Ditopic Binding by a Self-Assembled Receptor: Metal in a Structural and Functional Role

Jinho Lee and Alan W. Schwabacher*

Department of Chemistry, Iowa State University
Ames, Iowa 50011

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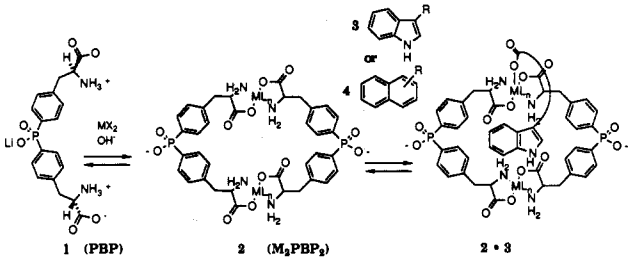
The exceptional behavior of biological molecules is due to the effects of unexceptional functional groups acting in concert. Model systems that reproduce the effects of cooperativity responsible for biomolecular activity are of interest, particularly those applying different types of interactions.[1-7] We have recently reported that complexes of Ni^{2+} or Co^{2+} with bis amino acid PBP **1**, designed to self-assemble[8,9] to form receptor **2**, mediate transport of aromatic hydrocarbons through an aqueous membrane.[10] Here we demonstrate that the metal can play a structural and a functional role: the hydrophobic pocket of these receptors and the metal itself cooperate to bind substrates bearing both aromatic and polar functional groups (Scheme 1).

The metals provide a variable template to link readily-prepared[11] diarylphosphinates, with a connectivity similar to that of our covalently-linked macrocyclic binding sites.[12,13] In these M_2PBP_2 complexes,[10] the metal role of assembling subunits and encouraging the active conformation parallels a common role of metals in protein and nucleic acid structure. However, metals are important to the functions as well as the structures of diverse biomolecules.[14] We asked whether the metal could also play a dual role in our simple structures.

We have measured the affinity of our Co_2PBP_2 receptor for a series of guests **3** and **4**. The values, determined by NMR (Figure 1) on these paramagnetic complexes, are given in Table 1. A substituent at the 3-position of the indole (**3b**,**c**) causes more than a order of magnitude drop in the binding affinity compared to that of indole **3a**, implying a steric interference with the optimal indole binding geometry. Acetyltryptophans **3d**,**e**, with anionic side chains, bind with another order of magnitude lower affinity. This is consistent with *repulsion* by the doubly

Scheme 1



anionic host. Removal of the acetamide substituent from **3d** or **3e** gives **3g**, which, despite the presence of the C3 substituent and the anionic sidechain, binds 37 times more strongly, almost as well as indole itself. Indoleacetic acid **3f** and indolebutyric acid **3h** bind still more tightly, as do several substituted naphthalene derivatives. As we see enhanced binding of anionic substrates by an anionic receptor, we can rule out ion-pairing as a driving force. Addition of the carboxylate, tethered through a three-carbon link in **3h**, provides 10–14-fold stronger binding, depending on which neutral 3-substituted indole (**3b** or **3c**) is taken as the standard. With the optimal side chain for naphthalene, **4c** binds 2.5 times more strongly than does the neutral aromatic **4a**.

We suggest that these data are consistent with ditopic binding: ligation of the cobalt by carboxylate in those complexes where the carboxyl group is appropriately positioned upon binding of the aromatic portion. In support of this notion, we point out that in all cases where there is an anomalously high affinity for an anionic ligand, and in only those cases, the NMR resonances for the α and β protons of the PBP are broadened to the point of undetectability upon addition of substrate. The geometric requirements of ditopic binding moderate the preference for 1- over 2-substitution of naphthalene: **4c** is preferred over the isomeric **4f** by 67-fold, while the related **4b** is preferred over its isomer **4e** by only 2.8-fold. (These are changes in opposite directions compared to the **4a**:**4d** ratio of 7.2.)

A ditopic receptor should prefer binding to substrates that allow both binding interactions. The extent to which the two interactions cooperate to achieve such selectivity can be described in terms of an "effective molarity" (EM) for the carboxylate groups of bound substrate in the presence of the metal.[15,16] Estimating the binding constant of a carboxylate ion to the charge-neutral Co^{2+} center as $K_D \sim 1.2$ M, (the K_3 of acetate for Co^{2+}),[17] the EM for binding of the indole and carboxylate moieties of **3h** is EM = 17 M.[18] This value, though well below the 10^8 M estimated limit,[19] is quite large compared to that of most synthetic receptors.[20] Nonetheless, it is an underestimate of our EM because it does not take into account the repulsion by the anionic receptor: correction (by $K_{(3c)}/K_{(3d)} = 7.3$) gives an EM of 120 M. If this analysis is correct, then the modest ~10-fold selectivity for **3h** over **3b** or **3c** is due to nice geometric placement of an intrinsically extremely weak attraction. A metal–ligand interaction comparable in strength to the hydrophobic affinity would yield selectivity for bifunctional substrate binding proportionally larger.

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Table 1. Binding of Guests to Co_2PBP_2 in 200 mM pD 9 Borate[a]

guest	R	$\Delta\delta_{max}$ (ppm)[b] H_α	H_β	H_{Ar1}	H_{Ar2}	K_D (M)
3a	H	−21	14	3.1	1.0	$(1.18 \pm 0.11) \times 10^{-3}$
3b	$CH_2CH_2NH_2$	−26	16	3.2	1.1	$(1.60 \pm 0.14) \times 10^{-2}$
3c	$CH_2CH(NHAc)CONH_2$	−25	14	2.0	0.8	$(2.18 \pm 1.20) \times 10^{-2}$
3d	L-$CH_2CH(NHAc)CO_2^-$	−33	16	2.1	0.9	$(1.57 \pm 0.23) \times 10^{-1}$
3e	D-$CH_2CH(NHAc)CO_2^-$	−28	14	2.2	0.8	$(1.55 \pm 0.19) \times 10^{-1}$
3f	$CH_2CO_2^-$	*c*	*c*	2.2	0.7	$(1.94 \pm 0.51) \times 10^{-3}$
3g	$(CH_2)_2CO_2^-$	*c*	*c*	2.5	0.9	$(4.21 \pm 0.77) \times 10^{-3}$
3h	$(CH_2)_3CO_2^-$	*c*	*c*	2.8	1.1	$(1.55 \pm 0.45) \times 10^{-3}$
4a	1-OH	−22	15	3.3	1.0	$(4.15 \pm 0.27) \times 10^{-4}$
4b	1-$CH_2CO_2^-$	*c*	*c*	2.4	0.8	$(1.02 \pm 0.13) \times 10^{-3}$
4c	1-$OCH_2CO_2^-$	*c*	*c*	2.7	0.7	$(1.65 \pm 0.52) \times 10^{-4}$
4d	2-OH	−22	15	3.1	1.1	$(2.97 \pm 0.06) \times 10^{-3}$
4e	2-$CH_2CO_2^-$	*c*	15[d]	2.5	0.7	$(2.85 \pm 0.70) \times 10^{-3}$
4f	2-$OCH_2CO_2^-$	−27	16	3.0	0.9	$(1.11 \pm 0.20) \times 10^{-2}$
4g	2-CO_2^-	−28	14	2.5	*e*	$(4.17 \pm 0.57) \times 10^{-2}$
4h	1,5-$(OCH_2CO_2^-)_2$	*c*	*c*	*c*	1.7	4.62×10^{-5}

[a] Host chemical shifts on titration with guest were fitted by nonlinear least squares to $\delta = \delta_0 + (\Delta\delta_{max}/2H_0)(S - (S^2 - 4H_0G_0)^{1/2})$ where $S = H_0 + G_0 + K_D$. K_D is the dissociation constant, H_0 is the total host concentration added to the sample, G_0 is the corresponding guest concentration, δ is the observed chemical shift of the host, δ_0 is the shift of free host, and $\Delta\delta_{max}$ is the extrapolated shift of fully complexed host. Error limits are given as 95% confidence, assuming independence of values determined by each signal. [b] Reference 24. [c] Broadened to unobservability. [d] Extrapolated from the first few points before disappearance, assuming the K_D determined from other resonances. [e] Obscured by guest signals.

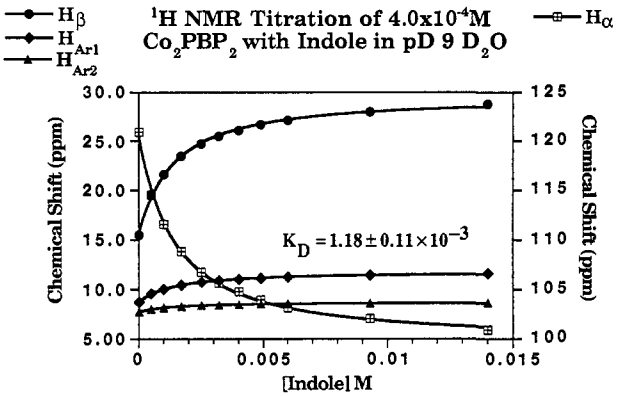

Figure 1.

The various data provide a series of tests a model for the binding geometry must meet. A great many stereoisomers at the metals are possible; many appear unsuitable for receptor formation, but many cannot be ruled out. The changes in indole shifts on binding also fit the 1:1 binding equation to yield the same dissociation constant ($(7.1 \pm 5.3) \times 10^{-4}$ M), supporting the stoichiometry of the receptor composition and its complex with indole. However, in stark contrast to the downfield shifts of the PBP protons on exposure to Co^{2+}, all indole protons are shifted upfield on binding.[21] This may represent a structurally significant difference in the orientation of the guest and the host hydrogens to the partially filled metal orbitals.

We have obtained information on stereochemistry at the metal centers by measurement of the binding of **4h** to Co_2PBP_2. If the naphthalene binds so as to position itself symmetrically in the middle of a symmetrical host cavity, a second carboxylate could bind to the second cobalt, adding at least as much to the binding affinity as the first carboxylate did (more, because of the probable lesser entropy to be frozen out by the second binding event[22]). The 1,5-disubstitution precludes binding of both carboxylates from the same face of the macrocycle, as a probable C_2 conformation would require. In fact, each carboxylate enhances binding: **4h** binds 4 times more strongly than **4c**, which binds 2.5 times more strongly than **4a**. If the second carboxylate did not assist the binding, we would predict that charge repulsion would cause lower affinity for **4h** than **4c**. This suggests that ligation by both carboxylates is possible, but we believe that a different geometry is enforced in this case because $\Delta\delta_{max}$ for H_{Ar2} is significantly (5.4 standard deviations) greater for **4h** than for the other substrates.

Indoleacetic acid **3f** is a plant growth hormone, an auxin, as are several of the other species bound in this study.[23] The structural requirement of our receptor for a naphthalene-sized aromatic group with a carboxylate side chain is analogous to the more strongly binding auxin receptor. This indicates that the simple means we have presented are sufficient to encode recognition of physiologically significant structures.

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(21) $\Delta\delta_{max}$ values for indole (ppm): H_2, = −6; H_3, = −14; H_4, = −15; H_5, = −9; H_6, = -7; H_7, = −15.

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