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Allosteric binding of amino alcohols and diamines by dimeric zinc biladienone

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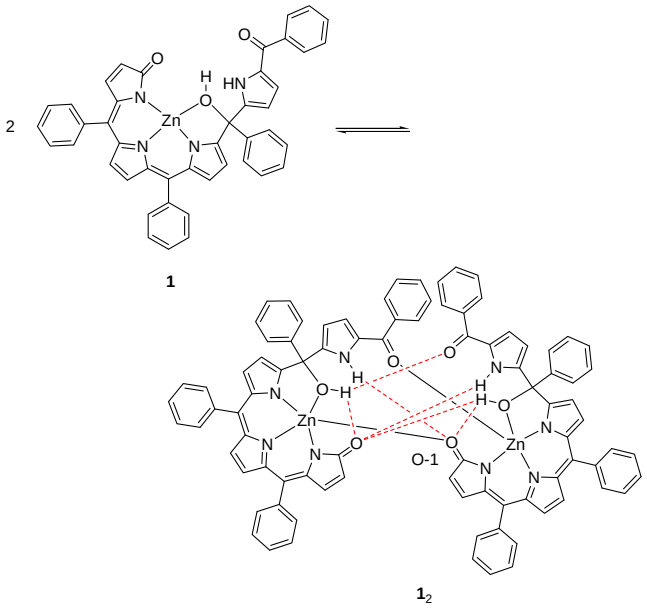
ABSTRACT

A zinc complex of biladienone forms a dimer in toluene and dichloromethane. Binding of two molecules of amino alcohols and diamines to the dimer occurs cooperatively, with the first binding constant being two orders or three orders of magnitude smaller than the second binding constant. Simple amines such as butylamine did not show such cooperative binding. We suggest the mechanism of cooperative binding, where the amino or hydroxyl group in amino alcohols or diamines prevents the dimer dissociation to result in the less tight binding for the first guest, while the binding of the second ligand caused dimer dissociation to release strain in the intermediate complex.

Allosteric effects on the ligand binding are the key concept of biological regulations, and the concept can be extended to the design of functional molecules in diverse fields.[1] The effects have been successfully modeled by an abiotic receptor molecule, where conformational changes of a dimeric receptor having two binding sites,[2] caused by the binding of the first ligand, would affect the structure of the second binding site, resulting in the cooperative binding. The binding of the first ligand is driven by a strong interaction, while modulation of the second binding site is caused by a weak interaction.[3] Recently, another type of allosteric control, where a dimer-monomer equilibrium is involved, attracts interest as seen in biofunctions of kinases and antibiotics.[4] In this Letter, we show that a zinc complex of biladienone acts as an allostric receptor due to the dimer–monomer equilibrium.

Zinc biladienone **1** was prepared according to the literature.[5,6] ESI mass spectra of **1** showed a peak at $m/z = 1457$ (MS fits predicted isotope cluster for $C_{88}H_{61}O_6N_8Zn_2$, $2M+H^+$) as well as at 709 (MS fits predicted isotope cluster for $C_{44}H_{29}O_2N_4Zn$, $M-OH^-$) in both toluene and $CHCl_3$. The previous studies[5b] demonstrate that **1** exists as a dimer in toluene and as a monomer in THF (Scheme 1). The ^1H NMR studies (vide infra) indicated that the dimer dissociates to a monomer when complexed with amines.

A crystal of **1** was obtained from THF-hexane and was analyzed by X-ray crystallography.[7] The zinc ion was coordinated by three pyrrolic nitrogens and one OH group in a square planar fashion, and was further coordinated axially by the other molecule of **1** to form an asymmetric dimer (Fig. 1).[8] The dimer was formed by a combination of 15*R*-**1** and 15*S*-**1** with the lactam oxygen and the keto carbonyl oxygen coordinated to the zinc. The NH hydrogens in the D-ring pyrrole are hydrogen bonded to 1-O of the other zinc biladienone. The two tripyrrolic planes of **1** are perpendicular to



Scheme 1. Monomer-dimer equilibrium of **1**. Hydrogen bonds in $\mathbf{1}_2$ are shown in dotted lines.

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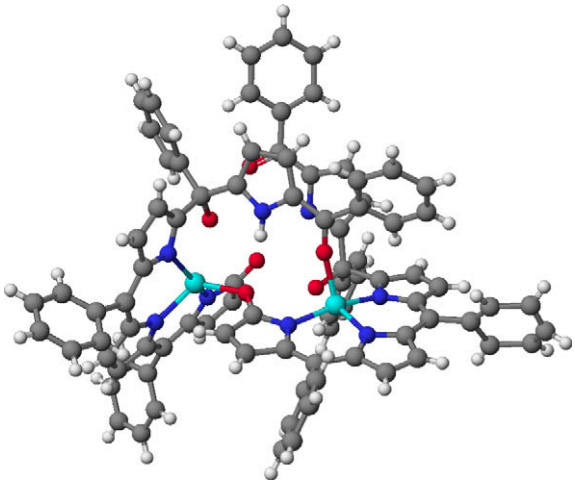

Figure 1. Structure of asymmetric dimeric **1** in the crystal.

each other, indicating that there is no interaction between the two chromophores. This can account for a similar electronic spectrum in toluene (dimer) and in THF (monomer). The crystal packing viewed along the *c*-axis is shown in Figure 2. There are tunnels along the *c*-axis, which are filled with solvents.

The binding of various amines to receptor **1** in solution was investigated by monitoring the visible spectral changes as a function of the concentration of amines. We reported that amines are bound to zinc bilindiones through Zn–N interaction.[9] Upon addition of benzylamine to a toluene solution of **1**, the absorption maximum at 624 nm was shifted to 639 nm. When the absorbance was plotted against the amine concentration, we observed either a simple saturation curve or a sigmoidal binding curve depending on the amines and the solvents. We classified the binding behavior into two types: type I with a simple saturation binding curve and type II with a sigmoidal binding curve. Type II binding curve implies that the binding equilibrium cannot be described by a single equilibrium, but should involve at least one intermediate species. In Figure 3 the representative curve of absorbance changes in the zinc biladienone band as a function of 2-aminoethanol concentrations is shown.

To estimate binding constants, we assumed two binding models as shown in Scheme 2. In type I, binding of a guest to dimeric host caused host–guest 1:1 complex in a single step. In type II, binding of a guest to dimeric host produced an intermediate, H_2G, and further addition of guest caused binding of another guest to H_2G to produce host–guest 1:1 complex.[10] Binding constants were determined by curve-fitting according to these models. For type II model, the equation for the concentration of free host, [*H*],

$$\left([\mathrm{H}]_\mathrm{T} - 2\frac{K_3}{K_1K_2}[\mathrm{H}]^2 - [\mathrm{H}]\right)\left(1 + \frac{K_3}{K_2}[\mathrm{H}]^2 + K_3[\mathrm{H}]\right) - [\mathrm{G}]_\mathrm{T}\left(\frac{2k_3}{K_2}[\mathrm{H}]^2 + K_3[\mathrm{H}]\right) = 0$$

was solved numerically, where $[H]_T$ and $[G]_T$ are the total concentrations of host and guest, respectively. Once the concentration of free host was determined, concentrations of other species such as H_2G, HG, and H_2 can be calculated. Using these concentrations, the binding constants and molar extinction coefficients of each species were optimized to reproduce the observed binding isotherm by nonlinear least square method. The binding constants K, K_1, K_2, and K_3 are listed in Table 1. The standard deviations for K were 3-5%, while the standard deviations for K_1, K_2, and K_3 were much larger, typically as large as 100%, so that only the order of magnitude of these values should be considered to be accurate.

Simple amines such as butylamine and benzylamine showed type I binding both in CH_2Cl_2 and in toluene, while all amino alco-

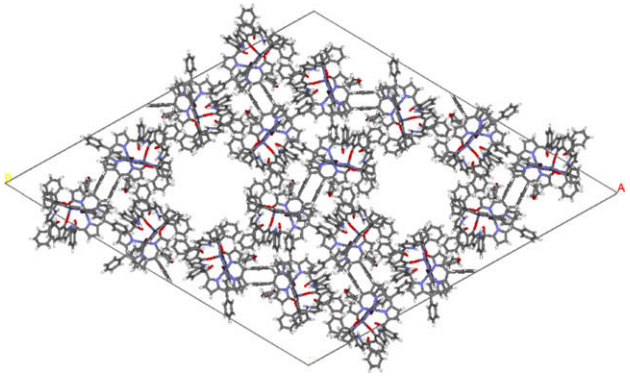

Figure 2. Crystal packing of $\mathbf{1}_2$ viewed along the *c*-axis.

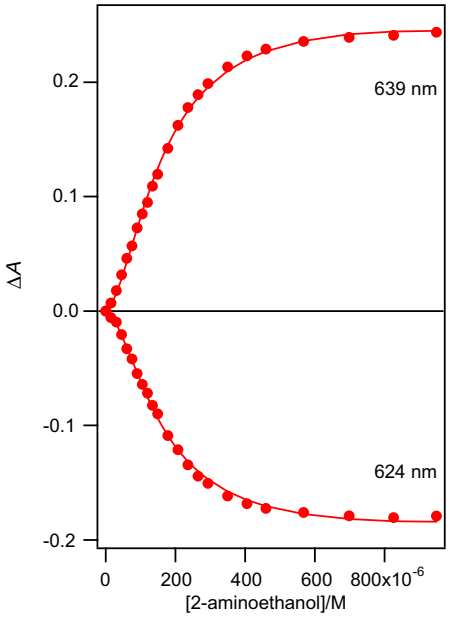

Figure 3. Absorbance changes of zinc biladienone **1** in toluene as a function of 2-aminoethanol concentration. $[\mathbf{1}]_T = 5 \times 10^{-5}$ M, 298 K. Solid curves show the calculated absorbance according to type II binding.

type I

$$\mathrm{H_2} + 2\mathrm{G} \underset{}{\overset{K}{\rightleftharpoons}} 2\mathrm{HG}$$

type II

$$\mathrm{H_2} + \mathrm{G} \overset{K_1}{\rightleftharpoons} \mathrm{H_2G} \overset{K_2}{\rightleftharpoons} \mathrm{HG} + \mathrm{H}$$

$$\mathrm{H} + \mathrm{G} \overset{K_3}{\rightleftharpoons} \mathrm{HG}$$

Scheme 2. Binding of amines (G) to the dimeric host (H_2). Type I model assumes one-step binding without cooperativity, while type II assumes multi-step binding, offering the possibility of cooperative binding.

Table 1
Binding constants and Hill coefficients n of amines at 298 K

	Solvent	Type	K (M^{-1}) or K_1 (M^{-1})	K_2 (M)	K_3 (M^{-1})	K_3/K_1	n
Butylamine	CH_2Cl_2	I	3940				1.0
	Toluene	I	5350				1.4
Benzylamine	CH_2Cl_2	I	535				1.0
	Toluene	I	216				1.2
2-Aminoethanol	CH_2Cl_2	I	2920				1.0
	Toluene	II	447	2.6×10^{-5}	1.7×10^{5}	370	1.6
3-Amino-1-propanol	CH_2Cl_2	I	18,300				1.1
	Toluene	II	39,200	1.6×10^{-6}	1.0×10^{7}	260	1.7
Ethylenediamine	CH_2Cl_2	II	758	4.2×10^{-5}	1.8×10^{6}	2430	1.9
	Toluene	II	1930	2.1×10^{-5}	3.5×10^{6}	1810	1.5
Imidazole	CH_2Cl_2	I	26,800				1.1
	Toluene	II	1110	1.1×10^{-4}	1.6×10^{6}	1470	1.7
DBU	CH_2Cl_2	II	2580	4.2×10^{-5}	1.5×10^{7}	5900	1.9
	Toluene	II	1.7×10^{5}	1.3×10^{-6}	2.5×10^{8}	1350	2.0

hols and diamines showed type II binding in toluene, and some of them showed type II binding in CH_2Cl_2. The binding constant K_1 of type II binding is smaller than the binding constant K_3, implying that the first binding of amine is a thermodynamically unfavorable process. For type II binding, the value of K_2 is small: the first binding of guest occurs without dissociation of **1**. Since the zinc coordination sites are partially occupied by dimerization, it is reasonable that the first binding is a thermodynamically unfavorable process. If the guest is a simple amine, the binding of the first guest caused dissociation of the dimer, so that we cannot observe any allosteric binding. However, if the amine has a functional group such as OH or NH_2 in the side chain, the functional group may inhibit dimer dissociation. To realize an allosteric system, both strong interaction and weak interaction should contribute to binding and to the subsequent structural changes.[11] The strong interaction is the zinc–nitrogen-coordinating interaction and the weak interaction is hydrogen bonding between guest and host. Hill coefficient n[12] less than 1.4 can be classified as type I, while n larger than 1.5 can be classified as type II.

The ^{1}H NMR of $\mathbf{1}_2$ in $CDCl_3$, CD_2Cl_2, and toluene-d_8 exhibited 16 resonances of β-pyrrole protons, consistent with the asymmetric homodimer structure, and the two binding sites of the dimer should have different affinity toward a guest. However, if the binding of the first guest caused dissociation of the dimer, we should not observe any allosteric binding even if the two biding sites are non-equivalent. Thus, the binding of a guest to the dimer without dimer dissociation is the key to allosteric binding. The ^{1}H NMR in THF-d_8 and methanol-d_4 as well as in $CDCl_3$ in the presence of amines showed only 8 resonances of β-pyrrole, indicating that dimeric **1** dissociates when an external ligand binds.

The chemical exchange between the dimer of **1** and the **1**-amine complex is slower than the NMR time scale at 298 K: the phenyl ortho proton of $\mathbf{1}_2$ splits into two resonances upon addition of guest. In the previous study of the binding of amines to zinc bilindiones, the exchange was fast even at 223 K, and we only observed the averaged signals.[13] The slow kinetics can be attributed to the dimer–monomer equilibria, where the dimer formation from the monomer may be slow.

The effects of solvent on allosteric binding are unique features of the system. Toluene favors allosteric binding (type II), while dichloromethane favors non-allosteric binding (type I). One distinct difference in the ^{1}H NMR of **1** in toluene and in $CDCl_3$ was that the NH (or OH) protons appeared in the downfield region at 11.1 and 12.8 ppm in toluene, while these protons appeared in the 7–8 ppm range in $CDCl_3$. Therefore, the hydrogen bonding of the NH proton is much stronger in the dimer in toluene than in $CDCl_3$. However, fluorescence spectroscopic studies revealed different behavior in toluene and CH_2Cl_2. Fluorescence of **1** was dependent on the solvent polarity: **1** fluoresces in polar solvents such as THF, acetone, and methanol, while not in non-polar solvents such as benzene, toluene, and hexane.[5b] Relative intensity of fluorescence of **1** in toluene, CH_2Cl_2, and THF was 2/60/100 at the concentration of 1.1×10^{-5} M, implying that the dimer in CH_2Cl_2 readily dissociates, while that in toluene does not dissociate at a lower concentration. If the dissociation of dimer leads to type I binding, it is reasonable to predict that type I binding is favored in CH_2Cl_2.

In conclusion, we demonstrate that the coordination and hydrogen bonding energy for formation of self-assembled homodimer is used as a source of allosteric and cooperative binding of diamines and amino alcohols. The functional group in the side chain in guest prevents dimer dissociation, leading to allosteric effects.

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7. X-ray structure determination of $\mathbf{1}_2$·THF: All data were measured on a Rigaku/MSC Mercury CCD diffractometer with graphite-monochromated Mo Kα radiation. The structure was solved by Patterson methods (SIR2002). Refinement was carried out with full-matrix least-squares on F^2. Crystal data for $C_{92}H_{68}N_8O_7Zn_2$:Mr.1536.36, crystal size $0.50 \times 0.30 \times 0.20$ mm, trigonal, space group R_3 (No. 148), $a = 58.903(15)$, $c = 12.877(4)$ Å, $V = 38691(18)$ Å^3, $Z = 18$, $\rho_{calcd} = 1.181$ g cm^{-3}, λ(Mo Kα) = 0.71069 Å, $F(000) = 14{,}256$, μ(Mo Kα) = 0.614 mm^{-1}, $T = 193$ K, $2\theta_{max} = 29.6°$. Of the 189,888 reflections collected, 24,103 were unique ($R_{int} = 0.335$). For 24,103 reflections with $I > 2.00\sigma(I)$, 958 parameters; $R(R_w) = 0.127(0.385)$. Min./max. residual

electron density −1.73/3.20 e Å^{-3}. Crystallographic data (excluding structure factors) for the structure reported in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-658611. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 0 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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10. One of the referees pointed out that the type II binding can be analyzed by two equilibria involving (1) dimer dissociation and (2) host–guest complex formation with a dissociated host:

$$H_2 \underset{}{\overset{K_D}{\rightleftharpoons}} 2H$$

$$H + G \underset{}{\overset{K_A}{\rightleftharpoons}} HG$$

However, we could not reproduce sigmoidal binding isotherms by numerical simulations based on the equilibria above. In the simulation, we chose conditions similar to those in Figure 3, and assumed that $K_A = 1 \times 10^5\ M^{-1}$, the host concentration, $[H]_{Total} = 5.0 \times 10^{-5}$ M, and the guest concentrations, $[G]_{Total}$, were varied from 5.0×10^{-6} to 4.5×10^{-4} M, to calculate the concentration of the host–guest complex. For the varying values of K_D^{-1}, from 10^4 to $10^7\ M^{-1}$, we could not obtain sigmoidal binding isotherm. Therefore, we analyzed our binding isotherms using equilibria shown in Scheme 2. The referee also suggested that the second equilibrium of conversion of H_2G to HG and H should be removed since K_2 is very small. We incorporated this equilibrium to show that H_2G is the true intermediate.

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