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A Novel Photographic Assay of 1-Phenyl-5-mercaptotetrazole Having a Formylhydrazino Moiety by Using Its Electron Injecting Activity toward Silver Bromide Grains

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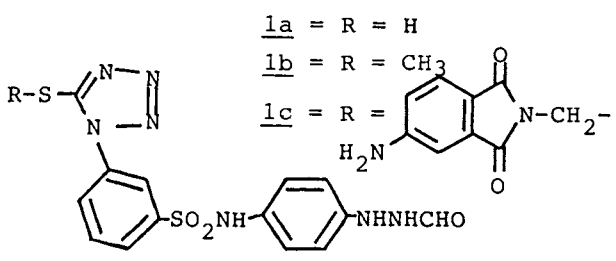
A novel photographic assay of 1-(3-[4-(2-formylhydrazino)-phenylaminosulfonyl]phenyl)-5-mercaptotetrazole is described. The present result provides the first example exhibiting a quantitative correlation between the logarithm of concentration of an organic reductant used instead of exposure to light and the optical density of the film after development. An applicability of this system for the determination of traces of enzyme is also suggested throughout several model experiments.

It is well accepted that the photographic process[1] includes a chemical amplification step induced by silver metal (Ag°) nuclei.[2] Exposure of a film to light is regarded as an injection process of photoelectrons toward silver ion (Ag^+). It produces Ag° nuclei in a silver halide grain in the film. The Ag° nuclei thus produced, even in very small amounts, exhibit a strong catalytic activity for the formation of silver image in the kinetically controlled[3] development, which depends on the light exposed fraction of Ag^+. Consequently, a linear relationship sets up between the optical density of the silver image and the logarithm of the intensity of light over limited exposure ranges,[4] as shown in Fig. 1. In the course of studies on the application of Ag° nuclei-catalyzed chemical amplification, one of our current interests is to demonstrate a new concept based on the formation of Ag° nuclei by using the reducing power of a certain kind of organic molecule instead of photoelectrons, that is possible determination of traces of an organic molecule in the photographic system. We report herein the highly sensitive photographic assay of 1-(3-[4-(2-formylhydrazino)phenylaminosulfonyl]phenyl)-5-mercaptotetrazole 1a.



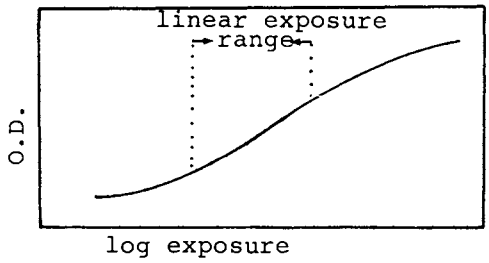


Fig. 1. Optical density-log exposure curve.

This assay requires an organic molecule (a nucleating reagent) for producing Ag° nuclei instead of exposure to light. Moreover, the presence of a linear relationship is essential for the logarithmic plot of concentration of a nucleating reagent as a function of the optical density of the film after development. We devised a photographic assay system using the negative film comprised of layers as illustrated in Fig. 2 for evaluation of the nucleating activity of a number of compounds. Typical procedure is as follows: Graded amounts of a compound in an aqueous solution (40 mm^3) were spotted onto a 5 mmϕ area of the negative films having an unexposed AgBr emulsion layer.[5] After standing for 5 min in order to complete the nucleation reaction at a dark room, these were developed in the alkali solution (pH =13) containing excess of 1-phenyl-3-pyrazolidone[6] and hydroquinone at 25 °C for 2 min, followed by fixing, rinsing with water, and drying. The optical densities of the films were then determined with a transmission densitometer.

A number of compounds have been examined in the viewpoint of their oxidation potentials and adsorptive activities toward AgBr. As a result, 1a[7] was found as one of the most efficient nucleating reagents. It is to be noted that a linear relationship clearly exists between the optical density of the film and the logarithm of concentration of 1a over the range from 4.0×10^{-8} to 8.0×10^{-10} M (line A in Fig. 3). Apart from the inherrent action[8] of 1a involved in the Ag° nuclei forming process, the decrease in the activity for 1b (mp 154-157 °C) indicated that the thiol moiety in 1a plays an important role[9] in this process (line B in Fig. 3).

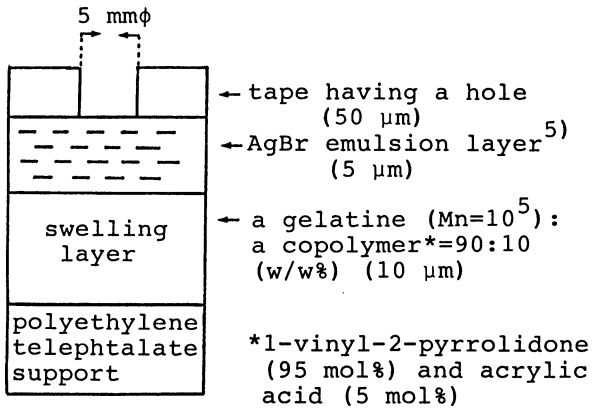


Fig. 2. Composition of layers for the photographic assay.

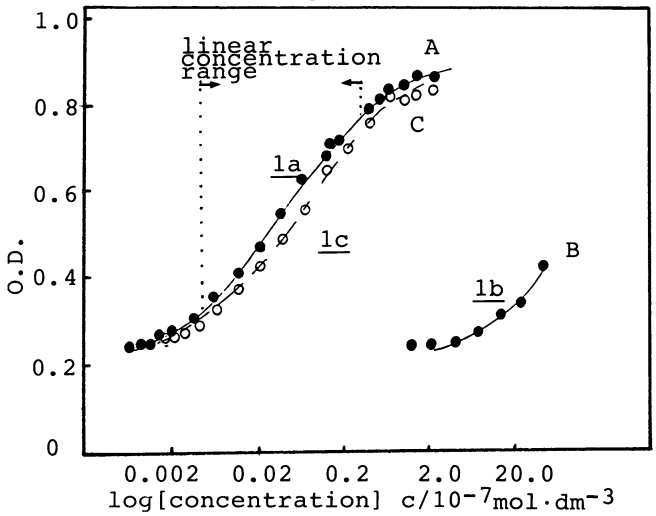


Fig. 3. The optical density-log[concentration] curve for 1a, 1b, and 1c.

This is the first example for the determination of traces of an organic compound by using the photographic process. The present result further suggested that 1a is applicable as a sensitive labeling reagent for the determination of traces of enzyme, when enzymatic reactions are incorporated in this assay process. An imaginary substrate 2 for the determination of α-amylase is represented in Fig. 4. Several experiments were carried out for providing the solutions to the problems present in the substrate 2, as described below briefly.

(1) How is 1a modified without loss of the nucleating activity?

(2) Does the biotine part exhibit an enough bioaffinity toward avidine for the separation of excess of the substrate 2 from the nucleating reagent liberated?

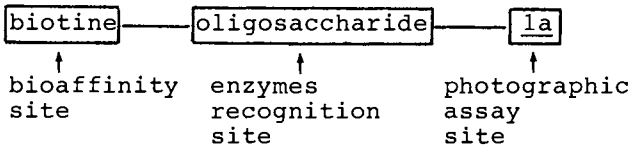


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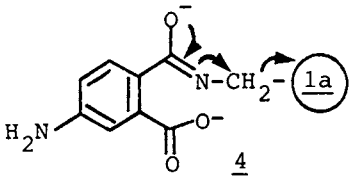
Fig. 4. An imaginary α-amylase substrate.

In the first place, 1a was transformed into an alkali labile precursor having an amino moiety 1c.[7] As expected, 1c, when treated in buffer (pH=13) at 36 °C, smoothly released 1a according to the pseudo-first order kinetics with $k = 2.05 \times 10^{-1}\ s^{-1}$ ($t_{1/2}$=3.5 s)(see a plausible intermediate 4). Its apparent nucleating activity in the spot test was also comparable to that of 1a (line C in Fig. 3). A model compound 3[7] was next prepared and the preliminary characterization of bioaffinity between 3 and avidine was examined.



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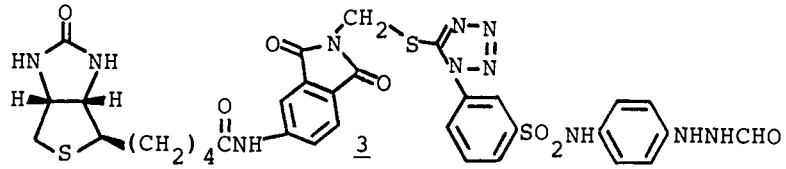

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Each solution of 1a, 1c, and 3 (4.0×10^{-5}M, 300 mm^3) was charged into the avidine-agarose gel (16 units per cm^3; 5 mmϕ, 1.5 cm long)[10] preequibrated with acetic acid containing 0.125 M pyridine (pH=6.0). After standing for 30 min, an eluting solution containing 0.5 M NaCl (300 mm^3) was passed and then the aliquots (40 mm^3) from the whole fraction (600 mm^3) was provided to the photographic test. The values of optical densities for 1a, 1c, and 3, when the eluents having two different ionic strength were used, are summarized in Table 1. Compounds 1a and 1c which are free from biotine gave the same values, respectively, as that of the direct addition[11] of 1a (2.0×10^{-5}M; 40 mm^3) onto the film. On the other hand, the value for 3 containing the biotine part was in excellent agreement with that for the case of a blank experiment.[12] These preliminary results indicate that the biotine part in the substrate 2 is possible to play a role in separation of excess of unreacted 2 from 1c liberated by catalytic action of enzymes.

Table 1. The optical density obtained by the photographic assay after treatment with avidine-agarose gel (5 runs)

Compound	Optical density of the negative film		
	NaCl concentration in the eluent		
	0.1 M	0.5 M	direct addition
1a	0.27±0.01	1.20±0.02	1.21±0.03
1c	0.28±0.01	1.11±0.01	1.15±0.01
3	0.29±0.01	0.40±0.03	1.15±0.01
Blank	0.35±0.02	0.38±0.04	0.35±0.01

In conclusion, we could constract a novel photographic assay based on the formation of Ag° nuclei by using compound 1a instead of exposure to light. It should be noted that a model study provided experimental justification to an imaginary substrate 2 for the photographic assay of α-amylase. The embodiment of α-amylase substrate and the enzymatic characterization will be presented elsewhere.[13,14]

References

1) For an excellent book, see: "The Theory of the Photographic Process," 4th ed, ed by T. H. James, Macmillan Publishing Co., Inc., New York (1977).
2) For the catalytic activity of silver metal nuclei, see: R. E. Maerker, J. Opt. Soc. Am., 46, 17 (1956).
3) Development (reduction of Ag^+) is usually carried out at 36 °C for 3 min.
4) O. H. Schade, Sr., J. Soc. Mot. Pict. Telv. Eng., 73, 81 (1964).
5) A tabular pure AgBr having a mean grain size of 1.8 μm and thickness of 1.6 μm; binder, gelatin (the isoelectric point is 4.9): polyacrylamide = 70 : 30 (w/w%), AgBr amount coated, 2.0 g/m^2; AgBr/binder = 2 : 5 by weight.
6) The first redox potential E_1 = -80 mV vs. SCE in 50 v/v% methanolic buffer (pH= 11.5). The pKa = 9.7 in 50 v/v% aqueous methanol, see: M. P. Youngblood, J. Am. Chem. Soc., 111, 1843 (1989).
7) The ^1H NMR spectra of 1a, 1c, and 3 exhibit the existence of unseparable two rotational isomers derived from restriction of the rotation between -NHNH and -CHO.
1a: mp 158 °C (dec); ^1H NMR (DMSO d_6+D_2O) δ 6.65 and 6.85 (each 0.4H, ABq, J=10 Hz), 6.68 and 7.00 (each 0.6H, ABq, J=10 Hz), 7.75-7.90 (2H, m), 8.06 (1H, s), 8.15-8.30 (1H, br), and 8.40 (1H, br s); MS m/z 391 (M^+); IR (KBr) ν 3500, 3300, 1680, 1620, 1610, 1520 cm^{-1}; Found: C, 42.92; H, 3.39; N, 25.23%. Calcd for $C_{14}H_{13}O_3N_7S_2$: C, 42.96; H, 3.35; N, 25.05%; The exact value of the redox potential could not get owing to the thiol moiety in 1a. The first redox potential E_1 of 1b = -270 mV vs. SCE in 50 v/v% methanolic buffer (pH=11.5).
1c: mp 152 °C; ^1H NMR (DMSO d_6+D_2O) δ 5.05 and 5.10 (total 2H, each s, $-NCH_2S-$), 6.70 and 6.92 (each 1.4H, ABq, J=10 Hz), 6.72 and 6.93 (each 0.6H, ABq, J=10 Hz), 6.95 and 7.55 (each 1H, ABq, J=8 Hz), 7.05 (1H, br s), 7.80-8.10 (5H, m), and 8.15 (1H, br s, -CHO); MS m/z 565 (M^+); IR (KBr) ν 3450, 3340, 3240, 1775, 1720, 1695, 1620, 1515 cm^{-1}; Found: C, 48.76; H, 3.42; N, 22.07%. Calcd for $C_{23}H_{19}N_9O_5S_2$: C, 48.85; H, 3.39; N, 22.29%.
3: mp 198 °C; $[\alpha]_D^{23}$+75.8 (c 0.34, MeOH/THF (3:1)); ^1H NMR (DMSO d_6+D_2O) δ 1.10-1.60 (6H, m), 1.95-2.20 (2H, m), 2.55 (1H, br s), 2.62 (1H, d, J=13 Hz), 2.80 (1H, dd, J=6 and 13 Hz), 4.04-4.15 (1H, br), 4.30-4.45 (1H, br), 5.20 (2H, br s, $-NCH_2S-$), 6.70-8.25 (11H, m), and 8.30 (1H, br s, -CHO); FAB-MS m/z 791 (M^+); Found: C, 50.13; H, 4.28; N, 19.50; S, 12.34%. Calcd for $C_{33}H_{33}N_{11}O_7S_3$: C, 50.06; H, 4.20; N, 19.46; S, 12.15%.
8) The true nucleating mechanism of a formylhydrazino moiety is still ambiguous, see: S. Moriuchi, N. Inoue, and S. Takada, Proc. 25th Autumn Meeting of the Society for Imaging and Technology, 1985, USA.
9) Any of mercaptotetrazole derivatives without a formylhydrazino moiety did not exhibit the nucleating activity in this system.
10) AVIDINE-AGAROSE(SIGMA®); One unit will bind 1.0 μg of d(+)-biotine, see: J. Turkova, "Affinity Chromatography," Elsevier Scientific Publishing Co., (1978).
11) Compound 1a, 1c, and 3 were spotted onto the film without chromatographic treatment, respectively.
12) The eluent containing only 0.5 M NaCl was spotted onto the film.
13) All compounds were re-purified by HPLC just before the assay.
14) The abbreviation "M" denotes mol dm^{-3} in the text.

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