Controlled Diffusion of Photoswitchable Receptors by Binding Anti-electrostatic Hydrogen-Bonded Phosphate Oligomers

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ABSTRACT: Dihydrogen phosphate anions are found to spontaneously associate into anti-electrostatic oligomers via hydrogen bonding interactions at millimolar concentrations in DMSO. Diffusion NMR measurements supported formation of these oligomers, which can be bound by photoswitchable anion receptors to form large bridged assemblies of approximately three times the volume of the unbound receptor. Photoisomerization of the oligomer-bound receptor causes a decrease in diffusion coefficient of up to 16%, corresponding to a 70% increase in effective volume. This new approach to external control of diffusion opens prospects in controlling molecular transport using light.

INTRODUCTION

Active control over molecular transport by synthetic systems is a topic of major contemporary interest. Recent progress has shown that motors, enzymes, or other energy-consuming nanostructures can effectively drive molecular transport in solution. Despite these advances, controlling transport of molecules in solution remains a challenging goal. One way to control transport is by modulating the diffusion rate of species in solution. Various theoretical proposals and experimental data have shown that increasing or decreasing the rate of diffusion can lead to directional transport when coupled with, for example, concentration gradients. Diffusion rates have been influenced using molecular photoswitches by altering self-assembled discrete structures or polymers. While diffusion NMR measurements are an established approach for characterizing supramolecular assemblies, the use of a switchable assembly to control diffusive transport is relatively unexplored.

Many small molecular receptors have been developed to selectively bind anions. Such binding may result in changes in the rate of diffusion of the receptor. If the binding properties could be modified by external stimuli, for example, by light, this could allow control of the rate of diffusion. Recently, some of us developed the first photoswitchable receptors exhibiting strong dihydrogen phosphate binding. These receptors were based on molecular motor and stiff-stilbene scaffolds containing urea anion-binding motifs. These hosts could be converted from a weakly guest-binding E to a strongly binding Z form using near-UV light (Figure 1a).

Some anions are known to associate through hydrogen bonds that are sufficiently strong to overcome electrostatic repulsion to form polyanionic species. This anti-electrostatic...
hydrogen bonding (AEHB) is common in the solid state for oxoanions such as HCO$_3^-$, HSO$_4^-$, and H$_2$PO$_4^-$ (Figure 1b,c).

While AEHB interactions have been identified in solid-state crystal structures, detection of unchaperoned anion dimers or oligomers in solution is challenging due to limited spectroscopic signatures, weak anion–anion bonds, labile protons, and rapidly exchanging bound species. These difficulties can be attenuated by the use of an anion-binding host to template hydrogen bonding (AEHB) interactions, leading to reports of HSO$_4^-$ dimers and oligomers in solution. Conductimetric and spectroscopic techniques have shown the formation of AEHB dimers of HCO$_3^-$ in DMSO$_2^2$ or DMSO$_2^2$ and suggested the possibility of higher order oligomers, however, to the best of our knowledge, the unassisted formation of AEHB oxoanion oligomers in solution has yet to be conclusively established. We anticipated that the use of photoswitchable anion receptors with AEHB dihydrogen phosphate oligomers would allow changes in diffusion to be controlled by light. Herein we report quantitative self-association data for the anti-electrostatic oligomerization of dihydrogen phosphate in DMSO at millimolar concentrations, association data for the anti-electrostatic oligomerization of dihydrogen phosphate in DMSO at millimolar concentrations, and as far as we know, this surprisingly strong process in a polar solvent is the first measurement of indefinite AEHB self-association. This model makes no assumptions about the structure of the self-assembly, which could be, for example, linear chains or globular-type assemblies, as suggested by the diversity of known solid-state structures containing H$_2$PO$_4^-$ clusters.

To modify the diffusion rate of a switchable receptor, we turned to the previously developed stuff stilbene, bisurea. The bis-styol derivative 1 (Figure 1a) was synthesized to take advantage of the convenient methyl $^1$H NMR signal for diffusion NMR experiments (see SI-3). The association constants for H$_2$PO$_4^-$ and OAc$^-$ were measured by NMR titrations and fitted to 1:2 [H$_2$PO$_4^-$][OAc$^-$] binding models, with results comparable to those previously reported for the nonsubstituted phenyl derivative when studied over the same guest concentration range. Binding studies at higher concentrations of H$_2$PO$_4^-$, however, resulted in slightly different association constants when fitted to the same binding model (SI-S7.8–S7.10) illustrating that competition due to self-association of H$_2$PO$_4^-$ complicates binding models for association constants. For example, a recent report of H$_2$PO$_4^-$ binding found different binding constants and stoichiometries when measured at different concentrations by UV–vis absorption or NMR spectroscopy, likely due to H$_2$PO$_4^-$ aggregation. This problem will also apply to all other H$_2$PO$_4^-$ binding studies measured at millimolar or higher concentrations.

Diffusion coefficients vary approximately proportionally to the inverse cube root of molecular volume, $V^{-1/3}$ [24]. Therefore, the 50% decrease in $D(H_2PO_4)$ as the H$_2$PO$_4^-$ concentration is increased from 2 to 300 mM suggests an 8-fold increase in effective volume over this concentration range. We propose this decrease in $D(H_2PO_4)$ is a result of the formation of AEHB oligomers of H$_2$PO$_4^-$ in solution.

A simple model for supramolecular oligomerization is the isodesmic model, in which the addition of each monomer to an oligomer occurs with the same association constant $K_2$ (See SI–SS.1). Combining this model with an inverse-cube relationship between $D$ and oligomer size (See SI–SS.2 for derivation and details) gives a model for the concentration dependence of the measured $D$ of a molecular species undergoing reversible oligomerization:

$$D = D_0 \frac{1}{K[A]_0} \times (K[A])^{-1/3}$$

where $Li(z)$ is the polylogarithm function, $D_0$ is the diffusion coefficient of the monomer, $K$ is the isodesmic association constant, $[A]_0$ is the total concentration, and $[A]$ is the concentration of the free (monomeric) species which can be obtained from $K$ and $[A]_0$. Nonlinear regression of eq 1 onto the measured diffusion coefficients of H$_2$PO$_4^-$ gave $K = 120 \pm 32$ M$^{-1}$, surprisingly close to the reported dimerization constants of H$_2$PO$_4^-$ in pure DMSO (180 M$^{-1}$ measured by $^{31}$P NMR [25], 51 M$^{-1}$ measured by ITC [15]). Our measured $K$ corresponds to median complexes composed of 4 or 10 H$_2$PO$_4^-$ subunits at 50 or 300 mM concentrations, respectively (Figure 3a, Figure S11), and as far as we know, this surprisingly strong process in a polar solvent is the first measurement of indefinite AEHB self-association. This model makes no assumptions about the structure of the self-assembly, which could be, for example, linear chains or globular-type assemblies, as suggested by the diversity of known solid-state structures containing H$_2$PO$_4^-$ clusters.

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formation of simple ditopic [HG] complexes12c or even decreases in measured size between host Z-1 (b) and E-1 (c) bis-urea hosts to form ditopic [H(G)] or [H(G)] complexes. (d) As E-1 possesses divergent urea binding sites, larger supramolecular structures can form from H2PO4− chains linked by E-1 hosts.

Figure 3. Example supramolecular assemblies present in solution. (a) Anti-electrostatic dihydrogen phosphate (H2PO4−) oligomers, Gn, form from monomeric phosphates. These oligomers are bound by anion-binding Z-1 (b) and E-1 (c) bis-urea hosts to form ditopic [H(G)] or [H(G)] complexes. (d) As E-1 possesses divergent urea binding sites, larger supramolecular structures can form from H2PO4− chains linked by E-1 hosts.

Table 1. Changes in Diffusion Coefficients of Pure Isomers and 1:1 Mixed Solutions of E-1 and Z-1 in the Presence of 50 mM NBu4−-H2PO4−

| entry | [H2PO4−] (mM) | [E-1] (mM) | [Z-1] (mM) | D(H2PO4−)5/10−10 (m2 s−1) | D(E-1)5/10−10 (m2 s−1) | D(Z-1)5/10−10 (m2 s−1) | D(NBu4)5/10−10 (m2 s−1) |
|-------|---------------|-------------|-------------|-----------------------------|-------------------------|-------------------------|-------------------------|
| 1     | 5             | 5           |             | 1.74 ± 0.03                 | 1.87 ± 0.01             | 2.50 ± 0.02             | 2.39 ± 0.01             |
| 2     | 50            | 5           | 5           | 2.16 ± 0.03                 | 1.93 ± 0.04             | 1.17 ± 0.03             | 1.39 ± 0.01             |
| 3     | 50            | 5           | 5           | 2.01 ± 0.03                 | 1.83 ± 0.08             | 1.12 ± 0.02             | 1.45 ± 0.03             |
| 4     | 50            | 5           | 5           | 1.97 ± 0.07                 | 2.05 ± 0.02             | 1.27 ± 0.03             | 2.52 ± 0.02             |

*DMSO-d6 with 0.5% v/v added water. 202 MHz 31P PGSTE, δ = 7 ms, Δ = 100 ms, g = 0−53.5 G cm−1. 500 MHz 1H PGSTE, δ = 4 ms, Δ = 50 ms, g = 0−53.5 G cm−1.

experiments reported here. In the presence of 50 mM [NBu4][H2PO4], the photostationary state under nonoptimal irradiation with a 405 nm LED comprised a E/Z ratio of 58:42 as measured by NMR integration.

The measured diffusion coefficients of E-1 and Z-1 (D(E-1) and D(Z-1)) in DMSO-d6 with 0.5% v/v added water are shown in Table 1. In the absence of H2PO4−, the extended E-1 isomer diffuses slightly more slowly (7%) than the more compact Z-1 (Table 1; entry 1 vs 2). Given the large difference in size between host 1 (MW = 529, approximately longest axis 13 Å) and the H2PO4− anion (MW = 97, radius 2.5 Å),31 we might have anticipated a modest decrease in average host diffusion coefficients at guest concentrations where near-complete complexation occurs based on measured binding constants.32

Instead, we observe a large decrease in measured D(1) that continues to decrease at concentrations above those predicted for near-complete complexation.33 After correcting for H2PO4−-induced viscosity changes (see SI-4),34 the measured D of pure E-1 or Z-1 in the presence of 50 mM [NBu4][H2PO4] was found to decrease by 33% (Δ(E-1)) or 26% (Δ(Z-1)) relative to D without [NBu4][H2PO4] (Table 1; entries 1 vs 4; 2 vs 5; Figure 4 for full [H2PO4−]-dependent diffusion data). This suggests greater than 2 or 3-fold increases in effective volumes of the Z-1 or E-1 hosts, respectively. These substantial decreases in measured D are too large to be explained by the formation of simple ditopic [HG] complexes32c or even [H(G)2] complexes, where G is an oligomeric assembly of n hydrogen-bonded H2PO4− subunits that forms as in the absence of host35 (Figure 3b,c see Figure S11 for modeling).12c

Host E-1 forms larger structures than host Z-1, despite having a lower binding constant.36 This observation is also supported by changes in the measured diffusion of H2PO4−, D(H2PO4−) (measured by 31P NMR), which decreases by 11% or 7% in the presence of 5 mM of E-1 or Z-1, respectively.
The concentration of the host increased from 1 to 5 to 10 mM to form much larger complexes (Table 1; entries 4 vs 5). These observations suggest the hosts do not significantly decrease in Dff (Table 1, entry 3 vs 4; 3 vs 5), suggesting ion pairing is only a small but observable amount of the other isomer is also present (Table 1; entries 4 vs 6; 5 vs 6), suggesting minimal interactions between the E- and Z-isomers.

Relative to that of a solution of pure [NBu4][H2PO4], D(E-1) decreases on going from 5 mM Z-1 (−7%, Table 1, entry 5) to 5 mM E-1 (−11%, entry 4) to 5 mM Z-1 and E-1 (−15%, entry 6). D(H2PO4) for a solution of 2.5 mM E-1 and 2.5 mM Z-1 (−9%, entry 7) is also the average of that 5 mM solutions of each isomer (entries 4, 5). This also suggests the H2PO4− does not experience anything other than a statistical binding by the hosts, with surprisingly no evidence of cross-linking between different host isomers.

However, there is evidence that complexes are formed involving multiple host molecules of the same isomer. To test this, titration experiments with [NBu4][H2PO4] were conducted with solutions of 1:1 mixtures of E-1:Z-1 at 1, 5, and 10 mM total concentrations (SI-8.3). A small but observable decrease in D for both E-1 and Z-1 was found as the total concentration of the host increased from 1 to 5 to 10 mM (e.g., at 50 mM [NBu4][H2PO4]: Table 1, entries 6–8; also SI-S8.3). This data supports the formation of structures involving multiple hosts, such as structures such as shown in Figure 3d.

Host 1 could also increase the effective size of polyionionic complexes by increasing ion pairing to the NBu4+ cations. This would result in a decrease in D(NBu4) with increasing host concentration, but only a 5% decrease in D(NBu4) is observed (Table 1, entry 3 vs 4; 3 vs 5), suggesting ion pairing is only a minor contributor. There is also no difference between D(NBu4) in the presence of E-1 or Z-1, despite E-1 forming much larger complexes (Table 1; entries 4 vs 5). These observations suggest the hosts do not significantly change ion pairing with the NBu4+ and oligomers of H2PO4−.

From the measured diffusion coefficients of free host (5 mM) and oligomeric H2PO4− (50 mM), we estimate the assemblies formed involve 1–2 molecules of 1 with 2–3 assemblies of oligomeric guest Gm (where n is the same as that formed at 50 mM [NBu4][H2PO4] in the absence of host, see SI–S9 for discussion of methodology).57 From the modeled size distribution of H2PO4− oligomers at 50 mM (Figure S11), this corresponds to complexes incorporating approximately 10 H2PO4− subunits with average molecular weights of 1.5–2.0 kDa. Together, these results indicate that H2PO4− anions not only form aggregates in polar and hydrogen-bond accepting solvents, but that these structures can associate to form larger assemblies with multiple hosts in solution.

As host 1 is a photoswitch, the E-1/Z-1 distribution of isomers can be controlled using light (SI-10). Reversible switching was investigated by UV–vis absorption; see Figure S46. By combining in situ irradiation within the NMR spectrometer with recently developed time-resolved diffusion NMR techniques,58 we simultaneously measured changes in concentration and diffusion coefficients of E-1 and Z-1 under 400 nm irradiation (Figure 5). The rapidly changing concentration during the early stages of the reaction causes the observed noise; see SI-S10.2 for more details.

Photoswitching of organic molecules is expected to result in differences in D, but such changes would typically be minor (e.g., the 7% difference between D(E-1) and D(Z-1) in the absence of anion guest (Table 1, entries 1 and 2). Switchable anion binding might give more control over the effective host D, but complexes with small anions (e.g., Cl−, OAc−, NO3−, HSO4−) might only cause modest changes in host D, as found for control experiments with acetate (8% and 4% decrease, respectively, in D(E-1) and D(Z-1) at 50 mM [NBu4][OAc]; SI-S8.2). The use of H2PO4− oligomers allows greater control over D: switching host 1 from Z-1 to E-1 causes a “molecular gear change” and a 16% decrease in measured D (Table 1, entries 4 and 5), suggestive of an approximately 70% increase in average molecular volume. This demonstrates substantial control over the diffusion rate of small molecules in bulk solution by coupling photococntrol of guest-binding to the ability of H2PO4− to form extended supramolecular structures.59 As 1 is a thermally stable (“P-type”) photoswitch56,60 these changes in D will persist in the dark.

**CONCLUSION**

In conclusion, we sought to use the switchable anion-binding properties of host 1 to achieve photocontrol of translational diffusion rates. Diffusion NMR allowed characterization of the thermodynamics of the anti-electrostatic self-assembly of the bare dihydrogen phosphate anion in solution, a long-suspected but previously uncharacterized phenomenon. We obtained a surprisingly high isosmotic association constant of Ks = 120 ± 32 M−1 for H2PO4− self-association, which corresponds to complexes of a median size of four (or 10) H2PO4− subunits at concentrations of 50 mM (or 300 mM) in wet DMSO. Anion binding studies with H2PO4− in DMSO therefore always involve competition between host–H2PO4− and H2PO4−–H2PO4− interactions,54b,17 which poses problems for fitting titration data (SI-7.11 for further discussion). Because of the limited solubility of our bis-urea receptor, this
study was conducted exclusively in wet DMSO. It would be reasonable to assume that in less polar solvents such as DMF, acetonitrile, or dichloromethane self-association of $\text{H}_2\text{PO}_4^-$ may be more significant. To test this, we measured the diffusion coefficients of $[\text{NBu}_4][\text{H}_2\text{PO}_4^-]$ in CDCl$_3$. Due to tight ion pairing the diffusion coefficients of the cation and anion were close over the range of 2–300 mM. A decrease in $D(\text{H}_2\text{PO}_4^-)$ of around 40% is observed over this concentration range, consistent with the formation of oligomers similar to that observed in DMSO-$d_4$ (see SI-12 for details). This result suggests ion pairing may disrupt the formation of even larger oligomers in noncompetitive solvents.

Combining the unusual anti-electrostatic oligomerization of $\text{H}_2\text{PO}_4^-$ with a photoswitchable anion-binding receptor allowed light to induce a “gear change” and sharply change the rate of receptor diffusion, equivalent to a 70% change in effective volume. Can control of diffusion via a spatially selective stimulus (such as light, as demonstrated here) drive directional transport of small molecular species and create concentration gradients? This remains an interesting open question.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.0c09072.

Experimental procedures, NMR spectra, scripts used to process diffusion NMR data (PDF)

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**Notes**

The authors declare no competing financial interest. Raw experimental data has been deposited on ChemRxiv and is available at DOI: 10.26434/chemrxiv.12298919.v1

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(33) For example, [H2PO4]− = 50 mM, [1] = 5 mM: Table 1, entries 4–8; SI-8. See SI-7 for speciation curves based on measured K1 values.

(34) Viscosities of [NBu4][H2PO4] solutions in DMSO-d6 were measured directly and fitted to a viscosity calibration curve, which was used to compensate for changes in measured D caused by viscosity. See the Supporting Information, S4, for details.

(35) Equivalent experiments using [NBu4][OAc] in place of [NBu4][H2PO4] do not result in similar changes in the measured D of the host, suggesting that the ability of H2PO4− to form extended hydrogen-bound chains is critical for the observed changes in measured D (see the Supporting Information, SI-8.2).

(36) Note that despite E-1 having a lower measured K1 for H2PO4− than Z-1, there is no free host left in both cases at 50 mM, see the Supporting Information, S9.

(37) This analysis does not rely on the accuracy of the isodesmic binding model and fitted parameters: the proposed [H−2(G−2)−4] average structure only requires the experimentally measured effective D(H2PO4). See the Supporting Information, S9, for details.

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