

liposomes were carried out at pH 6.4 in order to maintain the monomeric state of the membrane (at higher pH values, a slow spontaneous polymerization occurs). Polymerized liposomes of **1** showed the same permeability toward sucrose at pH 6.4 as that found at pH 8.5.

Dynamic Light Scattering. Size distributions were determined with a Nicomp Model 200 laser particle sizer equipped with a 5-mW helium-neon laser at an excitation wavelength of 632.8 nm. This instrument employs digital autocorrelation to analyze the fluctuations in scattered light intensity generated by the diffusion of liposomes in solution. The measured diffusion coefficient is used to obtain the average hydrodynamic radius and, thus, the mean diameter. Samples were filtered by using a 0.45- μm HV4 Millipore filter prior to analysis.

Film Balance Experiments. Surface pressure-area isotherms were recorded by using a MGW Lauda film balance, maintained at 25 °C,

and equipped with a computerized data acquisition station. All lipids were spread onto a pure water subphase (600 cm^2) that was purged with nitrogen, using a hexane/ethanol (4/1, v/v) solution. The concentrations of stock solutions of **1** and **2** that were used were 0.44 and 1.18 mg/mL, respectively. Monolayers were compressed under a nitrogen atmosphere up to the collapse point. The total quantity of lipid that was used in each experiment was 3.17×10^{-8} moles; the rate of compression was 60 cm^2/min .

Registry No. **1**, 116405-86-8; **2**, 116405-87-9; 12-(tetrahydropranyloxy)dodecanoic acid, 116405-88-0; 1-palmitoyl-*sn*-glycero-3-phosphocholine, 17364-16-8; 1-palmitoyl-2-(12-hydroxydodecanoyl)-*sn*-glycero-3-phosphocholine, 116405-89-1; lipoic acid anhydride, 91319-83-4.

Does a Stilbazolium Cation Adsorbing Poly(styrenesulfonate) Anion Form Micellelike Clusters?

Katsuhiko Takagi, Haruhiko Fukaya, and Yasuhiko Sawaki*

Contribution from the Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464, Japan. Received July 7, 1987

Abstract: Poly(styrenesulfonate) anion (PSS^-) is shown to aggregate with a large number of stilbazolium cations (Stz^+) which undergo an electron-relay chain photoisomerization in the presence of $\text{Ru}(\text{bpy})_3^{2+}$ sensitizer. The quantum yields for the *cis*-to-*trans* isomerization ($\Phi_{\text{cis} \rightarrow \text{tr}}$) were rather constant in the range of 50–110, irrespective of changing the solvent from water to acetone or of the degrees of polymerization of PSS^- from 100 to 5000. Adsorption and aggregation numbers of benzyltrimethylammonium ion (BTA^+), an organic cationic substitute for Stz^+ , on PSS^- were determined by a methylantracene quenching method. The results show that the thousands of Stz^+ ions bound to PSS^- are divided into small aggregates of micellelike clusters and that their aggregation numbers are constant in the range of 80–90, irrespective of the degree of polymerization of PSS^- . It was concluded that the electron-relay chain isomerization proceeds within one micellar cluster.

Interactions between polyions and organic counterions play an important role in a wide variety of biological and industrial systems.¹⁻⁴ The binding of organic materials to biopolymers or polyelectrolytes is a particularly interesting methodology to achieve artificial photosynthesis enabling a facile electron transfer between electron donors and acceptors.⁵ We have reported an electron-relay chain reaction utilizing anionic micelles^{6a} or colloidal silica gel surface for the $\text{Ru}(\text{bpy})_3^{2+}$ -sensitized *cis*-to-*trans* isomerization of stilbazolium cations (Stz^+).^{6b} The resulting high quantum yields of 50–100 are suggestive of a possibility for developing a so-called photomultiplier, provided that the length of electron relay could be further increased by applying polyionic fields. Poly(styrenesulfonate) anion (PSS^-), a polyelectrolyte, seems to be a good candidate for such fields with anionic sites available up to 5000.

Here, we report our unexpected results that thousands of Stz^+ molecules bound to the anionic polymer PSS^- are divided into micellelike clusters and that the electron relay is confined within one cluster.

Experimental Section

Materials. Tris(2,2'-bipyridine)ruthenium dichloride [$\text{Ru}(\text{bpy})_3^{2+}$](Cl^-)₂⁷ and *cis*- γ -styrylpyridine (*cis*- Stz)⁸ were prepared according to the literature methods. Sodium poly(styrenesulfonates) [(PSS)Na's] were either of the standard sample grade from Pressure Chemical Co. or from Toyo Soda Manufacturing Co. β -Phenethylammonium chloride, tetramethylammonium bromide, and 9-methylantracene of extra pure grade were used as received. Benzyltrimethylammonium chloride (BTAC) was prepared by the reaction of trimethylamine with benzyl chloride.

Preparation of *cis*- γ -Stilbazolium Poly(styrenesulfonate)[PSS^- -(*cis*- Stz^+)_n]. A 0.05 M solution of *cis*- Stz in methanol (80 μL) was precisely neutralized by adding an equivalent quantity of 0.01 M poly(styrenesulfonic) acid (PSSH) in water and stored as a stock solution in the dark. The concentration of PSSH was based on the sulfonic acid group involved, and the neutralization point was checked potentiometrically. Here, the PSSH solution was obtained according to the following procedures: 50 mg of (PSS)Na in water (~ 1 mL) was passed through a column (17 mm i.d. \times 200 mm length) packed with ca. 20 mL each of Amberlite IR and IRA and separated into fractions (ca. 10 mL). Fractions (no. 3–6) including PSSH were collected and diluted with ca. 50 mL of water to afford ca. 0.01 M PSSH solution. Its concentration was determined spectrophotometrically at 262 nm (ϵ 730) on a Shimadzu UV-265 spectrophotometer.

Photolysis of PSS^- -(*cis*- Stz^+)_n with $\text{Ru}(\text{bpy})_3^{2+}$. An aqueous solution of 0.4 mM (PSS^-/n)-*cis*- Stz^+ and 6.7 μM $\text{Ru}(\text{bpy})_3^{2+}$ sensitizer was

(1) (a) Hayakawa, K.; Kwak, J. C. T. *J. Phys. Chem.* **1982**, *86*, 3866. (b) Hayakawa, K.; Kwak, J. C. T. *J. Phys. Chem.* **1983**, *87*, 506. (c) Hayakawa, K.; Santerre, J. P.; Kwak, J. C. T. *Biophys. Chem.* **1983**, *17*, 175. (d) Molodkova, A.; Hayakawa, K.; Kwak, J. C. T. *J. Phys. Chem.* **1984**, *88*, 1930. (2) Satake, I.; Yang, J. T. *Biopolymers* **1976**, *15*, 2263. (3) (a) Dubin, P. L.; Davis, D. D. *J. Colloid Interface Sci.* **1983**, *95*, 453. (b) Leung, P. S.; Goddard, E. D. *Ibid.* **1985**, *103*, 47. (c) Chu, D. Y.; Thomas, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 6270. (4) Armstrong, D. W.; Stine, G. Y. *J. Am. Chem. Soc.* **1983**, *105*, 6220. (5) (a) Degani, Y.; Willner, I. *J. Am. Chem. Soc.* **1983**, *105*, 6228. (b) Willner, I.; Otvos, J. W.; Calvin, M. *Ibid.* **1981**, *103*, 3203. (c) Willner, I.; Yang, J.-M.; Otvos, J. W.; Calvin, M. *J. Phys. Chem.* **1981**, *85*, 3277. (d) Willner, I.; Degani, Y. *J. Chem. Soc., Chem. Commun.* **1982**, 761. (e) Willner, I.; Degani, Y. *Isr. J. Chem.* **1982**, *22*, 163. (f) Schanze, K. S.; Whitten, D. G. *J. Am. Chem. Soc.* **1983**, *105*, 6734. (6) (a) Takagi, K.; Aoshima, K.; Sawaki, Y.; Iwamura, H. *J. Am. Chem. Soc.* **1985**, *107*, 47. (b) Takagi, K.; Aoshima, K.; Sawaki, Y.; Iwamura, H. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1771.

(7) Burstall, F. H. *J. Chem. Soc.* **1936**, 173.

(8) Takagi, K.; Ogata, Y. *J. Org. Chem.* **1982**, *47*, 1409.

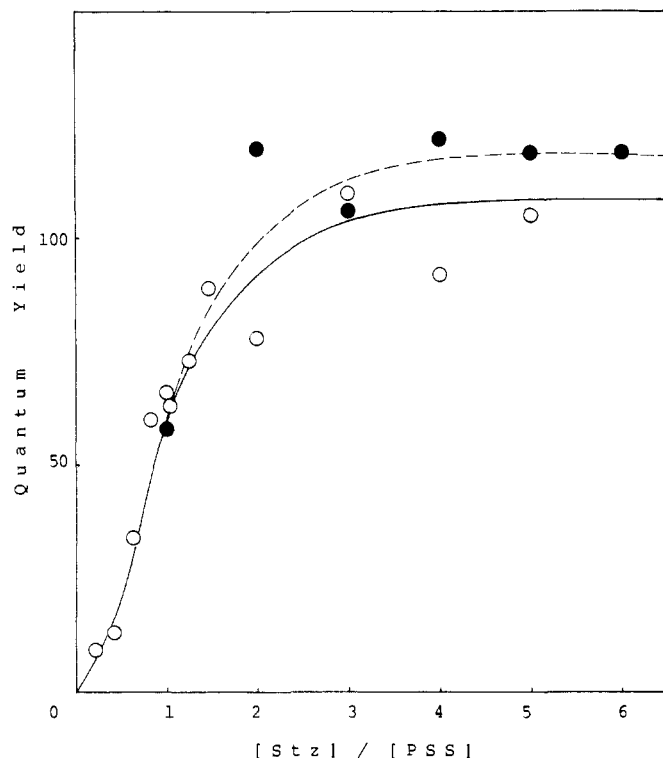


Figure 2. Dependence of the quantum yields of sensitized photoisomerization of *cis*-Stz⁺ on the ratio of [*cis*-Stz⁺] to [PSSⁿ⁻]; (O) for MW = $(1.0 \pm 0.2) \times 10^6$ from Toyo Soda Co. and (●) for MW = 1.06×10^6 from Pressure Chemical Co. conditions: [PSSⁿ⁻/n] = 0.4 mM; [*cis*-Stz⁺] = 0–2.4 mM; in the case where the ratios were greater than unity, *cis*- γ -stilbazolium *p*-toluenesulfonate was added; [Ru(bpy)₃²⁺] = 6.7 μ M; irradiated at 468 ± 5 nm in water under argon.

of 1/1 and 3/1 are summarized in the last two columns in Table I. Remarkably interesting as it is, the maximum yields remain constant at around 90–110 in spite of 50-fold variation in the number of *cis*-Stz⁺ molecules adsorbed on a PSSⁿ⁻ polymer chain. These observations suggest that *cis*-Stz⁺ ions adsorbed on the polymer chain do not form a singly aggregated form but are divided into small aggregates, i.e., clusters. In order to verify this hypothesis, the adsorption or aggregation number of *cis*-Stz⁺ ions on a PSSⁿ⁻ polymer chain was determined as stated below.

Extent of Adsorption of *cis*-Stz⁺ and Ru(bpy)₃²⁺ on PSSⁿ⁻. The degree of adsorption of *cis*-Stz⁺ ions was estimated according to the procedure reported by Whitten et al.¹⁰ except for the use of ZnTPPS as a fluorescence probe (λ_{\max} 606 nm) and summarized in the fourth column in Table I. The results show that *cis*-Stz⁺ ions bind to PSSⁿ⁻ anion sites quite efficiently (~90%) irrespective of the degrees of polymerization.

The degree of adsorption of Ru(bpy)₃²⁺ ions was estimated from the dissociation constant K_d for the complex of Ru(bpy)₃²⁺ and PSSⁿ⁻ (see eq 1). The K_d values obtained were $(1.88 \pm 0.08) \times 10^{-5}$ and $(6.39 \pm 1.16) \times 10^{-5}$ M in the absence and the presence of β -phenethylammonium ion, respectively, as a photochemically inert substitute for *cis*-Stz⁺. This means that 97% and 86% of the Ru(bpy)₃²⁺ ions are complexed with PSSⁿ⁻ anions in the absence and the presence of PhCH₂CH₂N(CH₃)₃⁺ ion, respectively. Thus, we could safely assume that most of the *cis*-Stz⁺ and Ru(bpy)₃²⁺ ions added are adsorbed on the polyanion sites under the reaction conditions.

Formation of Micellelike Clusters in Stz⁺-Adsorbed PSSⁿ⁻ Polymer Anion. An electron-relay chain isomerization is undoubtedly operative in the present polyelectrolyte system, as judged from the remarkably high efficiency ($\Phi_{c \rightarrow t} = 50$ –110). The quantum yields, however, deviate considerably from the number of *cis*-Stz⁺ ions adsorbed on one polymer chain of PSSⁿ⁻ (cf. the adsorption numbers and $\Phi_{c \rightarrow t}$ in Table I). Thus, percent isomerization of Stz⁺ ions on one polymer chain is ca. 26% for PSSⁿ⁻ with $n = 360$ but only 2% for the case of $n = 5100$. These results

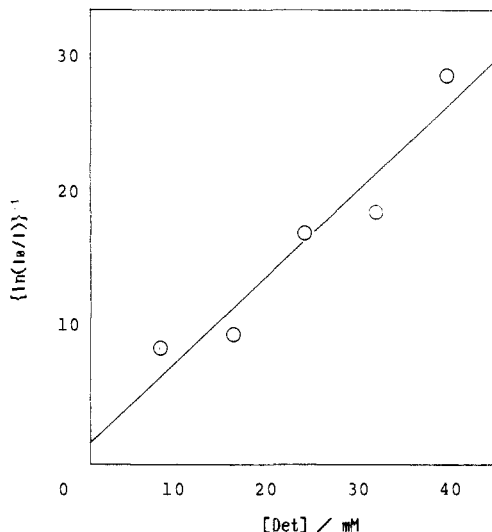
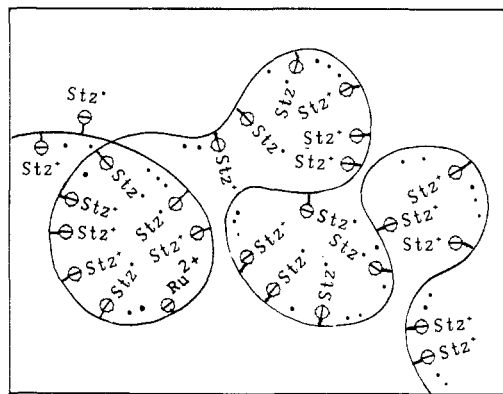


Figure 3. Plot of the reciprocal of $\ln(I_0/I)$ vs [Det] for the fluorescence quenching of Ru(bpy)₃²⁺ by 9-methylanthracene in water containing 10% MeCN. [Det] is the concentration of a monomer unit of PSSⁿ⁻-(BTA⁺)_n, i.e., [PSSⁿ⁻/n·BTA].

Scheme I. Schematic Model for Micellelike Clusters



suggest that the electron-relay chain isomerization is not widespread over the whole polymer chain of PSSⁿ⁻. A reasonable assumption is that the Stz⁺ ions adsorbed on a polymer chain are divided into a number of small clusters and the electron relay between each cluster is inefficient. This assumption could be substantiated as stated below.

The aggregation number N_A of Stz⁺ and PSSⁿ⁻ could be determined by the quenching of Ru(bpy)₃²⁺ fluorescence with 9-methylanthracene;^{1a} 10% aqueous acetonitrile was used as solvent to solubilize enough of the quencher.¹³ The luminescence quenching by 9-methylanthracene was done in the presence of various amounts of the PSSⁿ⁻-(BTA⁺)_n complex, BTA⁺ being a photochemically inert substitute for *cis*-Stz⁺. As shown in Figure 3, a linear relationship was obtained for the plot of reciprocal of $\ln(I_0/I)$ vs [Det] [i.e., [PSSⁿ⁻/(BTA⁺)_n]], satisfying eq 2. The aggregation numbers thus obtained are summarized in Table I. The result shows that N_A 's are constant in the range of 80–90, irrespective of the degree of polymerization (n). It is interesting to note the N_A values are in close coincidence with the isomerization efficiencies ($\Phi = 50$ –110). Previously, we confirmed that in a series of anionic micelle systems the electron-relay isomerization takes place in almost quantitative yields for all the Stz⁺ ions adsorbed and the quantum yields were practically the same as the N_A values.^{6a} Hence, the present results demonstrate that

(13) The aqueous solution of PSSⁿ⁻ and (*cis*-Stz⁺)_n ions could solubilize 9-methylanthracene; e.g., 0.248 mg of the anthracene was soluble in 100 g of an aqueous solution of 8.3 mM PSSⁿ⁻-(*cis*-Stz⁺)_n. Control experiments showed that the solubility of 9-methylanthracene was negligibly small without the two components; i.e., the solubility was less than 0.02 mg in the presence of either PSSⁿ⁻ or Stz⁺ ion.

Table II. Effect of Solvent on the Quantum Yields of Photosensitized Electron-Relay Isomerization^a

polym deg (n)	$\Phi_{c \rightarrow t}$		
	CH ₃ COCH ₃ ^b	CH ₃ OH ^b	CH ₃ CN ^b
c	0.45		
SDS ^d	0.55		
100 ± 50	42		
360 ± 120	47		
1700	61	91	93
2400 ± 500	66		
5100			98

^aIrradiated at 468 ± 5 nm under argon. Initial concentrations: [PSSⁿ⁻/n] = [cis-Stz⁺] = 0.4 mM; [Ru(bpy)₃²⁺] = 6.7 μM. ^bSolvents containing 10% H₂O. ^cAddition of equimolar HCl in place of PSSⁿ⁻. ^dSDS (sodium dodecylsulfate) was added in place of PSSⁿ⁻.

the poly(styrenesulfonate) anion chain and hydrophobic cation BTA⁺ form micellelike clusters and their N_A values are in the range of 80–90, irrespective of the degree of polymerization, as depicted in Scheme I. So far, dodecyltrimethylammonium bromide and PSSⁿ⁻ have been reported to form micelles, but their N_A values are less than 10.¹⁴ Hence, the present evidence for micellelike clusters is new and quite interesting.

Effect of Solvents. To our surprise, a similar enhancement of the electron-relay isomerization was also observed in organic solvents such as acetone, methanol, and acetonitrile (Table II). The chain isomerization occurs in organic solvents as efficiently as in water. This suggests that the PSSⁿ⁻-(Stz⁺)_n forms micellelike clusters even in organic solvents. This is in sharp contrast to the case of typical anionic micelles; i.e., micelles do not prevail in 90% aqueous acetone as exemplified by the quantum yield of less than unity in the presence of sodium dodecylsulfate (Table II). It was, however, impossible to measure the aggregation number of PSSⁿ⁻-(BTA⁺)_n in the organic solvents on account of the experimental restriction that 9-methylanthracene quencher is quite soluble in the solvents.

In conclusion, the present results suggest that Stz⁺ and PSSⁿ⁻ form a number of divided micellelike clusters, resulting in the electron-relay chain isomerization of cis-Stz⁺. This may provide an interesting model system for biopolymers and polyions.

Acknowledgment. We are grateful to Toyo Soda Manufacturing Co. and Mitsubishi Kasei Chemical Co. for their gifts of sodium poly(styrenesulfonates). We thank Professor Ichiro Noda for his helpful advice on the polyelectrolytes. This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

Registry No. PSS-cis-Stz, 116374-41-5; PSS-BTAC, 116350-20-0; Ru(bpy)₃²⁺Cl₂⁻, 14323-06-9.

(14) Abuin, E. B.; Scalano, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 6274.

Mechanism of Aromatic Hydroxylation in the Fenton and Related Reactions. One-Electron Oxidation and the NIH Shift

Tsunehiko Kurata, Yasumasa Watanabe, Makoto Katoh, and Yasuhiko Sawaki*

Contribution from the Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464, Japan. Received March 28, 1988

Abstract: Hydroxylation of substituted benzenes in the Fenton and peroxydisulfate oxidations has been studied mechanistically in relation to the NIH shift. One-electron oxidants such as Fe³⁺, Cu²⁺, and quinones increased the shift value effectively in aqueous or acetonitrile solutions. The shift values obtained were as high as 40–50% and dependent on both substituents (i.e., MeO ≪ Me, Cl, MeCO) and solvents. A high shift value was obtained also for the methoxylation, indicating unimportance of the arene oxide intermediate for the NIH shift. Oxygen reduced the shift effectively and sometimes was incorporated into product phenols with selective meta orientation. This means that oxygen abstracts a hydrogen atom from or adds to the oxycyclohexadienyl radical intermediate. It is concluded that the one-electron oxidation of the dienyl radical is the key step for the shift and its rates are dependent on substituents, oxidants, and solvents.

The NIH shift is a rearrangement of a hydrogen atom during enzymic aromatic hydroxylations,^{1–3} and significantly large shift values have been reported also in model cytochrome P-450 oxidation.⁴ The shift mechanism was originally understood by arene oxide intermediates,¹ but some results not compatible with the arene mechanism have also been reported.^{5,6} Recently, a rear-

rangement via cationic intermediates has been suggested by cytochrome P-450 model study⁷ or semiempirical MO calculation.⁸

As a nonenzymatic hydroxylation the Fenton oxidation is well-known and extensively studied.^{9,10} Recently developed are the regioselective hydroxylation of alcohols,¹¹ the nonclassical^{12a} or photo-Fenton reaction,^{12b} efficient hydroxylation of aromatics

- (1) Jerina, D. M.; Daly, J. W. *Science (Washington, D.C.)* **1974**, *185*, 573.
 (2) Hamilton, G. A. In *Molecular Mechanism of Oxygen Activation*; Hayaishi, O., Ed.; Academic: New York, 1974; p 405.
 (3) (a) Matsuura, T. *Tetrahedron* **1977**, *33*, 2869. (b) Sheldon, R. A.; Kochi, J. K. *Metal-Catalyzed Oxidation of Organic Compounds*; Academic: New York, 1981; p 254.
 (4) (a) Chang, C. K.; Kuo, M.-S. *J. Am. Chem. Soc.* **1979**, *101*, 3413. (b) Groves, J. T.; Nemo, T. E.; Myers, R. S. *Ibid.* **1979**, *101*, 1032. (c) Chang, C. K.; Ebina, F. *J. Chem. Soc., Chem. Commun.* **1981**, 778.
 (5) (a) Selander, H. G.; Jerina, D. M.; Piccolo, D. E.; Berchtold, G. A. *J. Am. Chem. Soc.* **1975**, *97*, 4428. (b) Preston, B. D.; Miller, J. A.; Miller, E. C. *J. Biol. Chem.* **1983**, *258*, 8304.
 (6) Hanzlik, R. P.; Hogberg, K.; Judson, C. M. *Biochemistry* **1984**, *23*, 3048.

- (7) (a) Groves, J. T.; Myers, T. S. *J. Am. Chem. Soc.* **1983**, *105*, 5791. (b) Collman, J. P.; Kodadek, T.; Brauman, J. I. *Ibid.* **1986**, *108*, 2588. (c) Traylor, T. G.; Iamamoto, Y.; Nakano, T. *Ibid.* **1986**, *108*, 3529.
 (8) Korzekwa, K.; Trager, W.; Gouterman, M.; Spangler, D.; Loew, G. H. *J. Am. Chem. Soc.* **1985**, *107*, 4273.
 (9) (a) Walling, C. *Acc. Chem. Res.* **1975**, *8*, 125. (b) Walling, C.; Johnson, R. A. *J. Am. Chem. Soc.* **1975**, *97*, 363.
 (10) Jefcoate, C. R. E.; Lindsay Smith, J. R.; Norman, R. O. C. *J. Chem. Soc. B* **1969**, 1013.
 (11) Groves, J. T.; Van Der Puy, M. *J. Am. Chem. Soc.* **1976**, *98*, 5290.
 (12) (a) Richer, H. W.; Waddell, W. H. *J. Am. Chem. Soc.* **1982**, *104*, 4630. (b) Fujihira, M.; Satoh, Y.; Osa, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 666.