Role of smart polymers in protein purification and refolding

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Many applications of proteins and enzymes do not require preparations of high purity.1-3 Non-chromatographic methods based upon precipitation, crystallization and partitioning in different phases have emerged as powerful alternative bioseparation strategies for obtaining proteins/enzymes for such applications.4,5 Affinity precipitation is one such technique.5-6 The technique is based upon the use of affinity ligands linked to reversibly soluble-insoluble polymers. Such polymers are also known as stimuli-sensitive or smart polymers. The affinity ligands could be the same as those used in more conventional affinity chromatography formats. The smart polymers are water-soluble polymers, which can be precipitated by a change in solution pH, temperature or addition of a simple chemical species such as Ca2+ or K+.

Reversing the condition would again convert the polymer into solution form (Fig. 1). The macro-(affinity ligand) i.e., smart polymer-affinity ligand conjugate could form an affinity complex with the desired protein (specifically, even in the presence of other undesirable proteins) in free solution. It could then be precipitated upon application of the suitable stimulus, dissociated and the desired protein recovered.

It was discovered that many commercially available synthetic polymers (e.g., Eudragit L-100 and Eudragit S-100) and naturally occurring polymers (like chitosan and alginate) could be used as smart macro-(affinity ligands) as such for a host of proteins.7-13 This was an important development in the context of protein bioprocessing in general and affinity precipitation in particular for many reasons. Most of these polymers were fairly inexpensive, non-toxic and easily available. Conjugation costs and fear of denaturation of protein (in its denatured form) with the polymer leads to the refolding of the protein.

Affinity precipitation is a non-chromatographic method which is useful for purification and refolding of proteins. Quite often, a stimuli-sensitive polymer can be identified which selectively binds to the desired protein. For separation, the protein can be recovered from the precipitate of the protein-smart polymer complex. In case of a refolding experiment, binding of the solubilized protein (in its denatured form) with the polymer leads to the refolding of the protein.

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Very early in the refolding area, it was observed that additives could facilitate refolding. One way of classifying such additives is in terms of molecular weight/size. In the list of small molecular additives, osmolytes (the compounds accumulated by water stressed organisms to stabilize proteins) were the early choices. Today this list extends to a very large number of classes including cyclodextrins16 and nanoparticles.17 The seminal work by Cleland et al.17 on polyethylene glycol facilitated refolding of denatured carbonic anhydrase suggested the possibility of using polymers as artificial chaperones. Very soon, as already mentioned, smart polymers were found to work well.18-20

How do these various additives work? Tsumoto et al.17 classified the small molecule additives as folding enhancers, refolding}
aggregation suppressors or even denaturants. However, it is likely that any additive functions in more than one way. With smart polymers, many papers have attempted to discuss the way these polymers facilitate refolding. Some have even used “molecular mechanism” or a very curious term “molecular-assisted refolding” in the titles. Unfortunately, none of these provide any clear insight. It is not clear whether “smartness” of the polymer has a role i.e., are smart polymers better than something like PEG? We find: (1) That all kinds of smart polymers viz. hydrophobic,11,24 analogous but less hydrophobic25 and hydrophilic polymers,9,12 work well as facilitators of protein refolding; (2) It looks as if a polymer which shows adequate binding would work well; (3) The binding itself is responsible for the refolding and kinetics of both processes.11,26 The precipitation step is merely for recovery and purification.

No matter what the exact mechanism is, it seems that if one searches enough, it would be possible to find a smart polymer for a specific refolding purpose. A 96-well plate format for screening polymers for this purpose has been recently described by our group.26 Earlier, we had shown that with a little ingenuity, it should be possible to use even starch as a smart polymer.25 Of about eight inclusion bodies tried, it was possible to find a suitable smart polymer for refolding in each case.25 It has also been shown that surface hydrophobicity of the polymer plays a role presumably via altering the binding constant for proteins. Microwave treatment seems to be a good tool to alter the surface hydrophobicity of these smart polymers. Hence, the approach of smart polymer facilitated protein refolding seems to be a promising approach as a general method. Given fairly high refolding yields coupled with the possibility of refolding at moderate concentrations of proteins, the method should attract wider attention. While inclusion bodies are believed to be relatively pure forms of proteins, inbuilt feature of “simultaneous purification” in a non-chromatographic format can be an additional advantage of the approach.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 1. Reversibly soluble-insoluble nature of alginate. The smart polymer alginate has blocks of guluronic acid and mannuronic acid moieties. Alguronic acid is soluble in aqueous medium as free carboxyl groups can interact with water molecules. Similarly, in case of p-hydroxy polymers like Eudragit and chitosan, the protonation of carboxyl groups and deprotonation of amino groups, respectively, makes the polymers insoluble in the aqueous medium.10
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