Enhancement of aromatic amino acid–nucleic acid base stacking interaction by metal coordination to base: fluorescence study on a tryptophan–Pt(II)–guanine ternary complex

Hisahiro Kawai, Mariko Tarui, Mitsunobo Doi, Toshimasa Ishida*

Department of Physical Chemistry, Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

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Abstract In order to investigate the effect of the Pt(II) ion on the stacking interaction between tryptophan and a guanine base, the quenching of Trp fluorescence was monitored for some systems in the absence and presence of the metal ion, and the association constants were obtained by the analysis of Eadie–Hofstee plots. All spectral data suggested that the stacking interaction is enhanced by the Pt(II) coordination to the guanine N7 atom. The result indicates the importance of the metal ion as a bookmark in the specific recognition of a nucleic acid base by an aromatic amino acid residue.

Key words: Platinum complex; Tryptophan; Guanine; Stacking interaction; Fluorescence

1. Introduction

The selective recognition of a specific nucleic acid base or base sequence of DNA or RNA by a protein is a fundamental biological process. The coupling of hydrogen-bond pairing to the periphery of the purine or pyrimidine base with a stacking interaction perpendicular to its plane is now accepted as an important interaction pattern that makes such a specific and precise recognition possible [1–6].

On the other hand, it is generally accepted that the stacking interaction itself is not strong enough to fix the most suitable partner for hydrogen-bond pairing, because the π-electron-accepting ability of the neutral nucleic acid base from a aromatic amino acid like tryptophan (Trp) is rather poor, compared with that of flavin or the pyridine coenzyme [7,8]. However, it has been shown [9,10] that the stacking interaction is prominently enhanced by the N-quarternization of nucleic acid bases, and this is mainly due to the lowering of the LUMO energy of the base, consequently leading to the reinforcement of the interaction with HOMO of aromatic amino acid. The N-quarternization of the nucleic acid bases occurs frequently in biological systems, as observed in the alkylation of DNA by alkylating agents or the partial protonation even in a neutral pH environment [11]. Thus, the aromatic amino acid residues in protein would play an important role in the specific recognition of such an N-quarternized nucleic base; for example, Trp residues in the eukaryotic initiation factor-4E are indispensable for recognizing the mRNA cap structure, which is characterized by an N7-methylated guanine base [12].

As another possibility to enhance the stacking ability between the neutral nucleic acid base and aromatic amino acid under usual biological conditions, the participation of metal ions could be considered. Since the lowering of LUMO energy of the nucleic acid base could be caused by removing the lone-paired electrons of the base nitrogen atoms, the coordination of a metal ion with respect to the nitrogen atom would exert the same effect as the N-quarternization for the stacking interaction with an aromatic amino acid. Since nucleic acids and nucleotides generally occur as complexes coordinated with metal ions and these are of importance for biological action, it is of special interest to examine this hypothesis. The present study was done to investigate to what extent the metal ion enhances the stacking interaction between tryptophan and nucleic acid bases. To our best knowledge, investigations from this point of view appear to be quite insufficient, although many works on binding modes of metal–nucleic acid base–amino acid ternary complexes have been reported so far [13–16]. Among many metal ions, Pt(II) was used to make clear the effect of metal ion for the stacking interaction of Trp with a nucleic acid base, especially guanine, because the Pt(II) ion has been found to coordinate predominantly to a fixed position of the base (the N7 atom for guanine base) [17,18]. In this work, Pt(II) was used as the form of $[Pt(dien)(H_2O)](ClO_4)_2$(dien-Pt) or [Pt(dien)(9-ethylguanine)]$(ClO_4)_2$(dien-Pt-GH) (Fig. 1).

2. Materials and methods

All solvents and starting materials were of the highest available purity and used without further purification. GH, dien-Pt and dien-Pt·GH were prepared as previously described [19–21]. The respective purities were checked by HPLC, ^{1}H-NMR, UV and fluorescent X-ray spectra.

Fluorescence spectra were measured on a Jasco FP-770F spectrometer (Nihon Bunko) using a Hg–Xe arc lamp, where a 10-nm slit and 1-cm path length were employed. The temperature of the sample solution was kept at 20°C by circulating the thermostatically regulated water. The fluorescence intensities of the Trp indole ring excited at 290 nm were measured at respective λ_{max} near 353 nm. Measurements were carried out four times and the data reported are the averages of these. The sample solution was prepared using 20 mM Tris-HCl (pH 7.6) or 20 mM citric acid–sodium citrate buffer (pH 2.5, 3.0, 3.5, 4.0 or 5.0). By adding aliquots of 7.5 mM nucleic acid component (base or nucleotide) with or without an equimolar dien-Pt to a 3 ml solution of 5.0 μM Trp, fluorescence tritation was performed under a concentration ratio of [nucleic acid]/[Trp] of 2.5 to 35; the dien-Pt was almost non-fluorescent in the region of measurement. All fluorescence measurements were corrected for sample dilution in the course of the tritation experiment.

*Corresponding author. Fax: (81) (723) 32 9929.

Abbreviations: HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; Trp, L-tryptophan; GH, 9-ethylguanine; dien, diethylenetriamine; dien-Pt, $[(dien)Pt(H_2O)](ClO_4)_2$; dien-Pt·GH, $[(dien)Pt(GH)](ClO_4)_2$

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The association constant (K_a) between Trp and nucleic acid component (base or nucleotide) was elavuated by the Eadie–Hofstee equation [22]:

$$\Delta F = -\frac{1}{K_a} \cdot \frac{\Delta F}{[\text{nucleic acid}]} + \Delta F_c$$

where ΔF is the difference between fluorescence intensities of Trp in the presence (F) and absence (F_o) of nucleic acid base ($\Delta F = F - F_o$), and ΔF_c is the difference of the Trp completely complexed with the nucleic acid. The value of K_a was obtained from the slope of the Eadie–Hofstee plot by least-squares linear regression analysis.

3. Results and discussion

Generally, the fluorescence of the Trp indole ring decreases by the stacking interaction with a nucleic acid base [23], due to an electron transfer in the excited state from indole to a purine or pyrimidine base (Fig. 2). Thus, the degree of this quenching could be used to estimate the strength of the stacking interaction between the Trp and nucleic acid base at their excited states.

3.1. Effect of platinum for interaction between Trp and purine nucleotide

In order to investigate the effect of Pt(II) on the stacking interaction of Trp–purine nucleotide (5′-GMP, 5′-AMP and 5′-IMP), the Trp fluorescence was measured as a function of the nucleotide concentration in the absence and presence of dien-Pt. As a result, reliable Eadie–Hofstee plots and K_a values were obtained only for 5′-GMP (Fig. 3a and Table 1); no reliable linearities in the plots were obtained for 5′-AMP and 5′-IMP, probably due to the weak interactions of these nucleotides with Trp, compared with that of 5′-GMP. Although the exact K_a values were not obtained, the quenching degree of Trp was constantly observed under a definite concentration of nucleotide; for example, the quenching at [nucleotide]/[Trp] = 14 was 54.0, 22.4 and 20.3% in the presence of an equimolar dien-Pt to the nucleotide, and 41.9, 12.6 and 10.3% in the absence of dien-Pt for 5′-GMP, 5′-AMP and 5′-IMP, respectively.

From the quenching degree of Trp, it could say that the interaction decreases in the order 5′-GMP > 5′-AMP ≈ 5′-IMP, and increases significantly by the presence of dien-Pt; the preference of the Trp indole ring for the interaction with a guanine base among different bases has also been observed by other methods [24]. Although the enhancement of the K_a value of Trp–GMP by dien-Pt is not significant as expected, the profiles of Eadie-Hofstee plots (Fig. 3a) are significantly different in the presence and absence of dien-Pt. Especially the difference between ΔF_cs is significant: 0.056 and 0.097 in the absence and presence of dien-Pt, respectively. This implies that the effect of Pt(II) on the Trp–GMP complex is stronger expressed by ΔF_c than by the K_a value, and a significant increase in ΔF_c could be due to the tightening of the complex by dien-Pt.

Table 1
Association constants (K_a) between tryptophan and a guanine analogue in the absence or presence of Pt(II)

Sample	K_a ($\times 10^3$, M^{-1})
Trp + 5′-GMP	3.8(1)
Trp + 5′-GMP + dien-Pt	4.4(1)
Trp + GH	3.0(3)
Trp + dien-Pt·GH	4.6(5)
Trp + dGTP	4.1(1)
Trp + dGTP + dien-Pt	7.6(6)
Trp + N7-deaza-dGTP	5.4(1)
Trp + N7-deaza-dGTP + dien-Pt	7.7(5)

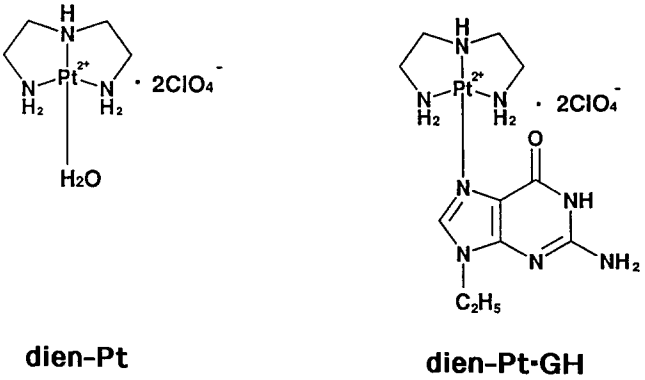

Fig. 1. Chemical structures of $[Pt(dien)(H_2O)](ClO_4)_2$ (dien-Pt) and $[Pt(dien)(9\text{-ethylguanine})](ClO_4)_2$ (dien-Pt·GH).

3.2. Effect of platinum on the interaction between Trp and a guanine base

The positive effect of Pt(II) on the Trp–GMP stacking formation could result from the direct coordination of Pt(II) with respect to the base and/or the bridging effect between both molecules *via* simultaneous coordinations to the Trp amino and carboxyl groups and the nucleotide phosphate group [13]. In order to make clear the contribution of the former case, the interaction of Trp with a guanine base was investigated in the absence and presence of Pt(II), where GH or dien-Pt·GH was used as the guanine base without or with Pt(II), respectively. Although the guanine base has several coordination sites [25],

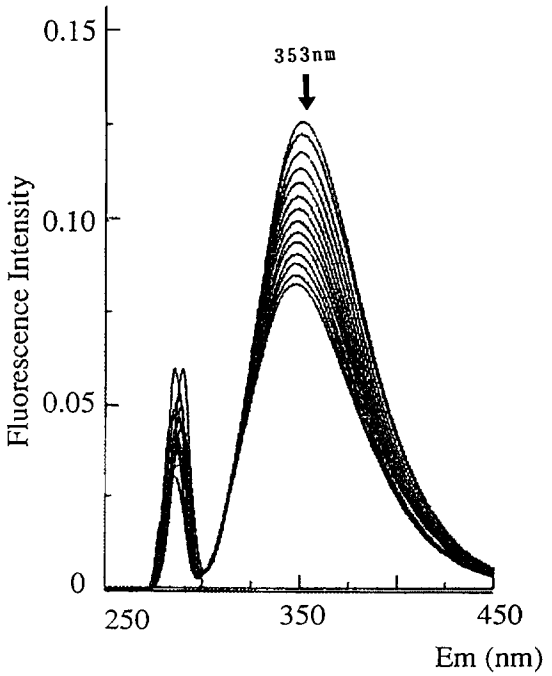

Fig. 2. Fluorescence quenching of Trp as a function of 5′-GMP (excitation = 290 nm).

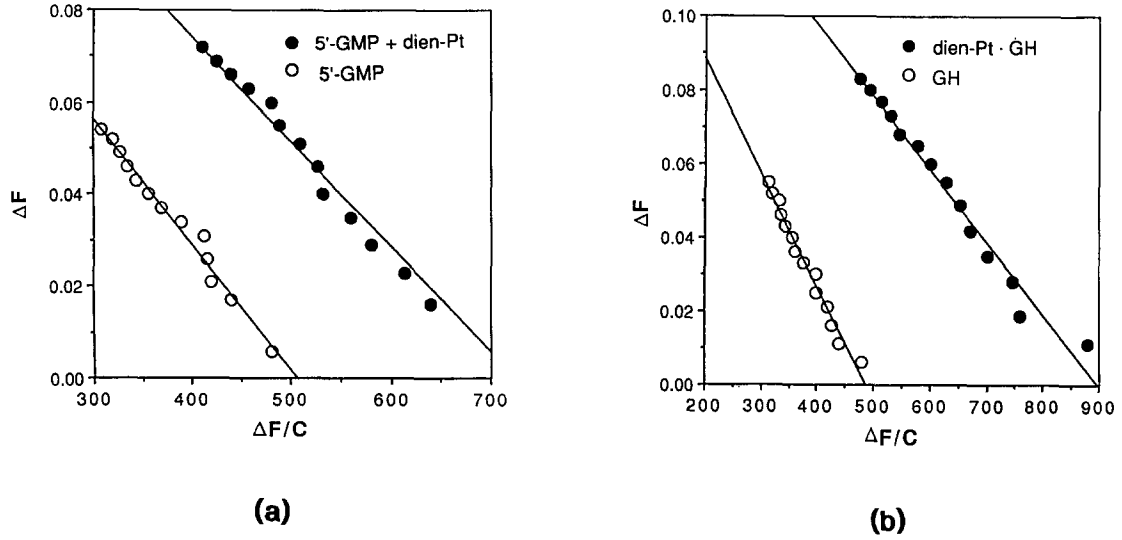

Fig. 3. Eadie–Hofstee plots of Trp fluorescence quenching as a function of (a) 5′-GMP or 5′-GMP + dien-Pt, and (b) GH or dien-Pt·GH (pH 7.6, 20°C).

it is well known that the predominant coordination of Pt(II) takes place at the N7 atom [26,27]. In the present dien-Pt·GH complex, ^{1}H-NMR spectra in D_2O solution (data not shown) suggested that the N7-platinated guanine base, as judged from that the chemical shift (7.70 ppm) of the GH H8 proton, was significantly shifted to the lower side (8.19 ppm) in the dien-Pt·GH complex, while the ethyl protons were not so influenced.

Based on the Trp quenching caused by the interaction with GH or dien-Pt·GH, the Eadie–Hofstee plots (Fig. 3b) were analyzed to give the K_a values (Table 1). The K_a values indicate that the enhancement of stacking interaction in the presence of Pt(II) is more significant than that in Trp–nucleotide interactions, and it is obvious the positive contribution of Pt(II) for the reinforcement of the interaction as a result of the direct coordination to guanine N7 atom; the stiffening of the Trp–GH complex by Pt(II) is also conceivable from the values of ΔF_c = 0.097 and 0.137 in the absence and presence of dien-Pt, respectively.

3.3. *Further characterization of the reinforcement of the Trp–guanine interaction by the base coordination of platinum*

The main coordination site of Pt(II) in the dien-Pt·GH complex was suggested to be the guanine N7 atom as shown by the ^{1}H-NMR spectra. Thus, it is interesting to investigate the effect of Pt(II) on the Trp–N7 protonated guanine interaction, because the protonation would inhibit the direct binding of Pt(II) to the N7 atom. Since the pK_a of the guanine N7 atom is about 3.3 [28], the association constant between Trp and guanine base was examined as a function of pH for GH and 5′-GMP in the absence and presence of Pt(II). From Fig. 4a and b it can be deduced that the K_a values are essentially independent on pH in the presence of Pt(II), while in the absence of Pt(II) these values increase in the acidic region of pH < 4.0.

Since the protonation of the guanine N7 atom causes the prominent stacking interaction with the Trp indole ring [9,10], the increase in the K_a value at an acidic pH < 4.0 is as expected, and consequently, it is obvious that no significant effect on pH

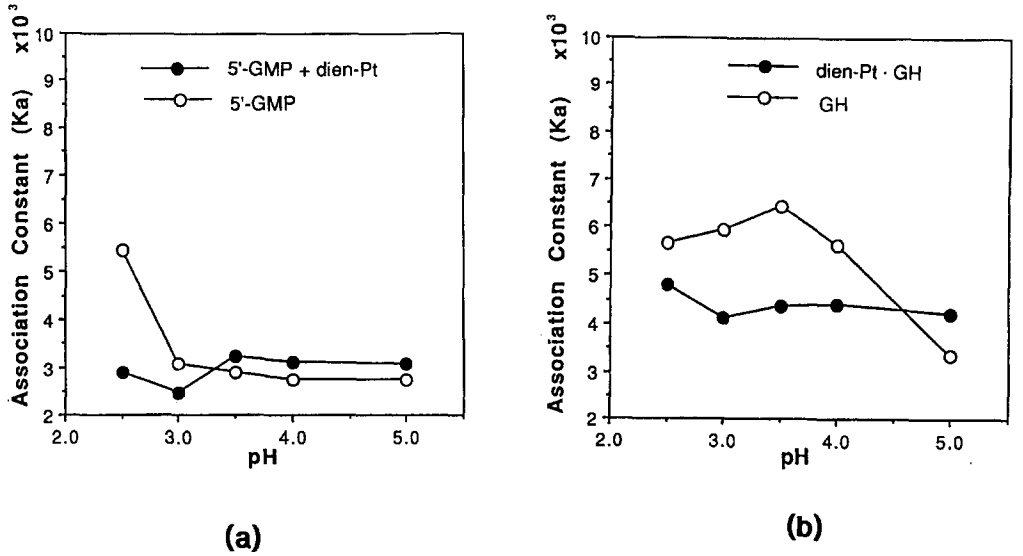

Fig. 4. Plots of association constants as a function of pH: (a) Trp–5′-GMP or Trp–dien-Pt + 5′-GMP, and (b) Trp–GH or Trp–dien-Pt·GH.

for the K_a value in the presence of Pt(II) is due to the direct coordination of Pt(II) to the guanine N7 atom, where Pt(II) inhibits the N7 protonation even at pH < 3.3. Also, the present result indicates that the N-quarternization is a little superior to the N-coordination for the reinforcement of the guanine–Trp stacking interaction.

The effect of the guanine N7-coordinated Pt(II) on the interaction with Trp was also investigated by comparing of the K_a values of Trp–N7-deaza-dGTP and –dGTP complexes (Table 1). Although the existence of the guanine N7 atom is rather inconvenient for the interaction with Trp, as judged from the K_a values, the enhancement of respective K_a values in the presence of Pt(II) is more prominent for Trp–dGTP ($\Delta K_a = 3.5$) than Trp–N7-deaza-dGTP (ΔK_a) = 2.3). This clearly shows the importance of the N7-coordination of Pt(II) for the stacking interaction between Trp and guanine base.

Because metal ions like Mg(II), Ca(II), Ni(I), and K(I) are present in the body in millimolar concentrations, nucleic acids and nucleotides generally occur as complexes coordinated with metal ions. Although the significance of metal binding to nucleic acids and to enzymes involved in processes related to DNA replication, translation and so on has long been recognized, the action of metal ions in these enzyme-catalyzed DNA biological functions is only partially understood. The present results clearly show that the stacking interaction between aromatic amino acids like Trp and nucleic acid bases like G is strengthened by the direct binding of a metal ion to the base. This implies also the role of metal ions as a bookmark for the nucleic acid–enzyme recognition in such a manner that the metal-coordinated base of DNA/RNA is able to more strongly interact with the aromatic amino residue of the enzyme than the uncoordinated one.

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