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. 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 (PrPSc), which is derived from normal, cellular protein (PrPC). A dramatic increase in the sensitivity and specificity of the CDI arose upon the discovery that PrPSc can be selectively precipitated from tissue homogenates by Na2H[PW12O40] 3− (1). This water-soluble salt features the nearly spherical trianion [PW12O40] 3−, which belongs to a broad class of polynuclear transition metal–oxo complexes known as polyoxometalates. The utility of such species in precipitating proteins nonspecifically has long been recognized. Further, certain polyoxometalates have been shown to interact with viral surface proteins, which, in the case of HIV, inhibits infectivity. 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 PrPSc 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 MgCl2·6H2O. 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 PrPC and PrPSc were measured using the time-resolved fluorescence signals from the specific antibody binding to native and denatured samples, as described previously. Initial experiments, in which the concentration of 1 was maintained at 0.31% w/v while the concentration of MgCl2 was varied, showed little impact on the amount of precipitated PrPSc but a correlation with the amount of PrPC and other proteins in the pellet. These results suggest that the addition of MgCl2 actually diminishes the selectivity of PrPSc precipitation. With no MgCl2 added, the relative amount of precipitated PrPSc was found to increase for concentrations of 1 above 0.31% w/v. Indeed, as shown in the upper left panel of Figure 2, PrPSc levels in the precipitate did not reach a maximum until 2.48% w/v. At the same time, PrPSc levels in precipitates from control brain homogenates were 50–110 times lower and showed no apparent trend.


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Selective Precipitation of Prions by Polyoxometalate Complexes

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Published on Web 09/17/2005

10.1021/ja055219y CCC: $30.25 © 2005 American Chemical Society
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 PrPSc 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. The general trend in which the PrPSc 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 PrPSc moieties to create larger aggregates, while at higher concentrations the PrPSc 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 PrPSc 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 PrPSc cleft sites. Using these compounds in the purification of prions for structural studies might enhance investigations of the conformation of PrPSc. 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.

Acknowledgment. This research was funded by NIH contract NS02328 and DOD grant NP020038. S.B.P. and J.G.S. have financial interest in InPro Biotechnology, Inc.

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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