HEMIN BINDING TO DNA WITH BIS-DENTATE ACRIDINE INTERCALATOR

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Hemin was covalently connected to two 9-aminoacridines (9AA) through its two propionates, and the binding properties of this bis-dentate compound (hemin(9AA)$_2$) to DNA were examined by visible absorption spectroscopy. The binding affinity of the hemin(9AA)$_2$ was found to be higher than that of the hemin, and this should be attributed to the two linked acridine moieties. The binding constant (K) and the number of binding sites per nucleotide (n) were estimated by Scatchard plot analyses. Though the order of the K value of hemin(9AA)$_2$ was similar to that of 9AA, the hemin(9AA)$_2$ was analyzed to have a smaller n value, the order of which was of about 10^{-4}. The small n value may reflect the sequence specificity of the bis-dentate hemin(9AA)$_2$ on binding to the DNA.

KEYWORDS hemin; acridine; intercalator; binding constant; binding site

The glycopeptide antibiotic bleomycin is widely used in the clinical treatment of human malignant diseases, and the principal target is recognized to be DNA in the cell.[1] The bleomycin action involves site-selective binding to double-helical DNA and oxygen-mediated scission of the DNA strands, which is catalyzed by the iron domain brought into proximity with the target.[2] Functional bleomycin models were synthesized by covalently connecting iron porphyrin complex with an acridine dye[3] or a pyrolysate of L-glutamic acid (Glu-P).[4] The iron atom in the former acts as an oxygen-activating group, while the latter is an intercalator to the double-helical DNA. The DNA scission abilities of these compounds were examined, and the preferred cleavages at GC and GT sequences are reported.[4] The specificity for the cleavage of DNA base sequences is quite similar to that of bleomycin.[5] The previous studies are, however, mainly intended for the examination of the DNA scission ability of the model compounds, and the binding properties have not been fully understood. To design drugs with high efficiency of DNA scission, molecular interactions between the drugs and the target DNA should be revealed. Here we have synthesized a hemin derivative with two acridine moieties which were covalently connected to the two propionate periphery of the hemin, and report binding properties of this bis-dentate compound (hemin(9AA)$_2$) with DNA by the use of absorption spectroscopy.

Protoporphyrin IX was obtained from Sigma and used without further purification. The hemin(9AA)$_2$ was prepared by the method reported elsewhere[3b] with slight modifications. In brief, the protoporphyrin was condensed with 9-aminoacridine (9AA, Nakarai) in the presence of water-soluble carbodiimide (Nakarai), and the desired product was purified by column chromatography over alumina (Merck, neutral). The product was then dissolved in DMF and refluxed with anhydrous $FeCl_2$.[6] The hemin(9AA)$_2$ was purified by column chromatography over alumina, and was found to give a single spot on TLC. The hemin(9AA)$_2$ was found to show blue fluorescence with UV irradiation and to have a Soret absorption band at about 390 nm, which are the characteristics of the 9AA and the porphyrin moieties, respectively.

In Fig. 1 are shown absorption spectral changes of the hemin(9AA)$_2$ by the addition of salmon testis (ST) DNA (Wako Chemicals). All of the absorption spectra were obtained in 10 mM sodium phosphate buffer (pH 7.0). It is clear that the spectra change with one set of isosbestic points on increasing amounts of

DNA. This indicates that the reaction proceeds in equilibrium between the free and DNA-bound hemin(9AA)$_2$. The Soret band increased, which is opposite to the hypochromic effect of porphyrin intercalators such as H_2TMPyP,[7] suggesting that the porphyrin moiety did not intercalate to the DNA. The absorbance changes at 387 nm on the addition of DNA were compared between the two hemin derivatives (Fig. 2). It is clearly shown that the hemin(9AA)$_2$ has higher binding affinity than the hemin, and half of the hemin(9AA)$_2$ seems to have bound to the DNA in the presence of about 15 mMP[8] DNA.

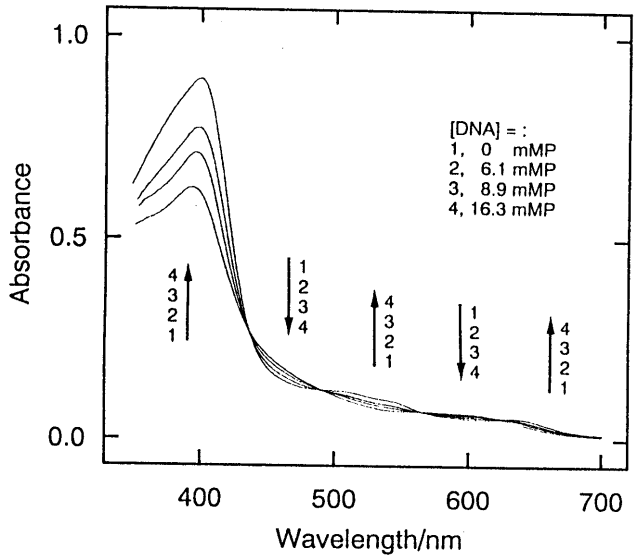

Fig. 1. Absorption Spectral Changes of Hemin(9AA)$_2$ by the Addition of ST DNA. DNA concentration (mMP); 1, 0; 2, 6.1; 3, 8.9; 4, 16.3.

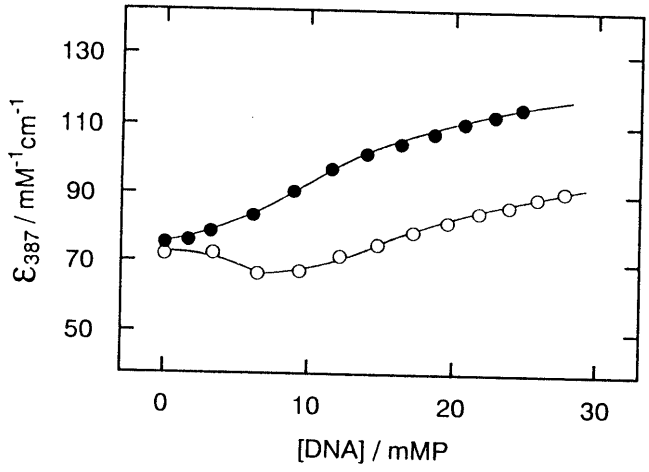

Fig. 2. Absorbance Changes of Hemin Derivatives by the Addition of ST DNA. The optical density was normalized to molar extinction coefficient of the hemin derivatives. Open circle, hemin; closed circle, hemin(9AA)$_2$.

The binding constant (K) and the number of the binding sites on the DNA per nucleotide (n) were estimated by the Scatchard analysis. In Fig. 3 (left), Scatchard plots for the binding of 9AA to the DNA are shown. The K and n values for 9AA were estimated to be 970 mM^{-1} and 0.24, respectively. Similar analyses were made for some of synthetic oligodeoxynucleotides, and the obtained values are summarized in Table I. The K values are found to be in the order GC > GG (CC) = AT > AA (TT), while the n values are nearly in the opposite order. Therefore, the 9AA appears to prefer intercalative binding into the GC sequence, although the number of binding sites will decrease.

The Scatchard plots for the hemin(9AA)$_2$ are shown in Fig. 3 (right), and K and n values were estimated to be 4800 mM^{-1}and 0.00043, respectively. Since the K value of the hemin(9AA)$_2$ was greater than that of the 9AA for ST DNA (Table I), both of the acridine moieties of the hemin(9AA)$_2$ should be concerned with its binding to the DNA. Some of the metal complexes of TMPyP are reported to bind to the surface of the DNA.[7] Therefore, the absorption spectral changes of the hemin(9AA)$_2$ (Fig. 1) should indicate the interaction between the iron atom and the DNA backbone on the DNA surface. This mode of interaction may in part be responsible for the greater K value of the hemin(9AA)$_2$.

On the other hand, the n value of the hemin(9AA)$_2$ was extremely low. Since the 9AA has a greater K value for the intercalative binding with GC sequences of the DNA (Table I), each of the two acridine moieties of the hemin(9AA)$_2$ should also prefer GC, and hence the hemin(9AA)$_2$ may prefer GCGC sequences. The relative occurrence of the GCGC to GC sequences will be estimated to be about $(1/4)^2$ = 0.0625, which could not fully explain the observed ratio of 0.00043/0.24 = 0.0018. The hemin(9AA)$_2$ has a short linking chain between the porphyrin skeletal and the individual acridine moiety, and hence the binding of the hemin(9AA)$_2$ should distort the DNA helical structure considerably. Therefore, the binding would be allowed only to specific DNA sequences with flexibility, and the observed low n value may reflect the sequence specificity of this bis-dentate hemin(9AA)$_2$. Preparation of molecules with longer linking chain

and higher affinity to the DNA is currently underway. Such molecules may possibly be utilized as a synthetic restriction "enzyme" for DNA scission.

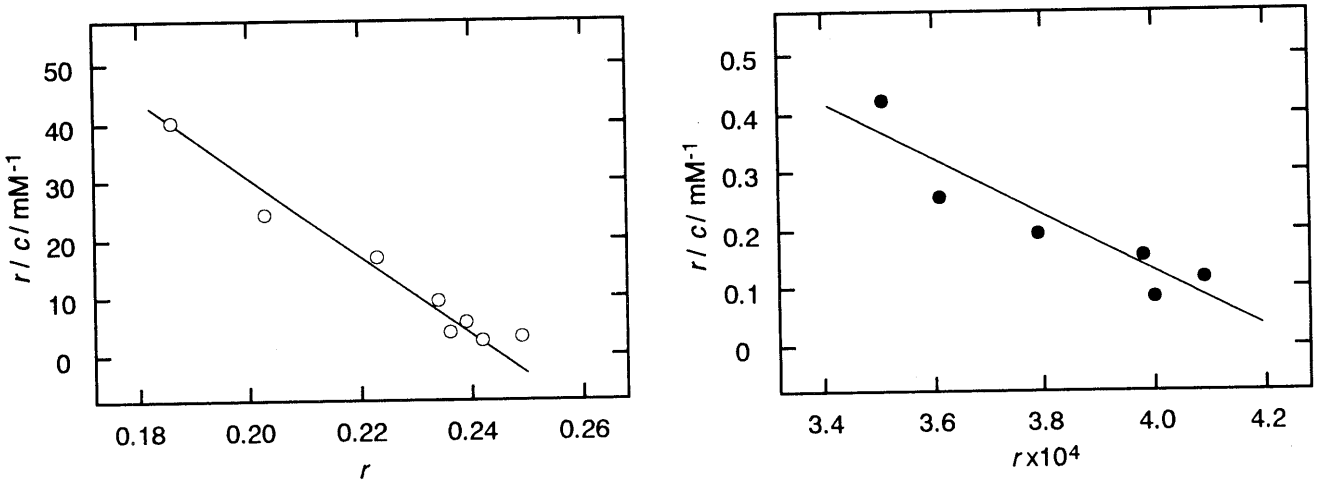

Fig. 3. Scatchard Plot Analyses for the Binding of 9AA (Left) and Hemin $(9AA)_2$ (Right) to ST DNA

Table I. The Binding Constants (K) and the Number of Binding Sites (n) of 9AA and Hemin$(9AA)_2$

Compound	DNA	K/mM^{-1}	n
9AA	Salmon Testis	970	0.24
9AA	Poly[(dG-dC)]·poly[(dG-dC)]	2300	0.43
9AA	Poly(dG)·poly(dC)	890	0.37
9AA	Poly[(dA-dT)]·poly[(dA-dT)]	900	0.59
9AA	Poly (dA)·poly(dT)	130	1.1
Hemin$(9AA)_2$	Salmon Testis	4800	0.00043

REFERENCES AND NOTES

1) S. M. Hecht (ed.), "Bleomycin: Chemical, Biochemical and Biological Aspects", Springer-Verlag, New York, 1979; L. F. Povirk, "Molecular Aspects of Anticancer Drug Action", ed. by S. Neidle, M. J. Waring, Macmillan, New York, 1983, pp. 157; M. A. Friedman, *Recent Results Cancer Res.*, **8**, 2835 (1980).
2) E. A. Sausville, J. Peisach, S. B. Horwitz, *Biochemistry*, **17**, 2740 (1978); H. Kuramochi, K. Takahashi, T. Takita, H. Umezawa, *J. Antibiot.*, **34**, 576 (1981); J. W. Lown, S. K. Sim, *Biochem. Biophys. Res. Commun.*, **77**, 1150 (1977).
3) a) J. W. Lown, S. M. Sondhi, C.-W. Ong, A. Skorobogaty, H. Kishikawa, J. C. Dabrowiak, *Biochemistry*, **25**, 5111 (1986); b) J. W. Lown, A. V. Joshua, *J. Chem. Soc., Chem. Commum.*, **1982**, 1298.
4) Y. Hashimoto, H. Iijima, K. Shudo, M. Itoh, *Nucl. Acids Res.*, **15**, 157 (1984).
5) M. Takeshita, A. P. Grollman, E. Ohtsubo, H. Ohtsubo, *Proc. Natl. Acad. Sci., U. S. A.*, **75**, 5983 (1978).
6) A. D. Adler, F. R. Longo, F. Kampas, J. Kim, *J. Inorg. Nucl. Chem.*, **32**, 2443 (1970).
7) R. E. McKinnie, J. D. Choi, J. W. Bell, E. J. Gibbs, R. F. Pasternack, *J. Inorg. Biochem.*, **32**, 207 (1988); H_2TMPyP denotes α,β,γ,δ-tetrakis(4-*N*-methylpyridyl)porphine.
8) DNA concentrations were expressed by the concentration of nucleotides and denoted by mMP units.

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