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Specificity and Negative Cooperativity in Dendrimer−Oxime Drug Complexation

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Supporting Information

Dendrimers are a family of nanometer-sized, spherical macromolecules that have inspired a multitude of chemical, biological, and biomedical applications.[1] In particular, poly(amidoamine) (PAMAM) dendrimers have a well-defined core−shell architecture characterized by repetitive cavities and branches suitable for guest molecule complexation.[1a,e] Therefore, PAMAM dendrimers have been frequently used to make complexes with small molecule drugs, oligonucleotides, and genetic therapeutics for drug delivery and gene transfer applications.[1c,2] Despite many extensive demonstrations of this approach to therapeutics, very few studies provide analysis of the molecular interaction between the dendrimer and the guest molecule in the nanoscale architecture.[3] We report here binding analysis and molecular models that are of fundamental importance to understanding such processes. Using oxime-based guest molecules, we discuss evidence that the individual binding events contributing to the complexation at a global level occur in a specific and negatively cooperative manner.

Pralidoxime (2-PAM) and obidoxime belong to a class of oxime antidotes developed for the treatment of organophosphate poisoning.[4] Both drugs have short durations of action that could, in principle, be extended by complexing the drugs to nanocarriers for increasing their circulation half-lives. In the first study we performed ^{1}H NMR titration experiments to locate structural determinants for the complex formation between a generation 5 (G5) PAMAM dendrimer and 2-PAM (Figure 1). Upon the addition of 2-PAM, only a few subsets of the dendrimer protons that belong to terminal branches (c, e_o, a_o) apparently shifted downfield as a function of the [2-PAM]/[D] ratio, while other inner protons remained almost unchanged. The relative magnitudes of such changes ($c > e_o > a_o$) suggest that binding of the guest molecules selectively occurs at the terminal branches of the dendrimer. On the guest side, the proton signal associated with 2-PAM shifted upfield as the ratio decreased.

Given the p$K_a$of 2-PAM (8.1[5]), lower than the pK_a of a terminal primary amine (9.0[6]−10.77[7]), we hypothesize that electrostatic interaction is the driving force for the drug complexation (Figure 2). We verified this hypothesis by measuring the ^{1}H NMR spectra of 2-PAM mixed with an equimolar amount of triethylamine (pK_a = 10.78) and of ethanolamine (pK_a = 9.50). Here, the Δ values for 2-PAM observed in a bound state ([2-PAM]/[D] = 10) are correlated with those from each of the mixtures (Figure S3). Furthermore, the ^{1}H NMR titration experiments performed with *N*-methylpyridinium chloride (MPC), a molecule that lacks such an aldoxime moiety, under otherwise an identical condition led to no evidence for the complexation (Figure S1). In contrast, the titration experiments performed with obidoxime resulted in the changes in chemical shifts that are consistent with those seen with 2-PAM (Figures S1 and S2). Thus, obidoxime, like 2-PAM, binds to the dendrimer through the electrostatic interactions. While the above experiments were carried out in a nonionic solution (D_2O), the same experiments performed for 2-PAM in a high ionic strength solution (PBS pH 7.4, I = 0.15) led to almost identical complexation trends (Figures S1 and S2).

We further explored the binding models proposed in Figure 2 by using other NMR techniques. First, 2D ^{1}H−^{1}H COSY and NOESY NMR experiments were performed for the dendrimer complexes with the oxime drugs (Figures S4 and S5). Notably, certain cross-peaks observed in the NOESY spectra are attributable to through-space intermolecular correlation, an evidence for spatial proximity ($d \leq 5$ Å) between the drug molecules and dendrimer branches (H_1-e_o,c,d for 2-PAM; H_2-e_o for obidoxime). Second, we studied hydrodynamic properties of dendrimer/2-PAM complexes by ^{1}H diffusion-ordered spectroscopy (DOSY) (Figure 3). Diffusion coefficients (D, $m^2\ s^{-1}$) determined for the complexes by fitting the peak-integration decay curves of the DOSY spectra (Figure S6) decrease relative to the dendrimer alone ($D = (7.49 \pm 0.30) \times 10^{-11}\ m^2\ s^{-1}$) and in response to the ratio [2-PAM]/[D]. The diffusion coefficients allowed to calculate hydrodynamic radii (R_h) for the complexes according to the Einstein−Stokes equation (eq 1, where η = viscosity of D_2O = 1.24×10^{-3} kg $m^{-1}\ s^{-1}$ and other parameters defined in the literature).[3a,c]

$$D = k_B T(1 - \kappa\varphi)/6\pi\eta R_h \qquad (1)$$

The dendrimer complexes display greater hydrodynamic radii than the free dendrimer (R_h = 2.35 ± 0.09 nm) in a drug concentration-dependent manner, which strongly supports the formation of specific complexes.

In our efforts to quantitatively understand individual binding events in ^{1}H NMR titration experiments (Figure 1 and Figure S1), we calculated the fractions of drugs bound at steady state and, complementarily, those of occupied binding sites. According to

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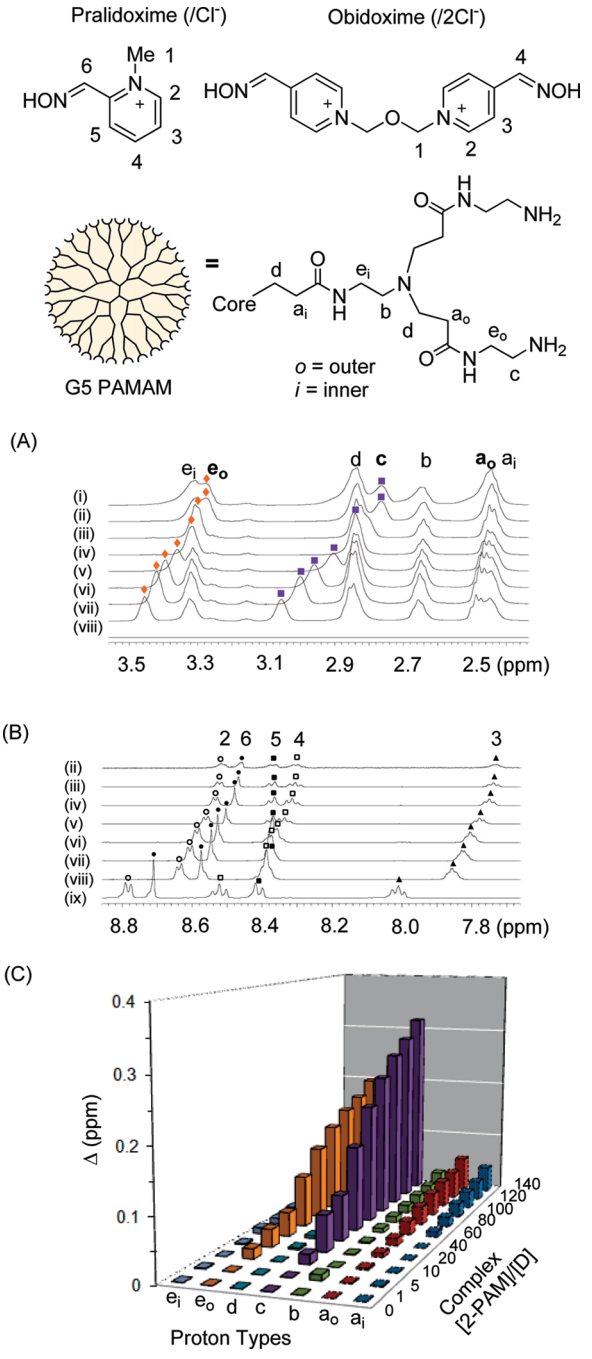

Figure 1. ^{1}H NMR titration experiments for a G5 PAMAM dendrimer and pralidoxime (2-PAM) in D_2O. ^{1}H NMR spectral regions for the unmodified, amine-terminated dendrimer (A) and 2-PAM (B). G5 dendrimer alone ([D] = 6.23×10^{-4} M) (i) and dendrimer−drug complexes prepared at [2-PAM]/[D] = 1 (ii), 10 (iii), 21 (iv), 42 (v), 63 (vi), 84 (vii), 125 (viii), and 2-PAM alone (ix). (C) Changes in chemical shift values (e.g., $\Delta = (\delta_{c,viii} - \delta_{c,i})$, ppm) for dendrimer protons plotted against [2-PAM]/[D] ratio.

the NMR responses, the present dendrimer−drug complexation belongs to a system undergoing fast on/off exchange and could be analyzed by eq 2, which determines fractions for the drugs bound (Figure 4; see Supporting Information for the details).[8] Unlike 2-PAM, obidoxime has a C_2 symmetry with two identical aldoxime moieties, and thus two modes of association

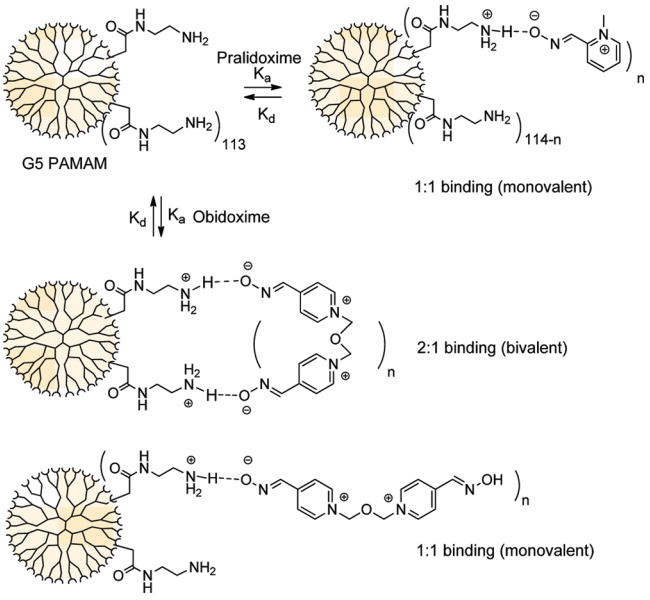

Figure 2. Models proposed for complexation of oxime drugs to G5 PAMAM dendrimer. The mean number (114) of terminal amines per dendrimer is determined by potentiometric titration where the molar amount of the dendrimer sample is calculated on the basis of its MALDI molecular weight (27 600 g mol^{-1}).

(monovalent, bivalent) were considered separately for analysis (Figure 2).

$$\delta_{obsd} = (Fr_{free} \times \delta_{free} + Fr_{bound} \times \delta_{bound}) \qquad (2)$$

Figure 4A illustrates that the number of bound oxime molecules increases as a function of the ratio [oxime]/[D]. Binding of 2-PAM in D_2O appears to be saturated at the level of 78 drugs bound per dendrimer ([2-PAM]/[D] = 145). The ionic strength of the medium affects the binding level such that 2-PAM molecules bound more in PBS than D_2O by up to 10 drug molecules per dendrimer. We believe such difference is attributable to structural and conformational flexibility of the dendrimer which is influenced by external factors such as solvent, pH, and ionic strength.[1a,9] Qualitatively, it is plausible that the presence of counterions associated with the dendrimer scaffold may open up dendritic branches by interrupting their intramolecular interactions and as a consequence relieve the degree of unfavorable steric congestion arising from drug binding. Remarkably, obidoxime shows a saturation behavior with its maximum (≈40 bound per dendrimer) reached at the lower ratio ([obidoxime]/[D] ≈ 80). Such a drug saturation curve corresponds with the dendrimer response curve (Figure S2). The changes (Δ) for the terminal branches reach the maximal level at a similar ratio ([obidoxime]/[D] ≈ 90 where ∼40 obidoxime molecules bound). In addition, its level is very comparable to that observed in experiments with the other drug ([2-PAM]/[D] ≈ 145 where ∼80 of 2-PAM molecules are bound). These findings are supportive of functional bivalency of obidoxime and consistent with the fractional analysis of occupied binding sites (θ) for obidoxime calculated on the basis of its bivalent model (Figure 4B). Scatchard analysis performed for each drug shows nonlinear decay, and thus, instead of calculating average affinity, the affinity distribution was estimated as a function of

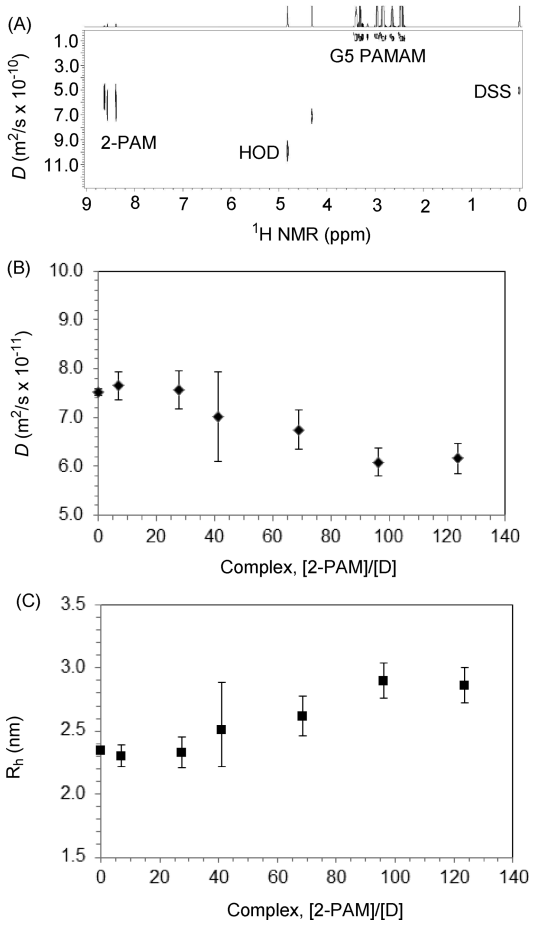

Figure 3. (A) A representative pseudo-2D DOSY plot for G5 PAMAM dendrimer in complex with 2-PAM ([D] = 6.04 × 10^{-5} M; [2-PAM]/[D] = 123.5). (B, C) Diffusion coefficients (D, m^2 s^{-1}), and hydrodynamic radii (R_h, nm) for G5/2-PAM complexes, each plotted as a function of [2-PAM]/[D] ratio. Diffusion coefficient determined for each complex in (B) refers to a mean value obtained from at least two independent sets of measurements, and the error represents the standard deviation from the mean value.

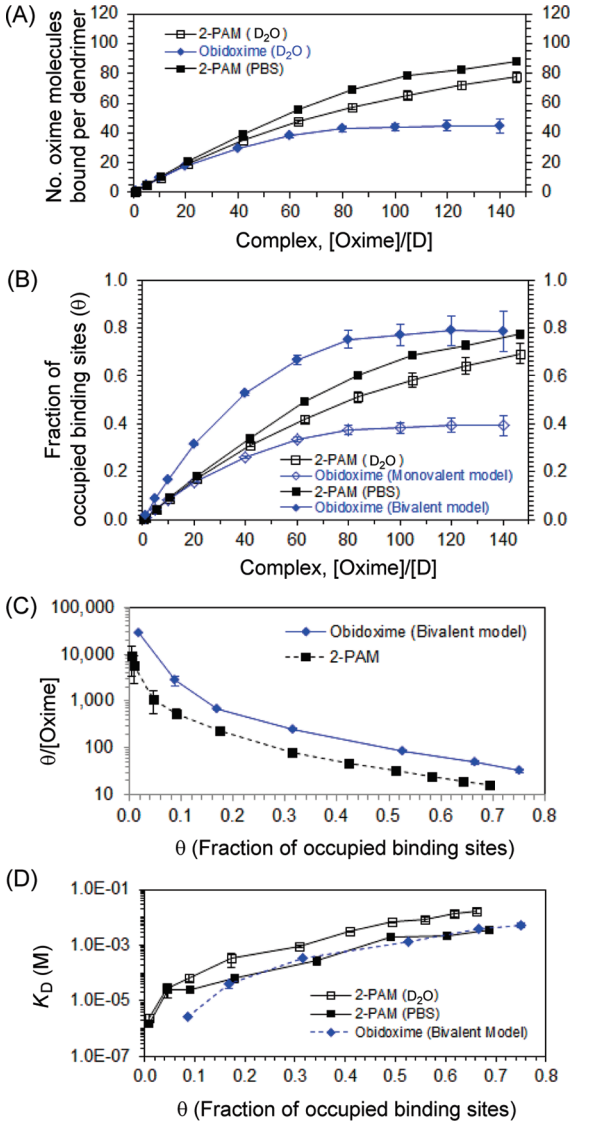

Figure 4. Quantitative analysis for the complexation of G5 dendrimer with two oxime drugs. (A) Number of bound molecules and (B) fraction of occupied binding sites (θ), plotted against the ratio [oxime]/[D]. (C) Scatchard plots for dendrimer−drug complexation in D_2O. (D) Steady-state dissociation constants (K_D) plotted as a function of θ.

θ (Figure 4C,D). Generally, affinities are greater for obidoxime than pralidoxime in D_2O, suggesting the difference in their modes of binding (bivalent vs monovalent). In addition, the affinities are higher ($K_D \sim 10^{-6}$ M) at lower binding fractions ($\theta < 0.1$) and decrease as more sites are occupied, indicative of repulsive interactions between successive binding events. The Hill coefficient (n) determined for each drug provides a quantitative index for such negative cooperativity with values of 0.58 (2-PAM) and 0.49 (obidoxime) (Figure S7). We believe that steric congestion plays a dominant role for this effect as suggested in a broad range of antibody−antigen recognition processes and specifically in the reactions catalyzed by metallodendrimers.[1g,10] However, it is in contrast to the proximity effects reported for other dendrimer-based catalytic reactions,[1d,f] suggesting that substrate binding in the catalytic dendrimer might be less sensitive to steric effects due to its rapid turnover.

This communication reports a useful new insight in host−guest interactions of PAMAM dendrimers as a synthetic multivalent receptor. Oxime guest molecules bind through specific interaction rather than random encapsulation, and in a negatively cooperative manner, pointing to the significant role of steric interactions within the dendrimer framework.

■ ASSOCIATED CONTENT

Supporting Information. An experimental section for materials and methods, full NMR spectral data (1D titration, $^1H-^1H$ 2D COSY, NOESY, DOSY), and Hill plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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