spectra of native purple membranes, particularly in the region 200–240 nm, and therefore they are unable to explain the discrepancy between experimental results and the seven-helix model.

**Registry No.** Octyl glucoside, 29836-26-8.

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# Nanosecond Fluorescence Studies of Noncovalent Interaction of Monomeric and Dimeric Intercalators with DNA


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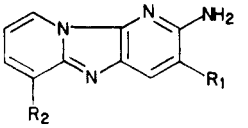
*Received March 8, 1985*


ABSTRACT: The noncovalent interaction of 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2) and its derivatives, which are potent mutagens isolated from L-glutamic acid pyrolysate, with calf thymus DNA was studied by steady-state and nanosecond fluorescence spectroscopies. The fluorescence of these compounds exhibits static quenching by noncovalent interaction with DNA. Fluorescence lifetimes of the free and intercalated states of these compounds were determined to be 9–10 and 0.5–1 ns, respectively. The bis-intercalative effect of the dimeric analogue of Glu-P-2, bis(Glu-P-2)spermine (2GP-SP), to DNA was also investigated. This 2GP-SP, which has two Glu-P-2 moieties at each end of spermine, indicates a strong intramolecular interaction exhibiting remarkable quenching of fluorescence spectrum and lifetime ($\tau$ = 3.5 ns) in the absence of DNA. In the presence of DNA, however, the 3.5-ns lifetime component of fluorescence disappeared, and a two-exponential decay of fluorescence ($t$ = ~10 and 1.5 ns) was observed at a DNA concentration of more than ~0.001 mM P, while the solution containing a very dilute DNA concentration (≤0.001 mM P) exhibits a three-component decay of fluorescence (1.5, 3.5, and ~10 ns). The potent bis intercalation of two moieties in 2GP-SP with an identical DNA molecule was suggested by the DNA-concentration dependence of these fluorescence lifetimes and their intensity.


The potent mutagens 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) and 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2), which were isolated from a




Glu-P-2, $R_1$ = H; $R_2$ = H
Glu-P-1, $R_1$ = H; $R_2$ = $CH_3$
3-Me-Glu-P-2, $R_1$ = $CH_3$; $R_2$ = H

pyrolysate of L-glutamic acid, (Yamamoto et al., 1978; Takeda et al., 1978), are metabolically active and react covalently with DNA (Hashimoto et al., 1979, 1980). In this case, the intercalation of these compounds with DNA was reported to be essential for the covalent reaction. The physicochemical interaction of these compounds with DNA has been investigated by absorption and fluorescence spectroscopies (Imamura et al., 1980a,b). Further, flow dichromism study suggested that Glu-P-1 and other derivatives are oriented parallel to the planes of base pairs of DNA (Imamura et al., 1980a,b). On the other hand, in some potent antitumor antibiotics such as echinomycin (Dell et al., 1975), luzopeptin (Arnold & Clardy, 1981), and carzinophilin (Terawaki & Greenberg, 1966) containing two heteroaromatic chromophores, bis intercalation of these two chromophores with DNA has been suggested by several kinds of analytical methods. In order to develop potential chemotherapeutic drugs, DNA polyintercalating agents such as bis(acridines) (King et al., 1982) and bis(methidium)sperimine (Dervan & Becker, 1978) have been studied by sedimentation and viscometric analysis and UV, fluorescence, and CD spectroscopies. The underlying principal of these investigations is that bis intercalation may afford the opportunity for improving both nucleotide-sequence selectivity and specificity for DNA. Therefore, dimeric analogues of intercalative Glu-P-2, bis(Glu-P-2)spermine (2GP-SP) and its analogues, were

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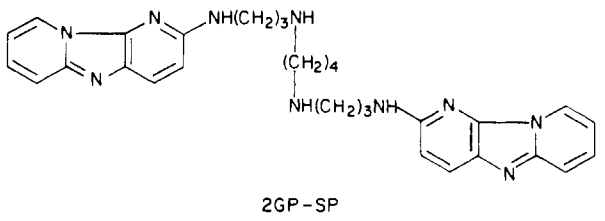


2GP-SP

synthesized (Lee et al., 1982). The remarkable potentiating effect of the intercalative binding of 2GP-SP to DNA was suggested.

This paper reports steady-state and nanosecond fluorescence studies of the intercalative interaction of Glu-P-1 and -2 and 2-amino-3-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (3-Me-Glu-P-2) with DNA. Further, the bis-intercalating compound 2GP-SP is reported to show potential bis-intercalating interaction with DNA. 2GP-SP shows remarkable intramolecular interaction in the buffer solution without DNA, exhibiting fluorescence quenching compared with Glu-P-2. In the presence of DNA, 2GP-SP exhibits double-exponential fluorescence decay with lifetimes of 10 and 1.5 ns. The long- and short-lifetime components are attributable to free (open conformer) and intercalated states of Glu-P-2 moieties of 2GP-SP. The amplitude of the latter 1.5-ns lifetime component is much greater than that of the former, and this ratio is almost independent of the DNA concentration above 0.01 mM P. From these facts, bis intercalation of two moieties of 2-GP-SP to an identical DNA molecule was suggested. Further, the binding of this compound to DNA was demonstrated to be not so much influenced by the ionic strength, while Glu-P-2 and -1 were recovered from their intercalated states with increasing ionic strength of solution ([NaCl] < 0.1 M).

## Experimental Procedures

The compounds Glu-P-1, Glu-P-2, and 3-Me-Glu-P-2 were prepared by the reported method. The dimeric analogue 2GP-SP as the hydrobromide form was synthesized by the method reported previously. Calf thymus DNA was obtained from Sigma. The concentration of DNA in 1 mM sodium phosphate–1 mM $Na_2EDTA$ buffer solution was determined by the intensity of the absorption spectrum ($\epsilon_{260nm}$ = 6400 per phosphate). Absorption and fluorescence spectra were recorded at room temperature (23–15 °C) on a Hitachi 323 spectrophotometer and on a MPF-4 spectrofluorometer, respectively. Fluorescence decays were determined by using a time-correlated single photon counting system (Ortec) with a nanosecond light pulser (PRA 510) through a monochromator (Ritsu MC 10). Fluorescence was observed at a right angle through appropriate cut-off filters. The double- and triple-exponential decays were analyzed by $I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + (1 - A_1 - A_2) \exp(-t/\tau_3)$, where $A_i$ and $\tau_i$ are preexponential factor and fluorescence lifetimes, respectively, and $A_1 + A_2 = 1$ for double-exponential decay. The data analysis for the equation was performed by a computer-simulated deconvolution method. The criterion for "best fit" was the minimum sum ($Q$) of the square of the difference between observed ($D_i$) and calculated ($D_i'$) data; an actual allowance was $Q < 0.0002$; $Q = \sum_i^n (D_i - D_i')^2/n$, where $n$ is the number of data (Itoh & Fujiwara, 1984).

## Results and Discussion

*Mono Intercalators.* Ultraviolet and visible absorption and fluorescence spectra of Glu-P-1 and -2 were measured in the absence and presence of DNA, as shown in Figure 1. The fluorescence of these compounds was remarkably quenched

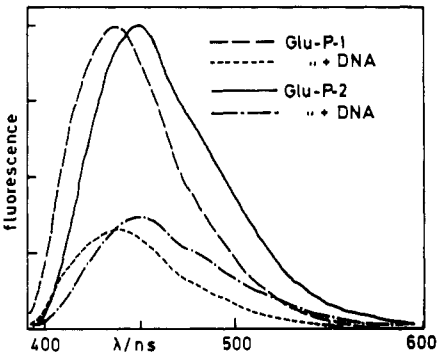


FIGURE 1: Fluorescence spectra of Glu-P-1 ($5.04 \times 10^{-7}$ M) and Glu-P-2 ($5.4 \times 10^{-7}$ M) in the absence and presence of DNA (2.5 mM P for Glu-P-1 and 9.7 mM P for Glu-P-2).

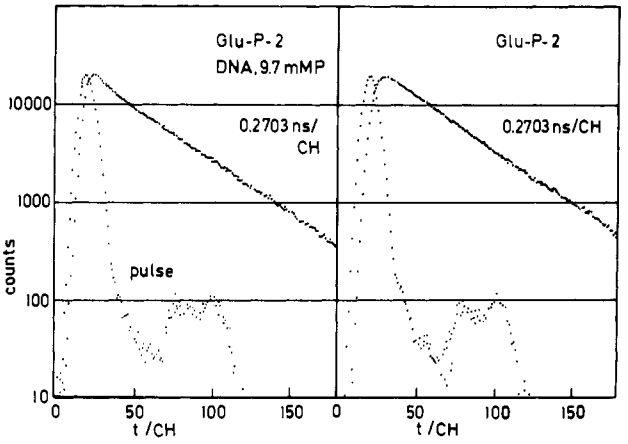


FIGURE 2: Fluorescence decay curves of Glu-P-2 in the absence and presence of DNA.

with a small spectral shift by increasing concentrations of DNA, while the absorption spectra exhibit a little decrease in intensity and red shift. In the absence of DNA, these compounds show exactly a single-exponential decay of fluorescence whose lifetimes are 9–10 ns. In the presence of DNA, the fluorescence decays were observed to show the double exponential expressed by the equation shown in the last section. The amplitude of the short decay component $A_1$ increases with increasing concentration of DNA. However, the decay times of both short and long decays of fluorescence are independent of the DNA concentration. The typical fluorescence decay curves for Glu-P-2 are shown in Figure 2. The concentration dependence of the fluorescence quenching and decay suggests that the fluorescence quenching by DNA is a static quenching and the short lifetime may be attributable to the intercalation of these compounds in DNA. The short lifetimes attributable to the intercalation of these compounds in the DNA molecule demonstrate that the lifetimes of 9–10 ns in the solution without DNA are remarkably quenched to 1–0.5 ns by the intercalation. The short lifetime components of these compounds in the intercalated states are somewhat different from each other; they are 1.0 ns for Glu-P-2, 0.6 ns for 3-Me-Glu-P-1, and 0.5 ns for Glu-P-1 (Table I). Since lifetimes of these compounds without DNA are almost similar each other (9–10 ns), the difference of the lifetimes seems to be attributable to the extent of the fluorescence quenching in the interaction. Further, the order of the quenching of fluorescence lifetimes is in good agreement with the binding characteristics of these compounds: Glu-P-1 > 3-Me-Glu-P-2 > Glu-P-2 (Imamura et al., 1980a,b).

Numerous fluorescence quenching studies of the DNA–proflavin and DNA–acriflavin complexes suggested that there

Table I: Fluorescence Lifetimes and Preexponential Factors ($A$) of Glu-P-1, Glu-P-2, and Me-Glu-P-2 Solutions Containing Several Concentrations of DNA[a]

| | [DNA] (mM P) | $A$ | $\tau_1$ (ns) | $\tau_2$ (ns) |
|---|---|---|---|---|
| Glu-P-1 | 0 | | | 8.62 |
| | 0.2 | 0.218 | 0.52 | 8.88 |
| | 0.5 | 0.31 | 0.53 | 8.95 |
| | 1.0 | 0.45 | 0.51 | 8.94 |
| | 2.5 | 0.55 | 0.53 | 9.00 |
| Glu-P-2 | 0 | | | 10.24 |
| | 2.5 | 0.28 | 1.02 | 10.41 |
| | 5.6 | 0.37 | 1.02 | 10.30 |
| | 9.7 | 0.57 | 1.03 | 10.37 |
| Me-Glu-P-2 | 0 | | | 8.85 |
| | 0.25 | 0.25 | 0.68 | 9.04 |
| | 0.5 | 0.31 | 0.64 | 9.03 |
| | 2.5 | 0.42 | 0.65 | 9.03 |
| | 4.0 | 0.45 | 0.60 | 9.03 |

[a] Concentrations of Glu-P-1 and derivatives are $5.0 \times 10^{-7}$ M. Errors are approximately ±5%.

Table II: Fluorescence Lifetimes and Preexponential Factors of Glu-P-1 ($5.0 \times 10^{-7}$ M) and DNA (1.0 mM P) Containing Several Concentrations of NaCl

| [NaCl] (M) | $A$ for $\tau_1$ component | $\tau_1$ (ns) | $\tau_2$ (ns) |
|---|---|---|---|
| 0 | 0.44 | 0.51 | 8.4 |
| 0.025 | 0.35 | 0.51 | 8.2 |
| 0.05 | 0.34 | 0.51 | 8.0 |
| 1.0 | 0.28 | 0.51 | 7.9 |
| 3.0 | 0.25 | 0.51 | 8.2 |

are two classes of strong binding sites in DNA (Tubbs et al., 1964; Schreiber & Daune, 1974). Duportail et al. (1977) reported that fluorescence decay of several acridine dyes upon intercalation to DNA was expressed by the double-exponential decay. Furthermore, Kubota & Motoda (1980) revealed that the fluorescence decay of 9-aminoacridine intercalated to DNA follows a three-exponential decay. In our system of Glu-P-1 and its derivatives and DNA, however, only one fluorescence decay of their lifetimes (0.5–1.0 ns) was observed for the intercalated states of these compounds in addition to decays of the nonintercalated ones. Imamura et al. (1980a,b) suggested that the intercalation of these compounds into the space of a dimeric guanine–cytosine base pair may take place and its intercalation is essential for covalent bonding of the activated mutagen with a guanyl moiety. Therefore, these very much quenched fluorescence lifetimes of these compounds intercalated in DNA may be ascribed to this intercalated state in the dimeric pair.

Lee et al. (1982) reported that the binding affinity of these compounds to DNA is very weak compared with ethidium bromide, known as a potent intercalator. Therefore, the effect of added NaCl concentration to the affinity of these compounds to DNA was measured by the fluorescence intensity and lifetime. The fluorescence intensities of Glu-P-1, Glu-P-2, and 3-Me-Glu-P-2 solutions in the presence of DNA, where the fluorescence is being strongly quenched, remarkably increase with increasing NaCl concentration. At a NaCl concentration of 1.0 M, the fluorescence spectra and intensities almost recover to those of the compounds in the solutions without DNA. Since this concentration of NaCl does not make any effect on the property of DNA, the increase of fluorescence intensity implies the recovery of these compounds from the intercalation in DNA. This consideration was further confirmed by the fact that the fluorescence lifetimes of these compounds in the intercalated and nonintercalated (free) states are independent of NaCl concentration, but the amplitude of the short-lifetime component $A_1$ due to the intercalation decreases with increasing NaCl concentration. Table II shows fluorescence lifetimes and preexponential factors ($A_i$) in Glu-P-1/DNA solutions containing several concentrations of NaCl. The fact implies that the intercalation of these compounds is rather weak binding compared with other potent intercalators such as 9-aminoacridine and ethidium bromide.

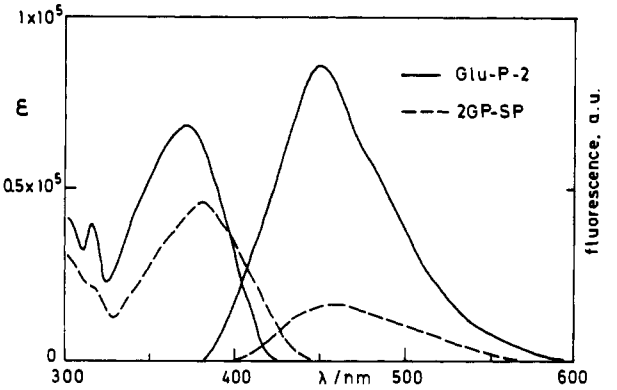


FIGURE 3: Absorption and fluorescence spectra of 2GP-SP in comparison with those of Glu-P-2 [ε for 2GP-SP is (molar extinction coefficient)/2].

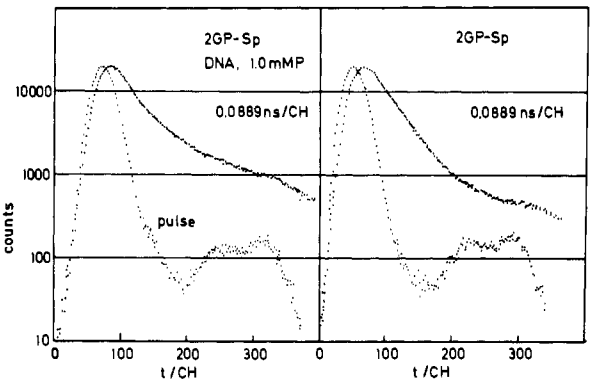


FIGURE 4: Fluorescence decay curves of 2GP-SP ($6.3 \times 10^{-7}$ M) in the absence and presence of DNA (1.0 mM P).

*Bis Intercalator.* The UV absorption spectrum of buffer solution of 2GP-SP shows an absorption band at 383 nm. Figure 3 shows the absorption spectrum of 2GP-SP in comparison with that of Glu-P-2. Since 2GP-SP has two Glu-P-2 moieties in one molecule and the intensity of the absorption band is illustrated as (molar extinction coefficient)/2, it is noteworthy that the absorption intensity for each Glu-P-2 moiety in 2GP-SP is approximately 67% of Glu-P-2. The weak absorption intensity may be attributable to a strong hypochromic interaction between two Glu-P-2 moieties in this compound. This solution exhibits a remarkably weak fluorescence spectrum at 440 nm, as shown in Figure 3. Relative fluorescence quantum yield of 2GP-SP is 0.19 of Glu-P-2. The fluorescence decay of this solution was measured to exhibit an entirely single-exponential decay with a decay time of 3.6 ns, whose fluorescence decay curve is shown in Figure 4. Since no concentration dependence of fluorescence spectrum and lifetime was observed, the fluorescence of Glu-P-2 moieties in 2GP-SP is being remarkably quenched by the intramolecular interaction between two moieties. This intramolecular interaction seems to suggest a model of the closed conformer of 2GP-SP.

Further, the fluorescence spectra of 2GP-SP in the several concentrations of NaCl were measured as shown in Figure 5. The intensity remarkably increases with increasing NaCl concentration. In the presence of NaCl, the fluorescence decays are expressed by a double exponential with decay times

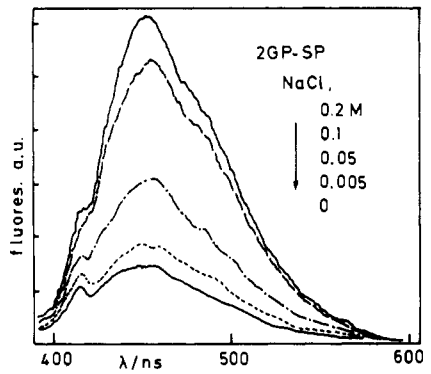


FIGURE 5: Fluorescence spectra of 2GP-SP ($3 \times 10^{-7}$ M) solution containing several concentration of NaCl.

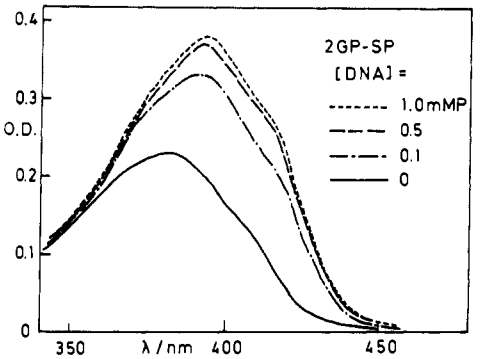


FIGURE 6: Absorption spectra of 2GP-SP solutions containing several concentrations of DNA.

Table III: Fluorescence Lifetimes and Decay Components of a 2GP-SP Solution Containing Several Concentrations of NaCl[a]

| [NaCl] (M) | $\tau_1$ (ns) | $\tau_2$ (ns) |
|---|---|---|
| 0 | 3.5 | |
| 0.05 | 3.6 (0.79) | 10.71 (0.21) |
| 0.1 | 3.3 (0.73) | 9.84 (0.27) |
| 0.2 | 3.6 (0.69) | 10.45 (0.31) |

[a] [2GP-SP] = $6.8 \times 10^{-7}$ M.

of 3.65 and 10.4 ns (Table III). The short-decay component due to the closed conformer decreases with increasing NaCl concentration. Since the long lifetime of 10.6 ns is very much similar to that of free Glu-P-2 mentioned above, the two lifetimes of 3.65 and 10.4 ns are ascribed to the closed form being self-quenched and the noninteracted Glu-P-2 moiety of 2GP-SP, respectively. From these results, it is demonstrated that the intramolecular interaction between two Glu-P-2 moieties may be released to the open conformer by strong ionic strength of solution. The fact implies that 2GP-SP is in an equilibrium between the intramolecularly interacted closed and noninteracted open conforms in the solution of intermediate ionic strength. In the weak ionic strength buffer, however, 2-GP-SP may be an entirely closed form exhibiting a single-exponential decay as previously mentioned.

In the presence of DNA, an absorption spectrum of 2GP-SP increases in intensity with somewhat of a red shift as shown in Figure 6, while that of Glu-P-2 exhibits little intensity change with a red shift as mentioned above. Taking account of the facts that the absorption spectrum and intensity of the intercalated state of Glu-P-2 were observed not to be different very much from those in the absence of DNA and that the absorption intensity for each Glu-P-2 moiety of 2GP-SP is only 67% of that of Glu-P-2 by the hypochromic interaction, the remarkable increase of the absorption intensity in the presence of DNA seems to be attributable to the increase of the intercalated moiety in 2GP-SP. The DNA concentration dependence of the absorption spectra shown in Figure 6 is in good agreement with this consideration. Therefore, 2GP-SP may release its intramolecular interaction in the presence of DNA, and its Glu-P-2 moiety may be intercalated in DNA.

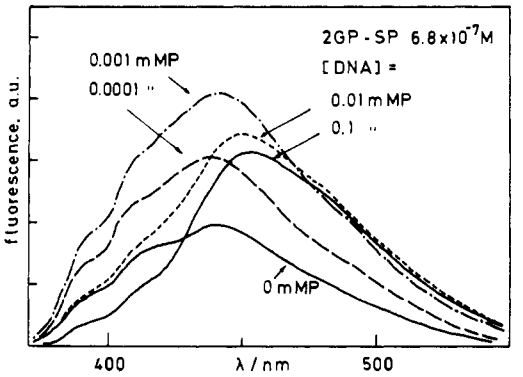


FIGURE 7: Fluorescence spectra of the 2GP-SP/DNA system; excitation wavelength was 360 nm.

Table IV: Fluorescence Lifetimes and Their Components (in Parentheses) of 2GP-SP Solutions ($6.3 \times 10^{-7}$ M) Containing Several Concentrations of DNA at Room Temperature

| DNA (mM P) | $\tau_1$ (ns) | $\tau_2$ (ns) | $\tau_2$ (ns) |
|---|---|---|---|
| 0[a] | | 3.46 (1.0) | |
| 0.0001[b] | 1.01 (0.38) | 3.56 (0.19) | 10.23 (0.42) |
| 0.001[b] | 1.35 (0.52) | 3.47 (0.21) | 10.23 (0.27) |
| 0.01[c] | 1.55 (0.83) | | 10.58 (0.17) |
| 0.1 | 1.41 (0.85) | | 10.66 (0.15) |
| 0.5 | 1.40 (0.86) | | 10.98 (0.14) |
| 1.0 | 1.49 (0.87) | | 10.33 (0.13) |
| 5.5 | 1.63 (0.87) | | 11.41 (0.13) |

[a] The decay curve was analyzed as a single-exponential decay. [b] The curve was expressed by a triple-exponential decay. [c] A double exponential.

On the other hand, the fluorescence spectra of 2GP-SP exhibit considerably complex features with increasing DNA concentration as shown in Figure 7; the spectra show a remarkable increase in intensity until [DNA] = 0.005 mM P and gradually decrease with a spectral red shift. However, no quantitative analysis of the intercalation can be carried out from these complicated fluorescence spectra. The fluorescence decays of 2GP-SP solutions with variable concentrations of DNA are very much suggestive for the intercalation of this compound as well as for the intramolecular interaction between two Glu-P-2 moieties. In the presence of DNA, the fluorescence decay of this solution is expressed by a double exponential as shown in Figure 4. The double-exponential decay affords two decay times of 1.5 and 10.5 ns, irrespective of DNA concentration more than 0.001 mM P, though the short-decay component ($A_1$) is a little dependent on the DNA concentration (>0.001 mM P) as summarized in Table IV. The fluorescence lifetimes of the intercalated and free states of Glu-P-2 were determined to be 1.0 and ~10 ns, respectively, as summarized in Table I. Therefore, the obtained lifetimes of 1.5 and 10.5 ns in 2GP-SP may be ascribed to the intercalated and free states of Glu-P-2 moieties of this compound, respectively. This fact implies that there is a open conformer whose Glu-P-2 moieties are intercalated in the presence of DNA, though this compound is in the closed form exhibiting a fluorescence lifetime of 3.5 ns in the absence of DNA. However, it is noteworthy that no 3.5-ns component attributable to the closed form of 2GP-SP was observed and the 1.5-ns component amounts to 0.83 in the DNA concentration more than 0.01 mM P. From these results, a striking feature

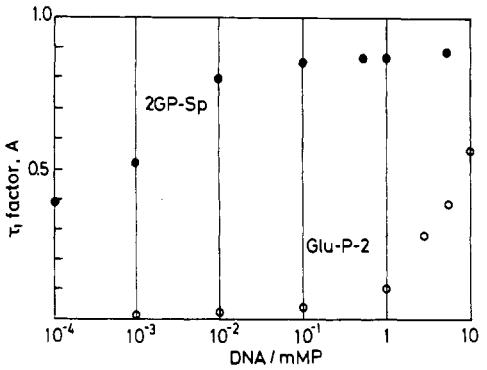


FIGURE 8: DNA concentration dependence of the short-decay component ($A$) in the fluorescence decay curve of the 2GP-SP/DNA system.

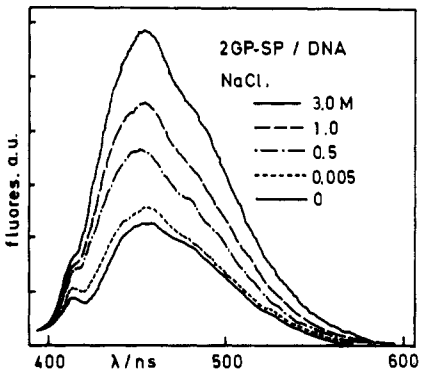


FIGURE 9: Effect of NaCl concentration on the fluorescence of 2GP-SP ($6.3 \times 10^{-7}$ M)/DNA (2.0 mM P).

may be considered in the interaction between 2GP-SP and DNA. The intramolecular interaction of 2GP-SP exhibiting a 3.5-ns fluorescence lifetime may be released to the open form by the strong interaction with a DNA molecule followed by the efficient intercalation of the Glu-P-2 moiety. If the intercalation of one moiety in 2GP-SP with DNA is similar to that of Glu-P-2 in structure and in equilibrium, the 1.5-ns component may be estimated to be at most ~0.4 (for one moiety in 2GP-SP) even at a high concentration of DNA from data shown in Table I. However, the 1.5-ns component in 2GP-SP/DNA is 0.81–0.87 throughout DNA concentrations of 5.5–0.01 mM P, as shown in Figure 8. This fact suggests that two Glu-P-2 moieties may be both intercalated by DNA (bis intercalation). Further, since A is not very much dependent on the DNA concentration, the bis intercalation seems to take place in the same DNA molecule. On the other hand, a small amount of 10-ns component in 2GP-SP/DNA may be attributable to the free moiety, as mentioned above. Taking account of the fact that the free moiety cannot be obtained in the absence of DNA, it seems that this free moiety is generated by the mono intercalation of another moiety in 2GP-SP. In the very dilute concentration of DNA less than 0.001 mM P, however, the fluorescence decay curves are more reliably expressed by a triple exponential rather than the double exponential, whose decay times are 1.5, 3.6, and 10 ns as summarized in Table IV. The 3.5-ns component attributable to the closed form of 2GP-SP, which seems to show no interaction with DNA, amounts to approximately 0.2 for a DNA concentration of 0.001–0.0001 mM P. In this very dilute DNA concentration, the concentration of 2GP-SP ($6.6 \times 10^{-7}$ M) is comparable with or less than the DNA concentration. Therefore, the observation of the triple-exponential decay mentioned above is reasonably consistent with this argument on both concentrations of 2GP-SP and DNA.

Lee et al. reported that the affinity of several intercalators toward DNA increase in the order of Glu-P-2 < EtBr < spermine < 2GP-SP. The weak affinity of monomeric Glu-P-1 and -2 was demonstrated by the effect of ionic strength of the solution mentioned above. Therefore, the effect of added NaCl to the 2GP-SP/DNA solution was determined. Figure 9 illustrates fluorescence spectra of the 2GP-SP/DNA solutions containing several concentrations of NaCl. Fluorescence intensity and 10-ns component (Figure 9) increase with increasing NaCl concentration. However, the extent of increase of the fluorescence intensity and 10-ns component is very small compared with those in Glu-P-1 and -2, and further, the short-lifetime component is not very much dependent on the NaCl concentration ($A$ = 0.87 for [NaCl] = 0 M and 0.77 for 3.0 M). These facts indicate the bis intercalation of 2GP-SP with a great affinity to DNA as suggested above. Since the intercalated state of the Glu-P-2 moiety in 2GP-SP does not seem to be different from those of Glu-P-1 and -2, the strong affinity of this bis intercalation of 2GP-SP might be ascribed to the strong binding of spermine with DNA.

## ACKNOWLEDGMENTS

Thanks are due to Setsuko Nakajima for determination and analysis of triple-exponential decay of fluorescence.

**Registry No.** Glu-P-2, 67730-10-3; 2GP-SP, 98105-11-4; Glu-P-1, 67730-11-4; Me-Glu-P-2, 75679-00-4.

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