BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 40 1693—1695 (1967)

The Chemiluminescent Polymer

Yoshimori OMOTE, Seiya OHMORI and Noboru SUGIYAMA
Department of Chemistry, Tokyo Kyoiku University, Otsuka, Tokyo

(Received January 10, 1967)

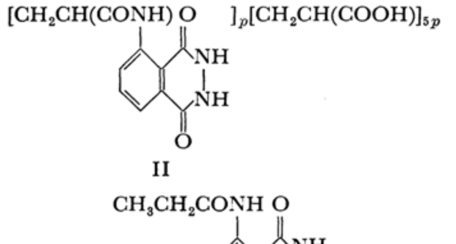
The synthesis and the chemiluminescence of poly(acrylylluminol-ir-acrylic acid) (II) are described. It was found that the reaction product of the chemiluminescence of II is roughly identical with the corresponding poly(acrylylaminophthalic acid-ir-acrylic acid). Also, it was found that II shows a stronger chemiluminescence than the monomeric *N*-propionylluminol (III) based on the same *N*-acylluminol unit in DMF-water (3 : 1 in vol), while II shows a weaker chemilluminescence in water.

Recently, the structure of an American firefly, luciferin, has been clarified by White *et al.*,[1] and the comparative study of bioluminescence and chemiluminescence has been promoted.[2]

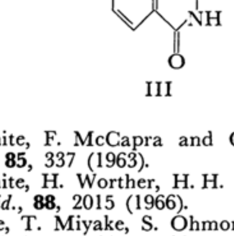
Generally, bioluminescence is the reaction carried out on an enzyme, which is a kind of biopolymer. Here a polymer containing luminol (3-aminophthalhydrazide) (I) as a part of the molecule is synthesized, and the relation between the chemiluminescence and the chemical structure is investigated.

Results and Discussion

The Chemiluminescence of Poly(acrylylluminol-acrylic Acid). Several methods of combining luminol with synthetic polymers were examined. As has been reported in a previous paper,[3] the present authors found a new method to acylate I in dimethylformamide (DMF). The end point of the acylation could be checked by the hypsochromic shift of I with an absorption maximum of about 350 nm in the UV spectrum. The acylation of I with polyacrylyl chloride was chosen as the method to prepare the polymer containing I as a *N*-acylated form. Polyacrylyl chloride was prepared by the polymerization of acrylyl chloride in dioxane, using azobisisobutyronitrile as an initiator.[4] Luminol was reacted with two equivalents of polyacrylyl chloride in DMF in the presence of pyridine. The product was then purified by reprecipitation from DMF and water. The elemental analysis of this purified product showed nitrogen percentage which agreed with the calculated value, the molar ratio of acrylylluminol units to free acrylic acid units being 1 : 5.

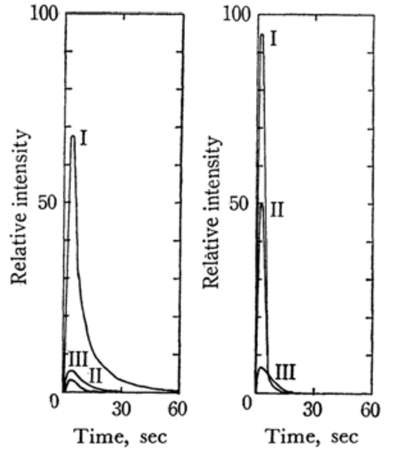


II



III

The chemiluminescence intensity curve of poly-



(A) (B)

Fig. 1. Chemiluminescence intensity curves of poly(acrylylluminol-acrylic acid) (II), *N*-propionylluminol (III) and luminol (I) in water (A) or in DMF-water (3 : 1 in vol) (B) using potassium ferricyanide as catalyst.
Final concentration: (A) sample, 5×10^{-5} mol/*l*, ((II): luminol unit), sodium hydroxide, 5×10^{-2} mol/*l*, potassium ferricyanide, 4.55×10^{-3} mol/*l*, hydrogen peroxide, 2.2×10^{-2} mol/*l*. (B) sample, 7.5×10^{-5} mol/*l*, sodium hydroxide, 2.5×10^{-1} mol/*l*, potassium ferricyanide, 2.28×10^{-3} mol/*l*.

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[1] E. H. White, F. McCapra and G. F. Field, *J. Am. Chem. Soc.*, **85**, 337 (1963).

[2] E. H. White, H. Worther, H. H. Seliger and W. D. McElroy. *ibid.*, **88**, 2015 (1966).

[3] Y. Omote, T. Miyake, S. Ohmori and N. Sugiyama, This Bulletin, **39**, 932 (1966).

[4] R. C. Schultz, P. Elzer and W. Kern, *Makromol. Chem.*, **42**, 189 (1961).

TABLE 1. FLUORESCENCE AND CHEMILUMINESCENCE OF POLY(ACRYLYLLUMINOL-ACRYLIC ACID) (II) AND *N*-PROPIONYLLUMINOL (III)

		Fluorescence ν_{max}, nm	Chemiluminescence ν_{max}, nm	UV absorption ν_{max}, nm
Poly (acrylylluminol-ir-acrylic acid) (II)	a)	415		331
	b)	485	480	300
N-Propionylluminol (III)	a)	412		332
	b)	500	480	332
Poly (acrylylaminophthalic acid-ir-acrylic acid)	a)	470		316
	b)	480		295
N-Acetylaminophthalic acid	a)	460		380
	b)	480		—

a) in DMF, 10^{-4} mol/*l*.
b) under the condition of the chemiluminescence, 7.5×10^{-5} mol/*l*.

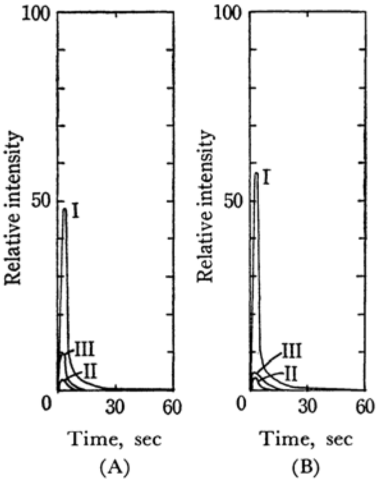

Fig. 2. Chemiluminescence intensity curves of poly(acrylylluminol-ir-acrylic acid) (II), *N*-propionylluminol (III) and luminol (I), in water using hemin (A) or hemoglobin (B) as catalyst.
Final concentration: sample, 5×10^{-7} mol/*l*, ((II): luminol unit), sodium hydroxide, 5×10^{-2} mol/*l*, hydrogen peroxide, 2.2×10^{-2} mol/*l*, hemin or hemoglobin, 2.5×10^{-6} mol/*l*.

$p:q$(acrylylluminol-ir-acrylic acid) (ir means irregularly combined) ($p:q=1:5$) (II) was measured and compared with that of *N*-propionylluminol (III) and I. III corresponds to the monomeric unit of II. Figure 1 shows the chemiluminescence of II and III, as well as that of I, in DMF-water (3 : 1 in vol), using potassium ferricyanide as a catalyst (B), or in water and hydrogen peroxide, using the same catalyst (A). II shows a stronger chemiluminescence than III in DMF-water, while II shows a weaker chemiluminescence than III in water.

Moreover, Fig. 2 shows the chemiluminescence of II and III in the presence of hemin or hemoglobin as a catalyst. Hemoglobin is the protein containing hemin as an active center. If the protein molecule shows any interaction with polymeric II, but none at all or less with monomeric III, the ratio of chemiluminescence of II to that of III in the presence of hemin should differ from that in the presence of hemoglobin. The higher ratio in B of Fig. 2 than in A seems to be due to the fact that the interaction between the protein part of hemoglobin and the polymeric structure of II is more favorable to the chemiluminescent reaction than the monomeric structure of III.

Chemiluminescence Spectra and Fluorescence Spectra. Table 1 shows the chemiluminescence maxima of II and III as well as the fluorescence maxima before and after chemiluminescence. For the sake of comparison, the fluorescence maximum of each corresponding substituted phthalic acid is also listed. If the only emitting species is the corresponding phthalic acid (IV), the fluorescence maximum of IV should accord with the chemiluminescence maximum of II as well as with the fluorescence maximum after the chemiluminescence of II. Such an accordance may be roughly recognized in Table 1.

Experimental

Polyacrylyl Chloride. Acrylyl chloride was prepared according to the method of Stempel *et al.*[5] A mixture of acrylyl chloride and dioxane, 1 : 1 in vol, was sealed in a glass tube and kept in a thermostat at 50°C for 12 hr, using azobisisobutyronitrile as an initiator. The solution of polyacrylyl chloride in dioxane thus obtained was used for the following reaction with luminol. The final concentration of acrylyl chloride units in the polymer solution was 6.15×10^{-3} mol/m*l*.

Poly(acrylylluminol-ir-acrylic Acid) (II). Two milliliters of a polyacrylyl chloride was diluted with DMF to 6.0 m*l*. After having been stirred and cooled in an ice bath, this solution, containing $2 \times 6.15 \times 10^{-3}$ mol acrylyl chloride units, was added to a solution of luminol, 6.15×10^{-3} mol, in 20 m*l* of DMF containing 0.5 m*l* of pyridine. After standing overnight the reaction mixture was vigorously stirred into an excess amount

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[5] G. H. Stempel, Jr., R. P. Cross and R. P. Mariella, *J. Am. Chem., Soc.*, **72**, 2299 (1950).

of water. Then the mixture was centrifuged, and the upper layer was removed. The precipitate was dissolved in 10 m*l* of DMF, and this solution was dropped again into an excess of water as above. This precipitation was repeated several times until the filtrate became free from unchanged luminol. Several kinds of II preparations were obtained by varying the concentration of the luminol solution. The representative preparations showed the following nitrogen percentages: Sample 1, N% 2.56 (Calcd for 20 : 1, acrylic acid unit : acrylylluminol unit, 2.70), Sample 2, N% 3.56 (Calcd for 15 : 1, 3.38), Sample 3, N% 8.12 (Calcd for 5 : 1, 8.09), and Sample 4, N% 11.42 (Calcd for 3 : 1, 11.20). Sample 3 was used for the following experiments. The IR spectra of these samples showed NH band at 3420 cm^{-1} and 1530 cm^{-1}.

***N*-Propionylluminol (III).** III, mp 278°C (decomp.). As III was a model of the monomeric unit of II, its UV spectrum was measured in the same solvent as was used for the reprecipitation of II. III, λ_{max}^{DMF} 333 nm (ε 8550), $\lambda_{max}^{DMF\text{-}H_2O(3:1\ vol)}$ 334 (7930), $\lambda_{max}^{0.1N\ NaOH}$ 326 (8350).

UV Spectrum of 3-Aminophthalic Acid. For the identification of 3-aminophthalic acid,[6] the UV spectrum was measured in several solvents. $\lambda_{max}^{0.1N\ NaOH}$ 305 nm (ε 2450), λ_{max}^{EtOH} 340 (4000), $\lambda_{max}^{DMF\text{-}H_2O(3:1\ vol)}$ 344 (2900), λ_{max}^{DMF} 345 (3800).

Measurement of Fluorescence and Chemiluminescence. The fluorescence spectra were measured at 30°C before and after chemiluminescence. All the chemiluminescence measurements were carried out at 30°C.

Solution of Poly(acrylylaminophthalic Acid-*ir*-acrylic Acid). Three milliliters of a solution of polyacrylyl chloride (6.15×10^{-3} mol unit) was reacted with an equivalent amount of 3-aminophthalic acid in DMF 10 m*l* in the presence of 0.25 m*l* of pyridine. The product was then purified by reprecipitation from DMF and water. The final product was proved to be free from 3-aminophthalic acid by a study of its UV spectrum in ethanol and DMF. The product was used as a DMF solution.

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[6] Y. Omote, T. Miyake, S. Ohmori and N. Sugiyama, This Bulletin, in press.