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Selective Precipitation of Prions by Polyoxometalate Complexes

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The rising number of cases of variant Creutzfeldt−Jakob disease, together with the discovery of two instances that probably originated from blood transfusions, has generated concern over the extent to which prions causing bovine spongiform encephalopathy have been transmitted to humans.[1] To detect human prions at the earliest stages of infection, we developed a conformation-dependent immunoassay (CDI) for the infectious isoform of the prion protein (PrP^{Sc}), which is derived from normal, cellular protein (PrP^{C}).[2] A dramatic increase in the sensitivity and specificity of the CDI arose upon the discovery that PrP^{Sc} can be selectively precipitated from tissue homogenates by $Na_2H[PW_{12}O_{40}]$ (**1**).[2,3] This water-soluble salt features the nearly spherical trianion $[PW_{12}O_{40}]^{3-}$, which belongs to a broad class of polynuclear transition metal−oxo complexes known as polyoxometalates.[4] The utility of such species in precipitating proteins nonspecifically has long been recognized.[4,5] Further, certain polyoxometalates have been shown to interact with viral surface proteins, which, in the case of HIV, inhibits infectivity.[6] To date, however, there have been no studies probing the nature of the interactions of polyoxometalates with prion proteins, and the mechanism of the selective precipitation of PrP^{Sc} by **1** is not understood. To gain insight into this conformational selectivity and improve the sensitivity of the CDI, we investigated the precipitation efficacy of a set of polyoxometalates that vary in composition, structure, and charge density (see Figure 1). Herein, we report our initial findings, identifying the most active solution species and noting a particular dependence on the size and charge of the anions employed.

The precipitation step of the CDI protocol utilizes a neutralized aqueous solution containing 0.31% w/v of **1** and 0.055% w/v of $MgCl_2 \cdot 6H_2O$. The impact of varying the concentrations of these two compounds was assessed by applying a direct CDI (without proteinase K) to the precipitates obtained from brain homogenates of uninoculated, control, and scrapie-infected Syrian hamsters. After incubation and centrifugation, the relative levels of PrP^{C} and PrP^{Sc} were measured using the time-resolved fluorescence signals from the specific antibody binding to native and denatured samples, as described previously.[2a] Initial experiments, in which the concentration of **1** was maintained at 0.31% w/v while the concentration of $MgCl_2$ was varied, showed little impact on the amount of precipitated PrP^{Sc} but a correlation with the amount of PrP^{C} and other proteins in the pellet. These results suggest that the addition of $MgCl_2$ actually diminishes the selectivity of PrP^{Sc} precipitation. With no $MgCl_2$ added, the relative amount of precipitated PrP^{Sc} was found to increase for concentrations of **1** above 0.31% w/v. Indeed, as shown in the upper left panel of Figure 2, PrP^{Sc} levels in the precipitate did not reach a maximum until 2.48% w/v. At the same time, PrP^{C} levels in precipitates from control brain homogenates were 50−110 times lower and showed no apparent trend.[7,8]

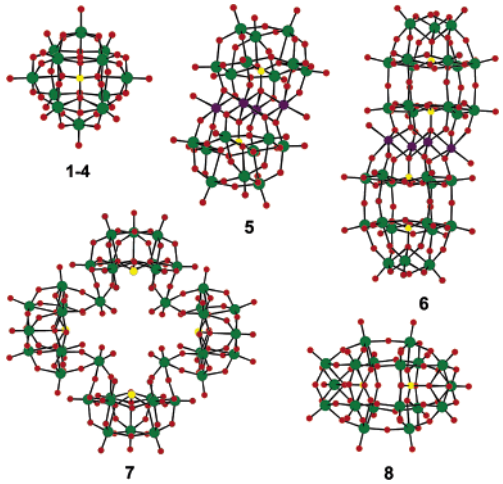

Figure 1. Solid-state structures for the polyoxometalate anions in compounds **1**−**8**: the Keggin structure of $[PW_{12}O_{40}]^{3-}$ (**1**), $[SiW_{12}O_{40}]^{4-}$ (**2**), $[BW_{12}O_{40}]^{5-}$ (**3**), and $[H_2W_{12}O_{40}]^{6-}$ (**4**); the double-Keggin structure of $[Zn_4(H_2O)_2(PW_9O_{34})_2]^{10-}$ (**5**); the double-Wells−Dawson structure of $[Zn_4(H_2O)_2(P_2W_{15}O_{56})_2]^{16-}$ (**6**); the wheel-like structure of $[As_4W_{40}O_{140}]^{28-}$ (**7**); and the Wells−Dawson structure of $[P_2W_{18}O_{62}]^{6-}$ (**8**). Green, purple, and red spheres represent W, Zn, and O atoms, respectively, while yellow spheres represent heteroatoms; H atoms are omitted for clarity.

To probe the role of polyoxometalate charge, similar precipitation experiments were performed using aqueous solutions of $Na_4[SiW_{12}O_{40}]$ (**2**), $K_5[BW_{12}O_{40}]$ (**3**), and $Na_6[H_2W_{12}O_{40}]$ (**4**). These salts contain anions bearing the same Keggin structure of $[PW_{12}O_{40}]^{3-}$, but with substitution of the central P^{IV} atom leading to an increasingly negative charge along the series $[SiW_{12}O_{40}]^{4-}$, $[BW_{12}O_{40}]^{5-}$, and $[H_2W_{12}O_{40}]^{6-}$. Overall, the results reveal trends comparable to that observed for **1**, except with some variation in the optimal polyoxometalate concentration (see Figure 2). Solutions of **2** and **3** showed maxima in PrP^{Sc} levels occurring at the much lower concentrations of 0.31% and 0.08% w/v, respectively. The selectivities attained, however, were not as high as those observed with 2.48% w/v of **1**. In the case of **4**, the results closely parallel those obtained for **1**, and a slightly greater selectivity is even achieved at the optimal concentration of 2.48% w/v.

Aqueous solutions of $K_{10}[Zn_4(H_2O)_2(PW_9O_{34})_2]$ (**5**), $Na_{16}[Zn_4(H_2O)_2P_2W_{15}O_{56})_2]$ (**6**), $Na_{27}[NaAs_4W_{40}O_{140}]$ (**7**), and $K_6[P_2W_{18}O_{62}]$ (**8**) were employed to test the effects of polyoxometalate size and structure on the selectivity of PrP^{Sc} precipitation. These salts contain much larger anions, exhibiting the prolate (**5**, **6**, and **8**) or wheel-like (**7**) structures depicted in Figure 1. Addition of solutions of **8** to brain homogenates resulted in the immediate nonspecific precipitation of large amounts of proteins, with concomitant formation of a blue supernatant solution indicative of polyoxometalate reduction.[4] In contrast, **5**−**7** suppressed PrP^{Sc} precipitation in a concentration-dependent manner (see Figure 2). Thus, very large polyoxometalates appear to inhibit PrP^{Sc} sedimentation.

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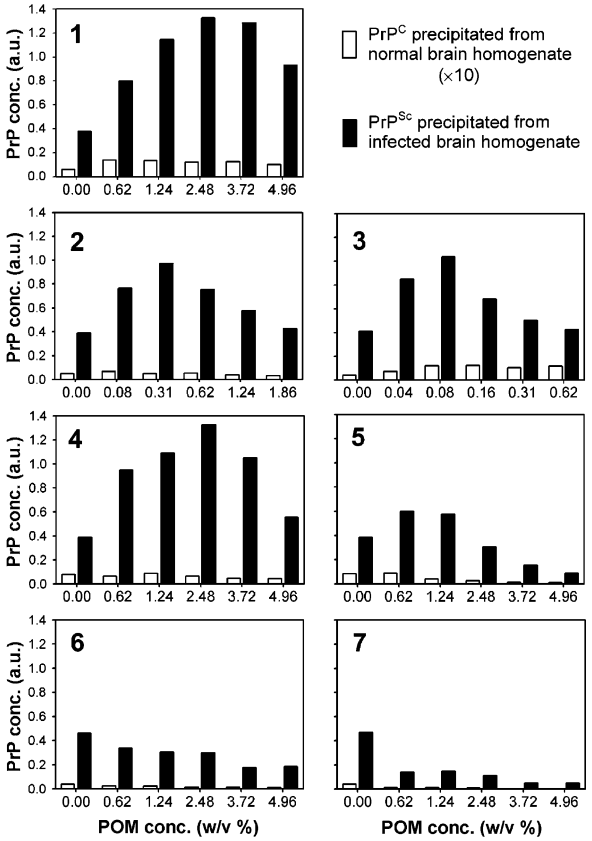

Figure 2. Relative concentrations of PrP^C and PrP^{Sc} in precipitates obtained from Syrian hamster brain homogenates, with variation in the concentration of the polyoxometalate (POM) salt employed. Values are relative to the concentration of PrP^{Sc} in a reference (ref) precipitate obtained using the standard CDI protocol, and were determined using the following expressions: PrP^C concentration $= D/(D_{ref} - N_{ref})$ and PrP^{Sc} concentration $= (D - N)/(D_{ref} - N_{ref})$, where D is the intensity of the fluorescence signal from Eu-labeled antibodies bound to prions upon denaturation and N is the intensity of the fluorescence signal from antibodies bound to native prions.

Whether this effect is due to the dissociation of pre-existing aggregates or denaturation of PrP^{Sc} with an accompanying change in prion titer demands more detailed investigation.

Spectroscopic studies were undertaken to establish the solution structures of the most selective precipitants (**1**−**4**). This was necessary because polyoxometalate anions, including those with the Keggin structure, are known to undergo degradation with changes in pH and/or buffer characteristics.[4,9] In each case, the experiments were performed on solutions containing all of the components present in the prion precipitation experiments, albeit with D_2O as a solvent in place of H_2O. For solutions of **1**, **3**, and **4**, the predominate species were identified as $[PW_{11}O_{39}]^{7-}$, $[HBW_{11}O_{39}]^{8-}$, and $[H_2W_{12}O_{40}]^{6-}$, respectively, by comparison of the ^{31}P, ^{11}B, and ^{1}H NMR spectra with the corresponding spectra for authentic, independently synthesized samples. In the case of **2**, the active anion was identified as $[SiW_{11}O_{39}]^{8-}$ via infrared spectroscopy. Importantly, a ^{31}P NMR spectrum collected upon addition of a working solution of **1** to a normal brain homogenate indicated no change in the form of the polyoxometalate. Thus, while the parent Keggin structure (see Figure 1) of $[H_2W_{12}O_{40}]^{6-}$ remains intact as the active precipitant in solutions of **4**, solutions of **1**−**3** instead contain lacunary complexes formed by the loss of a single $[WO]^{4+}$ unit from the parent structure. The trend in optimal polyoxometalate concentrations follows the order $[HBW_{11}O_{39}]^{8-} < [SiW_{11}O_{39}]^{8-} < [PW_{11}O_{39}]^{7-} = [H_2W_{12}O_{40}]^{6-}$, indicating an approximately inverse correlation with the charge density of the anion present in solution.

The foregoing results lead us to hypothesize that the observed conformational selectivity of polyoxometalates is due to size-specific electrostatic interactions between Keggin-type anions and multiple PrP^{Sc} oligomers. The opposite effect of larger polyoxometalate anions suggests that the binding site of the prion is a somewhat hindered cleft with one or more positively charged residues.[10] The general trend in which the PrP^{Sc} levels in the precipitates obtained with **1**−**4** rise to a maximum and then diminish with increasing polyoxometalate concentration is particularly interesting. This behavior is consistent with a model in which at low concentrations the polyoxometalate anions are capable of linking two or more PrP^{Sc} moieties to create larger aggregates, while at higher concentrations the PrP^{Sc} binding sites eventually all saturate such that no linking occurs. Consistent with this model, a higher binding affinity can be expected for the more highly charged anions of **2** and **3**, such that the maximum should occur at a lower concentration.

In summary, we have demonstrated the superior ability of Keggin-type polyoxometalate complexes to precipitate PrP^{Sc} selectively. On the basis of the concentration trends observed for such species, we propose an aggregation mechanism involving multivalent electrostatic interactions between the polyoxometalate anions and positively charged PrP^{Sc} cleft sites. Using these compounds in the purification of prions for structural studies might enhance investigations of the conformation of PrP^{Sc}. Additionally, they could possibly find application in the development of an immunoassay capable of detecting extremely low concentrations of infectious prions in blood and cerebrospinal fluid.

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Supporting Information Available: Full experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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