these conditions. Reaction 22 is therefore analogous to the decomposition of the ethyl radical, as might be expected.

Propylene and sulfur hexafluoride decreased the ethylene yield, while ammonia had no effect. The reasons are the same as those given for mechanisms I and II.

Ethane, *sec*-Butyl Alcohol, and 1,2-Propanediol. The increase of $G(C_2H_6)$ from 1.5 to 7.5 when the temperature was increased from 320 to 375° (Figure 2B) indicates that ethane was produced by a minor chain reaction in this temperature region. The yield was not affected by propylene but was reduced by ammonia at 350°, so cations appear to be involved in the chain. The mechanism will be investigated further.

The mechanisms of formation of *sec*-butyl alcohol and 1,2-propanediol are not known.

General Comment. In the radiation-sensitized pyrolysis of ethanol at 350° the three different modes of decomposition of ethanol, represented by mechanisms I, II, and III, all occurred to similar extents. The relative extents were I:II:III::2:2:1. In the normal pyrolysis at 525° the relative extents were 4:0:1,[5] whereas at 600°, only mechanism I was observed.[6] In both the normal and radiation-sensitized pyrolyses the initiation and termination reactions of the three mechanisms are intermingled, so this trend of the relative extents of the mechanisms with increasing temperature should be related to the relative activation energies of the chain-propagation reactions: $E_{\mathrm{Ip}} > E_{\mathrm{IIIp}} > E_{\mathrm{IIp}}$, where E_{Ip} is the activation energy of propagation of mechanism I, and so on. This is in agreement with observation, since $E_5 \geq 30$ kcal/mol, $E_{22} = 27$ kcal/mol, and $E_{17} = 20$ kcal/mol. A parallel implication of the trend is that the frequency factors of the propagation reactions are in the order $A_{\mathrm{Ip}} > A_{\mathrm{IIIp}} > A_{\mathrm{IIp}}$. This might be due to the relative values of the frequency factors of reactions 5, 22, and 17, or to the relative frequencies of production of the CH_3CHOH, CH_2CH_2OH, and CH_3CH_2O radicals, or both.

In agreement with the above discussion, extrapolation of the present results indicates that at about 420° the relative extents of the different mechanisms becomes I > III > II, and that the difference between their relative contributions increases with further increase in temperature.

Fluorescence of Zinc and Magnesium Etioporphyrin I. Quenching and Wavelength Shifts Due to Complex Formation[1]

David G. Whitten, Irene G. Lopp,[2] and Peter D. Wildes

Contribution from the Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27514. *Received July 29, 1968*

Abstract: Emission spectra of zinc and magnesium etioporphyrin I are red shifted and decrease in intensity on coordination with several ligands. These shifts parallel absorption spectral changes and are probably due to steric interactions between the ligand and the porphyrin π cloud. Ligand exchange for excited magnesium complexes is probably close to diffusion controlled. Nitrobenzene and other nitro compounds quench zinc and magnesium porphyrin fluorescence, probably through an excited complex which has some charge-transfer character. Studies of excited porphyrin-induced *cis–trans* isomerization of 4-nitrostilbene indicate that the zinc porphyrin triplet must be the precursor to the isomerizable species. The results suggest that zinc porphyrin triplets are better electron donors than the corresponding excited singlets.

Numerous investigations have dealt with energy transfer and electron transfer phenomena involving porphyrins and related compounds. Much of the interest of these systems centers around the mechanism of chlorophyll action and the role of other biologically important systems. It has been demonstrated that photoexcited porphyrins can serve either as electron donors[3–7] or electron acceptors[5–7] and that the porphyrin may or may not be altered permanently in the process. Excited porphyrins can also act as sensitizers in ordinary energy transfer processes.[8,9] Many of the phenomena concerned with chlorophyll and other porphyrins are very dependent on environment. Aggregated and coordinated species are common and it may well be such forms that are the reactive species in several photoreactions involving porphyrins.[10–13] Recent work has shown that amines,[14] conjugated dienes,[15]

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and other compounds (which lack low-lying excited singlet states) can quench fluorescence from low-lying singlets of aromatic hydrocarbons by processes not involving transfer of electronic excitation. Quenching by various substances could proceed *via* several different mechanisms, including charge or electron transfer. It has been shown very recently[16] that quenching may be accompanied by chemical change in which electronic energy may be converted into vibrational energy. Metalloporphyrins coordinate readily to a variety of substances including amines, heterocyclic bases, and other compounds with extended conjugation. Therefore, it seems likely that efficient intramolecular examples of one or more of the aforementioned processes may occur with excited porphyrin complexes. Accordingly, we have studied excited-state interactions between metalloporphyrins and coordinated ligands. In the present article, we report results of fluorescence studies on several complexes of zinc and magnesium etioporphyrin I.

Experimental Section

Emission spectra were recorded on an Aminco-Bowman spectrophotofluorometer using 1P21 and EMI 9558Q photomultiplier tubes. Fluorescence quenching studies were done in "true-bore" Pyrex ampoules degassed by three cycles of the freeze-pump-thaw method. Porphyrin concentrations were *ca.* 1×10^{-5} *M*.

Irradiations were carried out in a merry-go-round apparatus[17] on benzene solutions in Pyrex ampoules degassed by the freeze-pump-thaw method. Glass color filters were used to isolate the 5461 and 5770–5790 Å lines of a medium pressure mercury lamp.

Ultraviolet and visible absorption spectra were recorded on a Unicam or Cary 14 spectrophotometer.

Materials. Etioporphyrin I was prepared by the condensation of kryptopyrrole.[18,19] Zinc etioporphyrin I was prepared by the method of Calvin, *et al.*[19] Magnesium etioporphyrin I was made by the magnesium hexapyridine diiodide-pyridine method of Corwin, *et al.*[20] The porphyrins were purified by recrystallization and/or column chromatography. Purity of the etioporphyrin (*ca.* 99% or greater) was checked by liquid-liquid partition chromatography[21] and spectral techniques. *cis*- and *trans*-4-nitrostilbenes were prepared by the Wittig reaction of *p*-nitrobenzaldehyde and benzyltriphenylphosphonium bromide.[22] The *cis* and *trans* isomers were separated by fractional recrystallization from hexane. Benzene was purified by treatment with sulfuric acid followed by distillation from P_2O_5. Other materials except spectrograde solvents were purified by distillation or recrystallization.

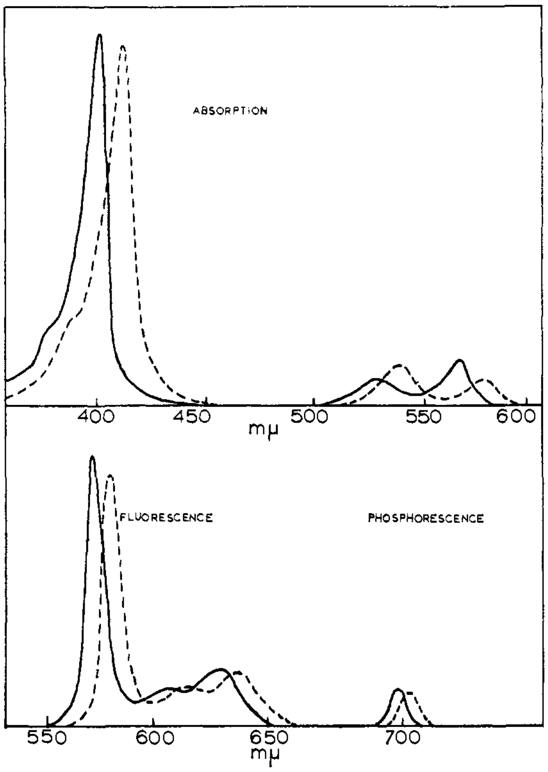

Figure 1. Absorption spectra (top) of zinc etioporphyrin in cyclohexane (——) and pyridine (– – – –) at 28° and emission spectra (bottom) of zinc etioporphyrin in EPA (——) and in EPA with pyridine (– – – –) at 77°K.

Table I. Position of λ_{max} in Zinc Etioporphyrin I Emission Spectra

Room temperature (~28°)		
Ligand	$\lambda_{max,fl}$, mμ	
None (methylcyclohexane)	570	
Benzene	572	
Ethanol	579	
Aniline	582	
Pyridine	582	
Triphenylphosphine	583	
Piperidine	584	
Liquid nitrogen temperature (77°K)		
Solvent	$\lambda_{max,fl}$, mμ	$\lambda_{max,phos}$, mμ
MCIP[a]	570	697
EPA[b]	573	699
Pyridine in EPA	576	705
Piperidine in EPA	578	708

[a] Methylcyclohexane–isopentane (5:1 by volume). [b] Ethyl ether–*n*-pentane–ethanol (5:5:2 by volume).

Results and Discussion

Coordinated Ligands. Absorption spectra of several metalloporphyrins are shifted to longer wavelength on coordination with ligands such as nitrogen and phosphorus bases. These shifts are accompanied by changes in the relative intensity of the peaks in the 500–600-mμ range. These changes have been ascribed to steric interaction of the ligand with the porphyrin π cloud.[23] We have found that emission spectra of coordinated zinc and magnesium etioporphyrins are also red shifted (Figure 1). Shifts in wavelength of the λ_{max} for both fluorescence and phosphorescence of zinc etioporphyrin I are given in Table I. The intensity of zinc etioporphyrin emission decreases upon coordination; for example, with pyridine, the fluorescence drops to 50% of its former intensity and the phosphorescence to 25%. However, the oscillator strength of the singlet–singlet transition decreases on coordination so that the change in the rate of fluorescence could be sufficient to account for the decrease: a similar process may be the cause of the change in phosphorescence intensity but no infor-

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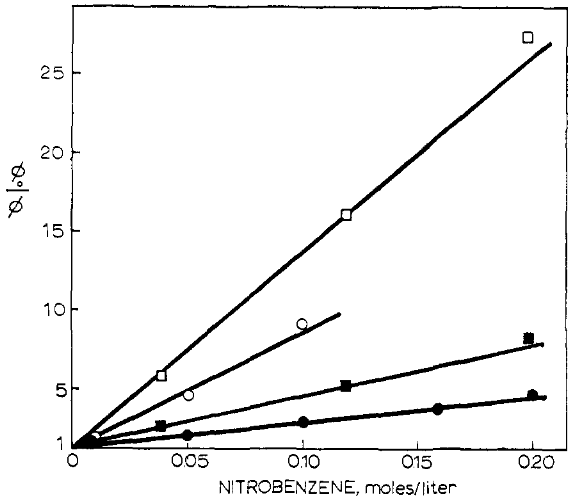

Figure 2. Typical Stern-Volmer plots for quenching of zinc and magnesium etioporphyrin fluorescence by nitrobenzene: ϕ_0/ϕ, the relative fluorescence intensity in the absence and presence of nitrobenzene; ●, zinc etioporphyrin in piperidine; ■, zinc etioporphyrin in ethanol; ○, magnesium etioporphyrin in piperidine; □, magnesium etioporphyrin in methylcyclohexane.

mation is available to confirm this. 4-Stilbazole and 1-(α-naphthyl)-2-(4-pyridyl)ethylene both have extended conjugation and give the same shift and intensity of porphyrin emission as does pyridine. This tends to confirm suggestions that the effects are due to a steric perturbation of the porphyrin π cloud rather than to a conjugative or energy transfer phenomenon.[24]

In previous studies,[23] it was found that only a slight excess of base such as pyridine was required to convert zinc porphyrins completely to the complexed form. Therefore, there was some uncertainty regarding the number of ligands in these complexes. We find that at concentrations of less than 1 *M* aniline, zinc etioporphyrin I in benzene solution gives two peaks in the near-ultraviolet: one for the uncoordinated form at 400 mμ and the other for the aniline complex at 412 mμ. An equilibrium constant (stability constant) of 47 ± 1 at 28° was determined by spectrophotometric methods.[26] A slope of 1.01 for a plot of log [complexed porphyrin]/[uncomplexed porphyrin] *vs.* log [aniline] indicates that the complex contains only one aniline per porphyrin. Spectral similarities indicate that most other nitrogen bases form only monocomplexes with zinc porphyrins.

Triphenylphosphine forms a complex with zinc etioporphyrin I; its Soret peak occurs at 420 mμ compared to 412 mμ maximum for the pyridine complex. Conversion to the complex is incomplete even in triphenylphosphine-saturated benzene. The Benesi-Hildebrand relationship[27] was used to determine the extinction coefficient for the complex; the stability constant at 28° was found to be 7.7 ± 0.2. A linear plot similar to that for aniline indicates that the complex contains one triphenylphosphine per porphyrin.

Solutions of ligands and zinc or magnesium porphyrins which have two near-ultraviolet peaks in their absorption spectra show two major peaks in their fluorescence spectra. Spectral overlap and other factors rendered a quantitative study of changes in the complexed-uncomplexed porphyrin distribution in the excited state unfeasible. However, certain qualitative observations can be made. Magnesium etioporphyrin I in pure pyridine is about 60% monopyridinate and 40% dipyridinate.[28] Fluorescence spectra show a preponderance of emission at longer wavelength, indicating a shift in favor of the dipyridinate in the excited state. For magnesium etioporphyrin I the fluorescence distribution is very nearly the same regardless of which Soret band is excited. Very rapid ligand exchange in ground-state magnesium porphyrin complexes is indicated by nmr studies.[29] Since the excited singlet lifetime for the magnesium porphyrin is *ca.* 10^{-8} sec,[30] our observations indicate the rate of exchange must be very nearly diffusion controlled. In contrast with magnesium, for zinc etioporphyrin I solutions, activation of the uncomplexed Soret results in emission chiefly from the uncomplexed form; activation of the complexed Soret promotes emission chiefly from the complex. Since the lifetime of excited zinc porphyrin singlets is close to that for magnesium,[30] our results indicate ligand exchange for excited zinc singlets is slower, by at least an order of magnitude. As noted earlier, the zinc porphyrin-triphenylphosphine complex absorbs at longer wavelengths than zinc etioporphyrin I complexes with nitrogen bases. However, emission from the zinc porphyrin-TPP complex occurs at 583 mμ, roughly the same location as that from the pyridine or piperidine complexes. This indicates that a more severe change in structure occurs for the zinc porphyrin-TPP complex on excitation than for the other complexes; probably the π bond between phosphorus and zinc is broken.

Electron-Deficient Quenchers. Livingstone and coworkers[31] found that fluorescence of chlorophyll and related compounds is strongly quenched by electron-deficient compounds such as quinone, aryl nitro compounds, and nitroso compounds. More recently, Gouterman[32] and others[33] have found that some of these same compounds form ground-state charge transfer complexes with several porphyrins. We have examined fluorescence quenching phenomena with compounds that form no ground-state complexes with porphyrin. Several mononitro compounds give no evidence for complex formation with zinc or magnesium etioporphyrins even at concentrations as high as 1 *M*. However, even 0.003 *M* nitrobenzene significantly quenches the porphyrin fluorescence. The unquenched portion of the fluorescence has the same shape and position as that in solutions without nitrobenzene; no new fluorescence is observed out to 750 mμ. Linear Stern-

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(24) The lack of fluorescence quenching by these ligands indicates that their highly efficient light-induced *cis-trans* isomerization in porphyrin complexes[25] must originate from the porphyrin triplet or some later obtained species.

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Volmer plots (Figure 2) were obtained for both zinc and magnesium etioporphyrin in several solvents.[34] The slope equals the product of the singlet lifetime, τ, and the rate constant for the quenching reaction, k_q. Singlet lifetimes are not available for etioporphyrin I or its metallo derivatives; however, fairly accurate k_q values can be obtained if one uses lifetimes for zinc and magnesium mesoporphyrin.[30, 37] Table II summarizes values for k_q under various conditions. The k_q values for magnesium etioporphyrin I are very near the diffusion controlled limit and show little solvent variation. Values for zinc are a little lower and show a general increase with solvent polarity. The lower apparent rates of quenching in the presence of piperidine and triphenylphosphine might be due to steric hindrance of approach of nitrobenzene to the excited porphyrin. However, it is also possible that shorter singlet lifetimes for these complexes mask equally efficient quenching. Sterically hindered nitro compounds (Table III) such as 1,2-dimethyl-3-nitrobenzene are less efficient quenchers. Strongly electron-donating groups render aryl nitro compounds less efficient as quenchers. Interestingly, we find that etioporphyrin fluorescence is unquenched by 0.1 *M* nitrobenzene in benzene and in methylcyclohexane but rather strongly quenched in the more polar solvent ethanol. This contrasts with ground-state change transfer phenomena[32, 33] where the free base porphyrins are complexed much more strongly than the zinc derivatives.

Table II. Quenching Constants (k_q) and Slopes of Stern-Volmer Plots with Nitrobenzene

Porphyrin	Solvent	Slope	k_q,[a] l. mol^{-1} sec^{-1}
Zn etio[b]	Ethanol	31	7.3×10^{9}
Zn etio	Methylcyclohexane	21	4.8×10^{9}
Zn etio	Piperidine	15	3.4×10^{9}
Zn etio	Benzene	15	3.4×10^{9}
Zn etio	Benzene + 1 *M* TPP[c]	12	2.8×10^{9}
Mg etio	Acetonitrile	160	1.4×10^{10}
Mg etio[d]	Ethanol	120	1.2×10^{10}
Mg etio	Methylcyclohexane	120	1.2×10^{10}
Mg etio	Benzene	120	1.2×10^{10}
Mg etio	Piperidine	63	5.7×10^{9}
Meso[e]	Ethanol	42	2.8×10^{9}
Etio[f]	Ethanol	55	3.7×10^{9}
Etio	Benzene	No quenching	
Etio	Methylcyclohexane	No quenching	

[a] The values of the singlet lifetimes used to calculate k_q are 11×10^{-9} sec for magnesium etioporphyrin, 4.3×10^{-9} sec for zinc etioporphyrin, and 15×10^{-9} for etioporphyrin and mesoporphyrin.[30] [b] Zinc etioporphyrin I. [c] Triphenylphosphine. [d] Magnesium etioporphyrin I. [e] Mesoporphyrin IX. [f] Etioporphyrin I.

Table III. Slopes of Stern–Volmer Plots for Zinc Etioporphyrin I with Several Quenchers

Quencher	Solvent	Slope
p-Benzoquinone	Benzene	55
p-Nitrotoluene	Ethanol	31
cis-4-Nitrostilbene	Benzene	29
p-Nitroanisole	Ethanol	26
p-Nitroaniline	Benzene	21
1,2-Dimethyl-3-nitrobenzene	Ethanol	~0.7[a]
Benzaldehyde	Ethanol	~1[a]

[a] Values this low probably indicate no quenching.

Obvious questions regarding the quenching phenomena pertain to the mechanism of the quenching and the fate of the porphyrin excitation energy. With regard to the former, it seems reasonable to conclude from the nature of the quencher that the initial interaction has some charge-transfer character. If we assume that initial contact of porphyrin excited singlet with quencher produces an excited complex with considerable charge separation, possibilities for decay of the complex include the following.

$$[\mathrm{P{-}Q}]^{1*} \longrightarrow \mathrm{P} + \mathrm{Q}^{3*} \tag{1}$$

$$[\mathrm{P{-}Q}]^{1*} \longrightarrow \mathrm{P}^{+} + \mathrm{Q}^{-} \tag{2}$$

$$[\mathrm{P{-}Q}]^{1*} \longrightarrow \text{chemical reaction} \tag{3}$$

$$[\mathrm{P{-}Q}]^{1*} \longrightarrow \mathrm{P} + \mathrm{Q} + \text{heat} \tag{4}$$

Singlet energies of the porphyrins (*ca.* 50 kcal/mol) are fairly close to triplet energies of quenchers such as benzoquinone (50)[17] and 4-nitrostilbene (50)[38] so that production of quencher triplet is feasible by this route if not by classical energy transfer from the porphyrin triplet. Similarly, electron transfer, reaction 2, is an attractive candidate since it has been amply demonstrated that photoexcited porphyrins can participate in electron transfer and redox phenomena with a variety of agents including benzoquinone.[3,5,6] In our studies we find very little permanent chemical change accompanying fluorescence quenching. Samples with nitrobenzene undergo slow decomposition on prolonged irradiation, perhaps by photoreduction of the porphyrin.[39]

As a means of determining the importance of reactions 1–3, we irradiated benzene solutions of 4-nitrostilbene (PNS) with zinc and magnesium etioporphyrin I using light absorbed only by the porphyrin-visible transitions. The only observable consequence of the irradiation was *cis–trans* isomerization of the olefin. Stationary states of 99.5% *trans* were obtained for both metalloporphyrins at several different concentrations. Quantum yields for isomerization were low in both directions; the energetically downhill $\phi_{c\rightarrow t}$ is much higher than $\phi_{t\rightarrow c}$. To our surprise we found that for zinc etioporphyrin I–*cis*-PNS solutions $\phi_{c\rightarrow t}$ (Figure 3) increases with concentration of PNS at very low concentrations, reaches a maximum, and then decreases at concentrations where fluorescence quenching is significant. The maximum value of $\phi_{c\rightarrow t}$, 0.02, was reached at an olefin concentration of 0.025 *M* where only about one-fourth of the excited singlets are captured. In contrast at an olefin concentration of 0.2 *M*, where about 80% of the singlets are captured, $\phi_{c\rightarrow t}$ has fallen to 0.004. $\phi_{c\rightarrow t}$ values were somewhat lower for magnesium etioporphyrin I solutions than for the zinc porphyrin and the variation with olefin concentration was much less pronounced. For magnesium the maximum value for $\phi_{c\rightarrow t}$ is 0.004; for 0.2 *M* olefin,

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(34) Positive deviations at high (*ca.* 0.2 *M*) quencher concentrations may be due to "static" or nearest neighbor quenching.[35,36]

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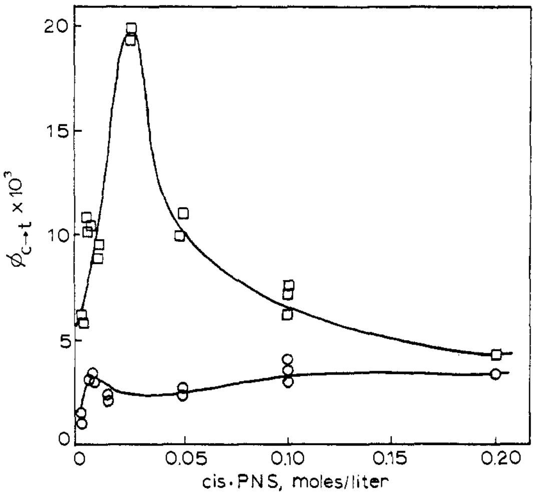

Figure 3. Variation of $\phi_{c\to t}$ with concentration of *cis*-4-nitrostilbene: ○, magnesium etioporphyrin; □, zinc etioporphyrin.

the highest concentration studied, $\phi_{c\to t}$ is 0.003. The differences between behavior of zinc and magnesium etioporphyrin are somewhat surprising, particularly since magnesium etioporphyrin is more readily quenched by nitro compounds than is zinc.

The foregoing results indicate that the porphyrin singlet is not the precursor to the isomerizable species in the zinc porphyrin–PNS system. Therefore, the isomerization probably proceeds *via* the porphyrin triplet. Reaction 1 and other reactions leading to triplet states from the singlet complex can be excluded. The fact that isomerization decreases with quenching of the porphyrin singlet *suggests* that reaction 2 must not be very important. Radical anions of the stilbenes are known to undergo very efficient isomerization and participate in chain processes.[40] In preliminary experiments we find that considerable *cis* to *trans* isomerization accompanies the chemical (potassium–glyme) generation of radical anions from *cis*-PNS. Although isomerization of PNS might be somewhat inefficient due to electron localization on the nitro group, some increase in isomerization with concentration would be expected if singlet quenching produced increasing concentrations of free ions.

Though the porphyrin triplet must be the precursor of the isomerizable PNS species, it is unlikely that electronic energy transfer is involved in the system. Triplet energies of the metalloporphyrin are lower than that of *trans*-PNS and probably much lower than that for *cis*. The *trans*-rich stationary states obtained in the isomerization strongly suggest a thermodynamic equilibration. *p*-Benzoquinone was found to quench strongly the isomerization even at low concentrations. For example, addition of 0.001 *M* *p*-benzoquinone (which produces no measurable fluorescence quenching) half-quenches the isomerization of 0.1 *M* *cis*-PNS with zinc etio. Our results suggest that either a complex originating from the triplet state with considerable charge separation or a radical anion of PNS is responsible for the isomerization. If the isomerization precursor is the radical anion of PNS, our results indicate that, at least in a nonpolar solvent such as benzene, the porphyrin triplet is more effective than the singlet in donating electrons even though its energy content is considerably lower.

Acknowledgments. We thank the Petroleum Research Fund, administered by the American Chemical Society (Grant 782-G1,2), and the U. S. Public Health Service (Grant No. GM 15,238-01) for support of this work.

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The Effect of Added Neutral Salt on the Isoionic pH of Proteins and Synthetic Polyampholytes[1]

Mitsuru Nagasawa and Ichiro Noda

Contribution from the Department of Synthetic Chemistry, Nagoya University, Chikusa-ku, Nagoya, Japan.

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Abstract: The method of determining the degree of ion binding on proteins from the pH shift of isoionic solution observed when a neutral salt is added is critically examined by comparing the ionization constants of carboxyl group on several proteins and synthetic polyampholytes. It is concluded that the pH shift of isoionic solution cannot always be considered to give the degree of ion binding.

It is often reported that some ions are bound by protein molecules in a neutral electrolyte solution. Several methods of determining the degree of ion binding have been proposed.[2,3] In particular, the method presented almost two decades ago[4] has been rather commonly used in assessing ion binding;[2,3] that is, the degree of ion binding is estimated from the small change in pH observed when a neutral salt is added to an iso-

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(1) This investigation was carried out, in part, in the laboratory of Professor A. Holtzer, Department of Chemistry, Washington University, St. Louis, Mo., where it was supported by Research Grant RG-5488 from the Division of General Medical Sciences, U. S. Public Health Service.

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