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Anion-Mediated Phase Transfer of Zinc(II)-Coordinated Tyrosine Derivatives

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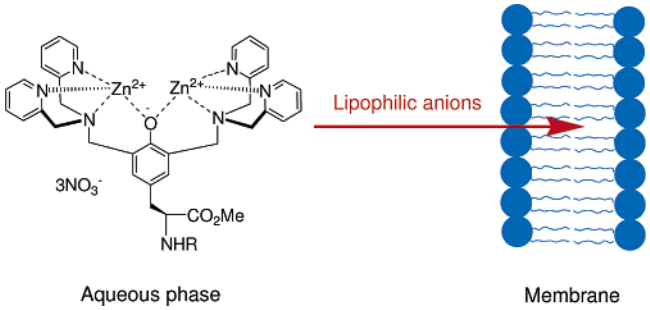
ABSTRACT



Tyrosine-derived Zn^{2+} coordination complexes and their fluorescent NBD conjugates are synthesized in a short, high-yielding procedure. The Zn^{2+} complexes are highly water soluble, but in the presence of sodium laurate they readily transfer into an octanol layer. Furthermore, the NBD-labeled bis-Zn^{2+} complex can partition into vesicle membranes containing anionic phospholipids.

Synthetic Zn^{2+} coordination complexes have been studied extensively as simplified models for various biological processes,[1] such as phosphate hydrolysis,[2] enzyme active-site recognition,[3] receptor antagonism,[4] peptidase mimicry,[5] and anion sensing.[6] 2,2′-Dipicolylamine (DPA) is an especially versatile ligand for Zn^{2+}, and Zn^{2+}−DPA complexes are known to exhibit supramolecular cooperative action. For example, dinuclear Zn^{2+}−DPA complexes can bind phosphorylated peptides in aqueous solution,[6a−c] while oligonucleotide conjugates of Zn^{2+}−DPA can selectively target DNA sequences[7] and hydrolyze RNA.[8] Phenol derivatives with ortho-substituted DPA units are also known to form dinuclear Zn^{2+} complexes that have an affinity for phosphate oxyanions in aqueous solution.[6d−f] Our interest in Zn^{2+}−DPA complexes stems from efforts to develop anion receptors that operate in aqueous solution or on the surface of bilayer membranes.[9] Our eventual goal is to design structurally complicated receptors, especially multivalent molecular

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recognition systems. This requires the development of versatile Zn^{2+}−DPA building blocks that can be readily attached to organic scaffolds or incoporated into oligomers. We were attracted to amino acid derivatives for the obvious reason that they can be incorporated into peptides. Here, we describe our first examples, namely, two Zn^{2+}−DPA derivatives of tyrosine. Specifically, we describe the synthesis of tyrosine-derived Zn^{2+} coordination complexes **1•Zn** and **2•2Zn** and their fluorescent conjugates **NBD**−**1•Zn** and **NBD**−**2•2Zn** (Figure 1). We also provide evidence for

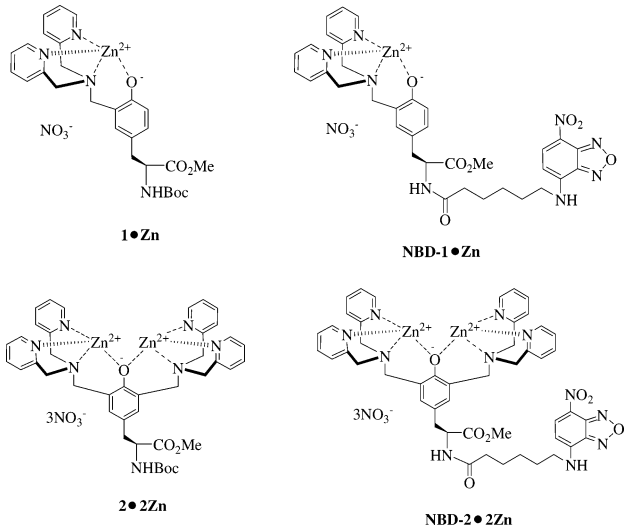
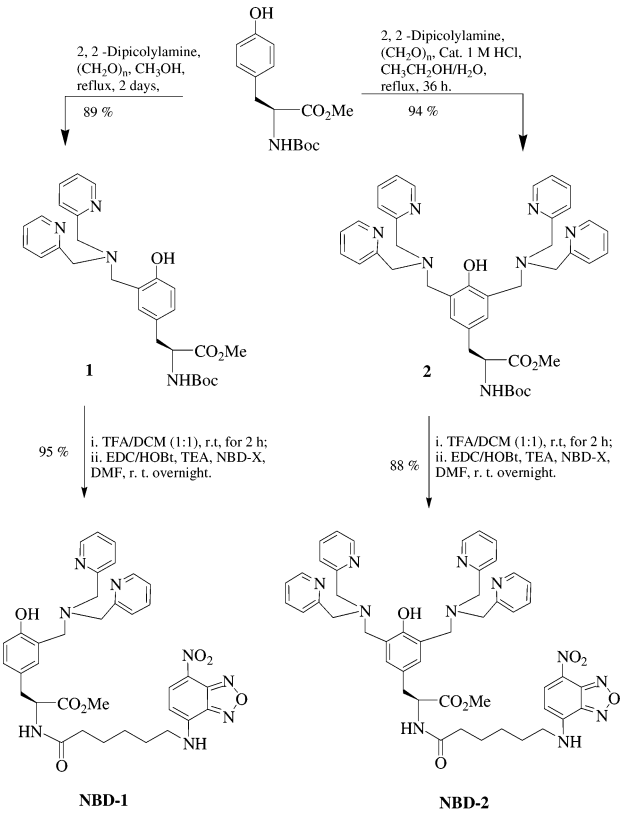


Figure 1. Structures of zinc(II)-coordinated tyrosine derivatives.

anion-mediated transfer of **1•Zn** and **2•2Zn** from water into a layer of octanol and the ability of **NBD**−**2•2Zn** to partition into vesicle membranes.

The introduction of one or two DPA units into the phenol ring of *N*-Boc-L-tyrosine methyl ester is easily achieved by Mannich reaction (Scheme 1).[10] Under neutral conditions, the mono-DPA derivative **1** is obtained in 89% yield, whereas acidic conditions promote the bis-DPA derivative **2** (obtained in 94% yield).[11] The *N*-Boc groups in **1** and **2** were removed by treatment with TFA, and the free amines were conjugated to 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino)hexanoic acid (NBD-X) to produce NBD-labeled tyrosine derivatives **NBD-1** and **NBD-2**. The coordination complexes **1•Zn**, **2•2Zn, NBD**−**1•Zn**, and **NBD**−**2•2Zn** were formed upon addition of an aqueous solution of $Zn(NO_3)_2$ (1 or 2 molar equiv, respectively) to solutions of the

Scheme 1



precursor ligands in methanol. As nitrate salts, the complexes are highly water soluble, but they can be stored conveniently as solids.

The recent reports of anion-mediated translocation of polyarginine across liquid and bilayer membranes[12] suggested to us that the phase transfer behavior of **1•Zn** and **2•2Zn** may depend on the presence and identity of the counteranions. Therefore, we examined the effect of sodium laurate ($C_{11}H_{23}CO_2Na$) on partitioning in biphasic water-octanol. To a solution of **1•Zn** in water (100 μM, 0.5 mL) was added an equal volume of octanol. After agitation for 30 s, the layers were separated and analyzed by UV absorption. As shown in Figure 2A, about 25% of the **1•Zn** was in the octanol layer. When the experiment was repeated in the presence of 1.2 molar equiv of sodium laurate, the octanol layer contained about 95% of the **1•Zn**. The presence of 3 molar equiv of sodium laurate induced complete partitioning of **1•Zn** into the octanol (see the Supporting Information).

The partitioning experiments were repeated with the dinuclear **2•2Zn**. Not suprisingly, the hydrophilic **2•2Zn** partitions almost completely into the aqueous layer, but in the presence of 1.2 molar equiv of sodium laurate per zinc the distribution is reversed; that is, the **2•2Zn** moves almost completely into the octanol phase (Figure 2B). It is clear that association of **1•Zn** or **2•2Z** with lipophilic laurate anion

(10) For recent examples of tyrosine modification, see: (a) Joshi, N. S.; Whitaker, L. R.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 15942−15943. (b) Schlick, T. L.; Ding, Z.; Kovacs, E. W.; Francis, M. B. *J. Am. Chem. Soc.* **2005**, *127*, 3718−3723.

(11) To the best of our knowledge, **1** has not been reported before, but the ethyl ester of **2** has been used as a redox component in synthetic mimics of photosystem II. See: Sun, L.; Burkitt, M.; Tamm, M.; Raymond, M. K.; Abrahamsson, M.; LeGourriérec, D.; Frapart, Y.; Magnuson, A.; Kenéz, P. H.; Brandt, P.; Tran, A.; Hammarstro1m, L.; Styring, S.; Kermark, B. *J. Am. Chem. Soc.* **1999**, *121*, 6834−6842.

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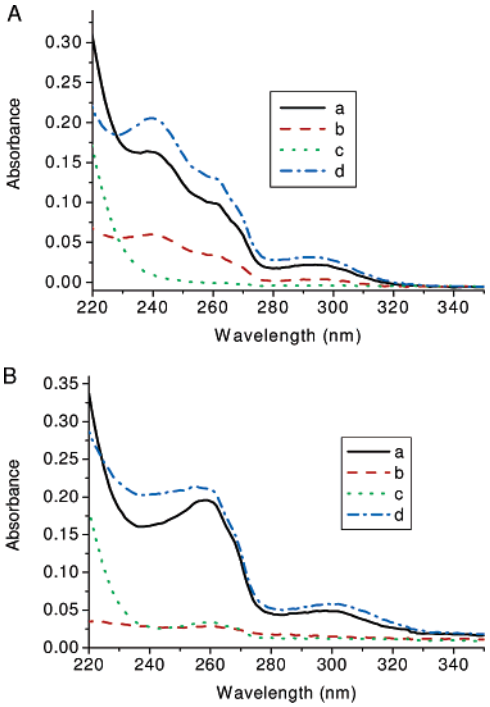

Figure 2. UV spectra of (A) **1•Zn** and (B) **2•2Zn** after partitioning between water and octanol in the absence and presence of 1.2 molar equiv of sodium laurate per zinc: (a) aqueous phase without sodium laurate; (b) organic phase without sodium laurate; (c) aqueous phase with sodium laurate; (d) organic phase with sodium laurate.

induces transfer of the complex into organic phase. Hydrophilic anions such as phosphate and chloride do not induce any phase transfer of **1•Zn** or **2•2Zn**.

The water/octanol partitioning results prompted us to evaluate the abilities of **1•Zn** and **2•2Zn** to interact with bilayer membranes. The fluorescent derivatives **NBD−1•Zn** and **NBD−2•2Zn** were prepared because previous work had shown how the NBD fluorophore can be used to measure binding and partitioning into vesicle membranes.[9b] The vesicle-partitioning assay is based on the ability of dithionite to chemically reduce the NBD fluorophore and quench its fluorescence. Sodium dithionite cannot cross vesicle membranes, so addition of the reagent to vesicle dispersions can only quench fluorophores that are in the external solution or exposed on the membrane surface. Any NBD-labeled compound that partitions into the vesicle membrane is protected from immediate quenching by added sodium dithionite (Figure 3).

The vesicle partitioning studies used anionic vesicles with two compositions, POPC/POPA 1:1 and POPC/POPS 1:1, where POPA is 1-palmitoyl- 2-oleoyl-*sn*-glycero-3-phosphatidic acid, POPS is 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine, and POPC is 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (Figure 4). Unilamellar vesicles (100 nm diameter, 25 μM total phospholipid, pH 7.4) were prepared by standard extrusion methods, and **NBD−1•Zn** and **NBD−2•2Zn** (C_{final} = 1 μM) were added to separate vesicle dispersions. After standing for 2 h, an aliquot from each

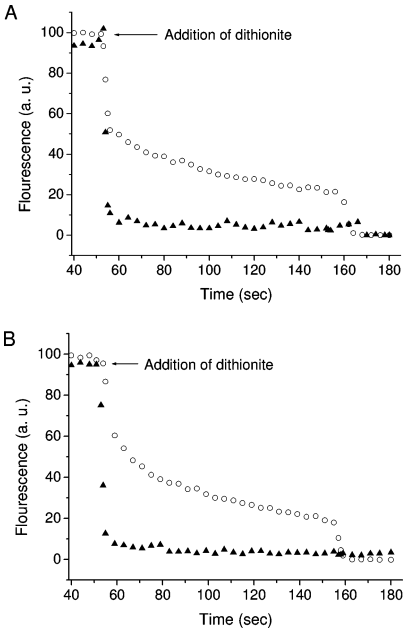

Figure 3. Protection of **NBD−1•Zn** and **NBD−2•2Zn** (1 μM) fluorescence by vesicles composed of (A) POPC/POPS 1:1 or (B) POPC/POPA 1:1. The vesicles (25 μM total phospholipid) were mixed with **NBD−1•Zn** (7) or **NBD−2•2Zn** (−) (1 μM). After standing for 2 h, an aliquot was removed from each sample and treated with sodium dithionite at assay time of 50 s and Triton-X 100 at assay time of 150 s.

sample was treated with excess sodium dithionite. As shown in Figure 3, the fluorescence of both samples containing **NBD−1•Zn** was completely and immediately quenched. In other words, the anionic vesicles were unable to protect the **NBD−1•Zn** from reacting with the dithionite.

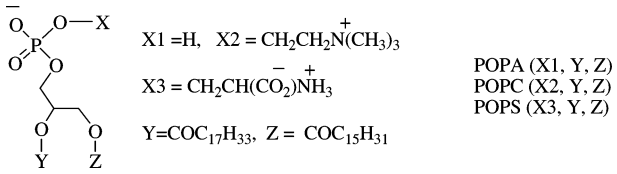

Figure 4. Phospholipids.

In the case of **NBD−2•2Zn**, there is clear evidence that anionic vesicles of both compositions can protect about half of the NBD conjugate from immediate reaction with dithionite. The **NBD−2•2Zn** quenching curves in Figure 3 show biphasic kinetics. Upon dithionite addition there is a sudden drop in fluorescence emission due to rapid reaction with the exposed NBD groups, then there is a slower rate of quenching which is attributed to either transport of the dithionite into the vesicles or reexposure of internalized NBD on the membrane surface. When the vesicles are lysed with Triton-X 100 the remaining fluorescence is immediately quenched. The fact that the anionic vesicles protect about half of the **NBD−2•2Zn** from dithionite quenching is strong

evidence that the **NBD−2•2Zn** associates with the vesicle membrane and subsequently equilibrates between the inner and outer surfaces.[13] The **NBD−2•2Zn** on the inner surface is protected from immediate reaction with the dithionite.

Further evidence that **NBD−2•2Zn** can associate with anionic vesicles was gained from fluorescence titration experiments. Previously, we have shown that the emission intensity of an NBD-labeled Zn^{2+}−DPA conjugate will increase if it associates with vesicle membranes.[9b] Therefore, we titrated separate samples of **NBD−1•Zn** and **NBD−2•2Zn** with anionic vesicles composed of POPC/POPS 1:1 and also with zwitterionic vesicles composed only of POPC. The titration curves in Figure 5 show that both coordination complexes have very weak affinity for the zwitterionic vesicles. However, the **NBD−2•2Zn** has a moderately strong affinity for the anionic vesicles with an apparent binding constant $(8.7 \pm 0.4) \times 10^4\ M^{-1}$, which is similar to previous results with closely related dinuclear Zn^{2+}−DPA complexes.[9b] Furthermore, the large change in fluorescence intensity upon vesicle binding is suggestive of a close interaction with the membrane. In the case of **NBD−1•Zn** the fluorescence hardly changes upon addition of the anionic vesicles, suggesting that the affinity is very weak, which explains why the **NBD−1•Zn** does not partition into anionic vesicles.

In summary, we have synthesized **1•Zn** and **2•2Zn**, two structurally related Zn^{2+}−DPA derivatives of tyrosine. We find that the presence of sodium laurate can induce these hydrophilic coordination complexes to transfer from water into octanol and that dinuclear complex **NBD−2•2Zn** can translocate through the bilayer membrane of anionic vesicles. The bilayer translocation mechanism is currently under investigation, and the results will be reported in due course. The data in hand indicates that oligomers and conjugates of **1•Zn** and **2•2Zn** are likely to have a range of useful recognition and transport properties.

(13) It is intriguing that closely related dinuclear Zn^{2+}−DPA complexes do not penetrate the membranes of anionic vesicles. See ref 9b.

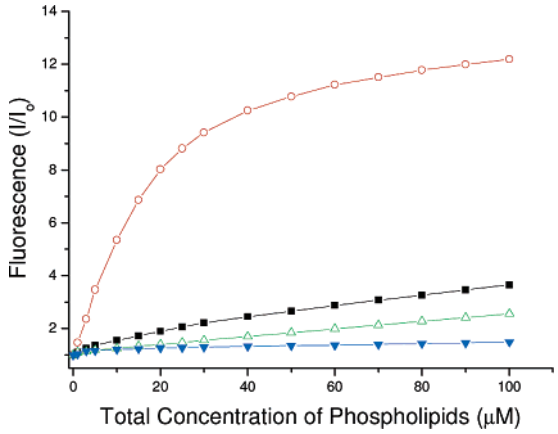

Figure 5. Fluorescence titration of **NBD−1•Zn** and **NBD−2•2Zn** (1 μM) with vesicles composed of POPC or POPC/POPS 1:1 in buffer (5 mM TES, 145 mM NaCl, pH 7.4). Change in **NBD−2•2Zn** fluorescence upon titration with 1:1 POPC/POPS vesicles (○) or POPC only vesicles (■). Change in **NBD−1•Zn** fluorescence upon titration with 1:1 POPC/POPS vesicles (△) or POPC-only vesicles (▼). The lines in the figure are for clarity; see the Supporting Information for curve-fitting.

Acknowledgment. This work was supported by the National Institutes of Health. The titration curves in Figure 5 were acquired by Roger G. Hanshaw.

Supporting Information Available: Synthetic procedures and spectroscopic data for all compounds, NBD-protection assay, and titration method. This material is available free of charge via the Internet at http://pubs.acs.org.

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