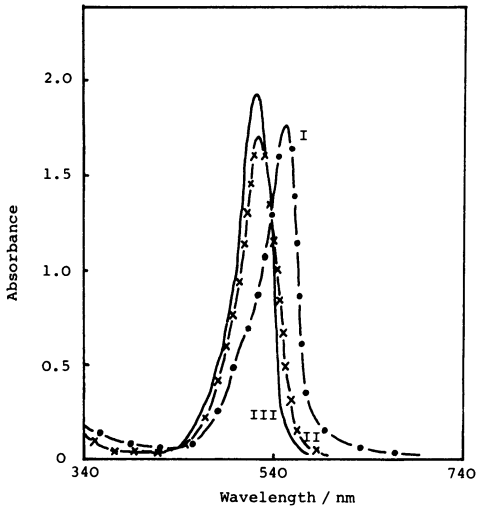

Fig. 1. Absorption spectra of eosin, eosin–silver(I), eosin–silver(I)–adenine, eosin–silver(I)–guanine, and eosin–silver(I)–phen solutions at pH 5.0.
Eosin: 2.5×10^{-5} mol dm^{-3}; silver(I): 5.0×10^{-5} mol dm^{-3}; adenine, guanine, and phen: 5.0×10^{-5} mol dm^{-3}; PVP: 1.0 cm^3 of 2.0% PVP solution/10 cm^3; Reference: water; curve I: eosin–silver(I)–adenine solution; curve II: eosin–silver(I)–guanine and eosin–silver(I)–phen solutions; curve III: eosin–silver(I) and eosin solution.

tions is also shown in Fig. 1.

The effect of dyes was studied by measuring the absorbance of Solution A against a dye solution. Eosin was superior to the other dyes tested in terms of sensitivity. The results are given in Table 1.

Only silver(I) was effective among the various metal ions: silver(I), cadmium(II), copper(II), zinc(II), iron (III), manganese(II), cobalt(II), cerium(III), lanthanum(III), thorium(IV), paladium(II), *etc.* The eosin–silver(I)–purine base ternary complex solution was fairly stable and was scarcely affected by any metal ions.

In the ternary complex systems among eosin, silver(I), and purine bases, the color reactions of various purine bases were examined. The difference in absorbance between the eosin–silver(I)–adenine solution and Solution B was larger than that of the other purine bases tested. The results are given in Table 2.

From the results described above, adenine was chosen for the purpose of fundamental investigations of the determination of purine bases, and the absorbance of Solution A at 560 nm against Solution B was measured in order to allow the determination of adenine.

Effect of pH. The effect of the pH on the color development was investigated with a solution containing 6.8 μg/10 cm^3 adenine. As is shown in Fig. 2, the ranges in which the maximum and nearly constant absorbance was observed at pH 4.8—5.4. Hence, the pH value of 5.0 with a 0.2 mol dm^{-3} acetic acid −0.2 mol dm^{-3} sodium acetate buffer solution was used for the pH adjustment.

Effect of Surfactants. Among the various surfactants tested, PVP(K-15), a nonionic surfactant, was best as a dispersion agent; the maximum and constant absorbance of Solution A was observed upon the addition of more than 0.75 cm^3 of a 2.0% PVP solution to the final volume of 10 cm^3. The results are given in Table 3.

Effect of Eosin and Silver(I) Concentrations. The effect of the amounts of eosin and silver(I) was exam-

Table 1. Effect of dyes

Dyes	Absorbance	at λ_{max}
Eosin	0.490	560
Erythrosine**	0.123	570
Phloxine**	0.153	575
Rose Bengal**	0.106	585
2,4,5,7-Tetrachlorofluorescein*	0.026	555
3′,4′,5′,6′-Tetrachlorofluorescein*	0	—
2,7-Dichlorofluorescein*	0.020	530
Fluorescein*	0	—
Pyrogallol Red**	0	—
Bromopyrogallol Red**	0.020	585
o-Hydroxyhydroquinonephthalein*	0	—
2,4,5,7-Tetrabromophenol-sulfonphthalein*	0	—
2,4,5,7-Tetrabromophenolphthalein*	0	—

Adenine taken: 6.8 μg/10 cm^3; dye: 5.0×10^{-5} mol dm^{-3}; silver(I): 1.0×10^{-4} mol dm^{-3}; PVP: 1.0 cm^3 of 2.0% PVP solution/10 cm^3; pH: 5.0; Reference: dye solution. * Synthesized by the published procedure[19]. ** Pure grade materials available commercially.

Table 2. Color reaction between some purine bases and solution B

Purine bases	(mol dm^{-3})	Absorbance	at λ_{max}
Adenine	5.0×10^{-6}	0.490	560
Guanine	2.0×10^{-5}	0.330	555
Hypoxanthine	5.0×10^{-5}	0.135	550
Purine	5.0×10^{-5}	0.140	555
6-Iodopurine	5.0×10^{-6}	0.310	560
6-Mercaptopurine	5.0×10^{-6}	0.125	555
1-Methyladenine	5.0×10^{-5}	0.015	555

Eosin: 5.0×10^{-5} mol dm^{-3}; silver(I): 1.0×10^{-4} mol dm^{-3}; PVP: 1.0 cm^3 of 2.0% PVP solution/10 cm^3; pH: 5.0; Reference: Solution B.

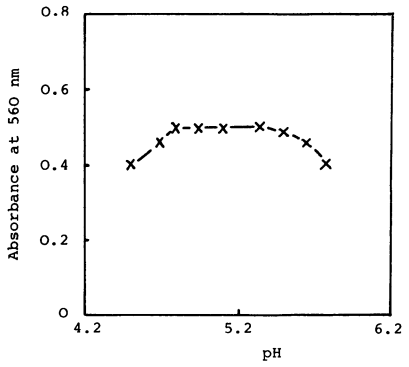

Fig. 2. Effect of pH.
Adenine: 6.8 μg/10 cm^3(5.0×10^{-6} mol dm^{-3}); silver (I): 1.0×10^{-4} mol dm^{-3}; eosin: 5.0×10^{-5} mol dm^{-3}; PVP: 1.0 cm^3 of 2,0% PVP solution/10 cm^3; Reference: Solution B.

TABLE 3. EFFECT OF SURFACTANTS

Surfactants	Absorbance at λ_{max}	
PVP(K-15)	0.490	560
PVP(K-30)	0.475	560
PVP(K-90)	0.438	560
Polyvinyl alcohol(n: 500)	0.370	555
Polyvinyl alcohol(n: 2000)	0.413	555
Poly(oxyethylene) sorbitan monolaurate	0.336	560
Gum arabic	0.320	550
Gelatin	0.257	550
Sodium dodecyl sulfate	0.124	545
Hexadecyltrimethylammonium chloride	0	—

Adenine taken: 6.8 μg/10 cm³; eosin: 5.0×10^{-5} mol dm^{-3}; silver(I): 1.0×10^{-4} mol dm^{-3}; surfactant: 1.0 cm³ of 2.0% surfactant solution/10 cm³; pH: 5.0; Reference: Solution B.

ined by varying the molar ratio of eosin to silver(I), the amount of adenine being kept constant. A maximum and almost constant absorbance was observed upon the addition of a 0.5—1.5 cm³ of 1.0×10^{-3} mol dm^{-3} silver(I) solution. The molar ratio of silver(I) to eosin in the complex was found by the molar-ratio method to be 2:1 in the presence of adenine.

Accordingly, all further work was carried out with 5.0×10^{-5} mol dm^{-3} eosin and 1.0×10^{-4} mol dm^{-3} silver(I) in the final volume.

Sequence of Addition and Stability. The maximum absorbance was obtained when silver(I) was finally added to an eosin-adenine solution. The color formation of the ternary complex did not occur instantaneously at room temperature (15—25 °C), about more than 120 min being required for a complete color reaction. The effect of the temperature on the color formation was examined by heating the mixed solution for 10—60 min at various temperature (30°, 45°, or 60 °C); the maximum and constant absorbance was obtained by heating for 20 min at 45 °C and cooling for 10 min in water. The absorbance was constant for at least 2 h at room temperature.

Calibration Curve. The calibration curve for adenine was constructed according to the standard procedure. It was found that Beer's law held in the concentration range of 1—10 μg of adenine in the final volume of 10 cm³. The apparent molar absorptivity at 560 nm was estimated to be 1.1×10^{5} dm³ mol^{-1} cm^{-1}. The sensitivity, according to Sandell's scale, was 0.0013 μg cm^{-2} for adenine. The relative standard deviation for five replicate determinations was 0.68% for 6.8 μg of adenine.

Effects of Foreign Substances. The effects of foreign substances on the determination of adenine were also examined. The coexistence of most metal ions did not interfere in a 100-fold excess over adenine. Among the various anions tested, the coexistence of small amounts of thiosulfate, thiocyanate, cyanide, and iodide, and the presence of large amounts of chloride gave negative errors because of reacting with silver(I). These anions could be conveniently eliminated by the addition of a silver nitrate solution as a preliminary step. Anions such as phosphate, nitrate, and organic acid ions did not interfere. The presence of small amounts of such purine bases as guanine, hypoxanthine, 6-mercaptopurine, and 6-iodopurine, and the coexistence of large amounts of such purine bases as purine, 1-methyladenine, and uric acid did interfere, though, xanthine derivatives such as caffeine and theophilline, and pyrimidine bases such as uracil and cytosine, did not affect the determination of adenine. Moreover, nucleosides and nucleotides, such as adenosine, 5′-adenine triphosphate (ATP), β-nicotinamide-adenine dinucleotide (NAD) and adenosine cyclic 3′,

TABLE 4. EFFECT OF FOREIGN SUBSTANCES

Substances	Added (μg/10 cm³)	Mole ratio (Substance/Adenine)	Absorbance at 560 nm
—	—	—	0.490
Fe(III), Sulfate	279.3	100	0.419
Cd(II), Nitrate	281.0	50	0.546
Zn(II), Nitrate	326.9	100	0.490
Mg(II), Nitrate	274.7	100	0.490
Cl^-, Sodium	35.5	20	0.327
I^-, Potassium	12.7	2	0.356
CN^-, Potassium	6.5	5	0.440
SCN^-, Potassium	5.8	2	0.417
$S_2O_3^{2-}$, Sodium	1.4	1/5	0.398
$H_2PO_4^-$, Potassium	485.0	100	0.490
Tartaric acid	750.5	100	0.490
Citric acid	960.6	100	0.490
Guanine	1.5	1/5	0.320
Hypoxanthine	3.4	1/2	0.400
6-Iodopurine	2.5	1/5	0.268
6-Mercaptopurine	1.5	1/5	0.395
Purine	12.0	2	0.450
1-Methyladenine	37.5	5	0.450
Uric acid	42.0	5	0.384
Theophylline	900.8	100	0.490
Uracil	560.5	100	0.490
Cytosine	555.5	100	0.490
Adenosine	1336.2	100	0.490
ATP	3025.0	100	0.490
NAD	3317.5	100	0.490
Cyclic AMP	1646.0	100	0.490
FAD*	865.6	20	0.417
Putrescine hydrochloride	44.1	10	0.343
Spermidine hydrochloride	36.3	5	0.410
Glutamine	730.8	100	0.490
L-Ascorbic acid	880.6	100	0.490
D-Glucose	900.8	100	0.490
Albumin(Human)	50.0	—	0.438
DNA(Herring)**	20.0	—	0.388

Adenine taken: 6.8 μg/10 cm³; eosin: 5.0×10^{-5} mol dm^{-3}; silver(I): 1.0×10^{-4} mol dm^{-3}; PVP: 1.0 cm³ of 2.0% PVP solution/10 cm³; pH: 5.0; Reference: Solution B.
* FAD, Flavin adenine dinucleotide; ** DNA, Deoxyribonucleic acid.

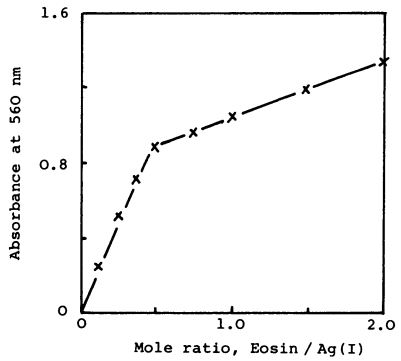

Fig. 3. Composition of [eosin: silver(I)] in the presence of adenine obtained by the molar-ratio method. Silver(I): 2.0×10^{-5} mol dm^{-3}; eosin: 2.0×10^{-4} mol dm$^{-3}\times$X cm^3/10 cm^3; adenine: 1.0×10^{-4} mol dm^{-3}; PVP: 1.0 cm^3 of 2.0% PVP solution/10 cm^3; pH: 5.0; Reference: Solution B.

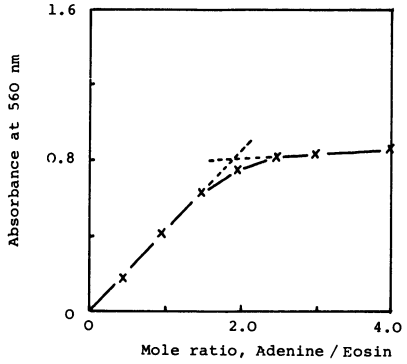

Fig. 4. Composition of [adenine: eosin] in the presence of silver(I) obtained by the molar-ratio method. Silver(I): 2.0×10^{-5} mol dm^{-3}; eosin: 1.0×10^{-5} mol dm^{-3}; adenine: 1.0×10^{-4} mol dm$^{-3}\times$X cm^3/10 cm^3; PVP: 1.0 cm^3 of 2.0% PVP solution/10 cm^3; pH:5.0; Reference: Solution B.

5′-monophosphate (cyclic AMP), also did not interfere in a 100-fold excess. Though such amines as putrescine, spermidine and tryptamine interfered in large amounts, amino acids, D-glucose, L-ascorbic acid, *etc.* did not interfere. The results are summarized in Table 4.

The Composition of the Complex. The mole ratio of silver(I) to eosin was established by the molar-ratio and continuous-variation methods. The results indicated that the mole ratio of silver(I) to eosin was 2:1. The results obtained by the molar-ratio method are shown in Fig. 3.

It was found that the adenine-to-eosin ratio obtained by the molar-ratio method was 2:1, as is shown in Fig. 4.

From these results, it is deduced that the ternary complex formed in this reaction system may be expressed as $(\text{adenine})_2(\text{Ag})_2(\text{eosin})$.

Color Reaction between Substances Containing Adenine and Solution C. The color reactions between some substances containing adenine and Solution C were also studied. No difference in color between these substances containing adenine and Solution C was observed. Therefore, liberated adenine was determined by the standard procedure after these substances containing adenine had been treated with acid-hydrolysis as a preliminary step. The results are given in Table 5.

TABLE 5. ANALYTICAL RESULTS OF SUBSTANCES CONTAINING ADENINE AS OBTAINED BY THE STANDARD PROCEDURE AFTER ACID-HYDROLYSIS

Substances	Recovery* (%)	
	Method A	Method B
Adenosine	99.1	—
ATP	98.7	—
NAD	84.0	—
Cyclic AMP	52.5	70.0
FAD	0	76.6

Eosin: 5.0×10^{-5} mol dm^{-3}; silver(I): 1.0×10^{-4} mol dm^{-3}; PVP: 1.0 cm^3 of 2.0% PVP solution/10 cm^3; pH 5.0; Reference: Solution B.

* Mean of 3 determinations; method A, boiled gently for 20 min in a 10% H_2SO_4 solution; method B, refluxed for 1 h in a 5% H_2SO_4 solution.

Though further investigation is necessary, this proposed method may also be useful in the determination of nucleosides and nucleotides.

Conclusion

The fundamental conditions for the color reaction of purine bases by ternary-complex formation with eosin and silver(I) have been discussed, and a spectrophotometric method for the determination of adenine, a purine base, utilizing this ternary complex has been established. This method could be used in the concentration range of 1—10 μg/10 cm^3 adenine at 560 nm. The apparent molar absorptivity in this procedure was estimated to be 1.1×10^5 dm^3 mol^{-1} cm^{-1} for adenine. The method is inferior to the fluorometric methods[16,17] in sensitivity, but it is about 10 times more sensitive than the method[18] utilizing the diazo-coupling reaction with the Brotton-Marshall reagent. This method is superior to those methods in terms of rapidity, simplicity, and reproducibility. In addition, this proposed method for the determination of adenine is scarcely affected by the coexistence of pyrimidine bases, such as uracil and cytosine, and the substances containing adenine, such as adenosine, ATP and NAD. The application to the determination of these nucleosides or nucleotides with a suitable preliminary treatment may be feasible. Therefore, the application to high-performance liquid chromatography for purine bases can also be expected.

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