Review of using diacetic functionalized polymers in solubilizing enzymes in organic solvents

Wenfa Ng

Citizen scientist, Singapore, Email: ngwenfa771@hotmail.com

Abstract

Enzymes have evolved to function in aqueous systems. However, a dichotomy exists in industrial biocatalysis where most of the substrates and products are more soluble in various organic solvents. Thus, it would be desirable to develop enzymes capable of dissolving and catalysing reactions in organic solvents. One approach to this end involves the directed evolution of enzymes to function in non-aqueous environments. But, this approach requires the generation of large mutant libraries difficult for small laboratories to effectively screen. Other approaches involve the modification of enzymes with hydrophobic groups such as hydrophobic polymers to aid in their solubilization in organic solvents. Working in this direction, Hijazi and coworkers reports in *Biotechnology and Bioengineering* the potential of using noncovalent interactions between poly(2-oxazoline) (POx) terminated with 2,2'-imino diacetic acid (IDA) to aid in the solubilization and conferment of higher enzymatic activity to a variety of enzymes in industrially relevant organic solvents: chloroform and toluene. Specifically, dynamic light scattering measurements revealed that IDA groups were important for aiding formation of soluble noncovalent polymer-enzyme conjugates (PEC) of molecular size, ~ 10 nm. In comparison, POx terminated with -OH group resulted in the formation of non-soluble aggregates of size ~ 140 nm. The approach was shown to be effective in solubilizing lysozyme, horseradish peroxidase (HRP), laccase, α-chymotrypsin, catalase and alcohol dehydrogenase to a concentration of 2 mg/mL, yielding clear solutions in chloroform and toluene. Furthermore, the stoichiometry of polymer binding to enzyme for solubilizing it was obtained and showed a general trend of higher polymer binding for higher molecular weight enzymes. Efforts in understanding whether polymer modification of enzyme or solubilization of PEC in organic solvents led to reduced enzymatic activity in water revealed that noncovalent conjugation of polymer to enzyme was generally well-tolerated but the same is not true for solubilization of PEC in organic solvent. Except for HRP, both laccase and α-chymotrypsin exhibited reduced enzymatic activity in water after extraction of PEC from organic solvent. Nevertheless, PEC of HRP, laccase and α-chymotrypsin exhibited enhanced activity in organic solvents compared to the native enzyme in the same solvents. Hence, the work demonstrated that noncovalent modification of enzymes by IDA terminated polymer is a feasible approach for aiding the solubilization of enzymes in organic solvents. Furthermore, PEC formed exhibited enhanced enzymatic activity compared to native enzymes in organic solvent; thereby, opening up the approach's potential use in non-aqueous enzymology. However, the reported work could be further augmented by more detailed exploration of toluene as an organic solvent for PEC as well as testing the feasibility of the approach is solubilizing more classes of enzymes in different organic solvents.

Keywords: enzyme-polymer conjugates, non-aqueous media, organic solvents, toluene, chloroform, enzymatic activity, dynamic light scattering, functional assays, denaturation, noncovalent,

Subject areas: biochemistry, biophysics, biotechnology, bioengineering, chemistry,

Organic solvents are important for solubilizing hydrophobic substrates and products in biocatalytic reactions. However, enzymes often have poor solubilities and activities in organic solvents; thereby, presenting a restricted set of enzymes for biotransformations, which limits the types of products possible. In *Biotechnology and Bioengineering*, Hijazi and co-workers described the use of noncovalent polymer enzyme conjugates (PEC) for improving the solubilities and activities of enzymes in organic solvent in a paper entitled: "Poly(2-oxozoline)s terminated with 2,2'-imino diacetic acid form noncovalent polymer-enzyme conjugates that are highly active in organic solvents". Building on previous covalent PEC,[1] the study revealed that poly(2-oxaline)s with 2,2'-imino acetic acid (IDA) groups could form noncovalent PEC that improved the solubilities and activities of enzymes in chloroform and toluene.

Poly(2-oxazoline)s (POx) with terminal IDA groups based on poly(methyl-2-oxazoline) (PMOx), poly(ethyl-2-oxazoline) (PEtOx) and a copolymer between poly(ethyl-2-oxazoline) and poly(heptyl-2-oxazoline) were synthesized and characterized by nuclear magnetic resonance (NMR) spectroscopy and size-exclusion chromatography (SEC). These polymers with differing hydrophobicity were shown to be useful for forming nano-sized noncovalent conjugates with a variety of enzymes such as lysozyme, horseradish peroxidase (HRP), laccase, α-chymotrypsin (CT), catalase, and alcohol dehydrogenase. These PECs showed higher solubilities in chloroform and toluene, up to 2 mg/mL. Visual observations and dynamic light scattering (DLS) analysis revealed that IDA terminal groups were important for attaching to cationic sides groups of lysine, arginine, histidine and tryptophan on enzyme surface and enabling enzyme dissolution in organic solvents compared to -OH terminal group. Specifically, large insoluble enzyme-polymer aggregates of size ~150 nm were obtained with PMOx terminated with -OH group for lysozyme and HRP. In contrast, soluble PECs of size ~ 10 nm were obtained with PMOx terminated with IDA groups for the same enzymes. Analysis of the relative ratio of polymer to enzyme (PER) needed to aid in enzyme dissolution in organic solvents revealed that higher PER was needed for higher molecular weight enzymes for PMOx and PEtOx. However, in the case of the copolymer, P(EtOx-co-HepOx)-IDA, similar PER were obtained for lysozyme, CT, and laccase even though they were of different molecular weight. Interestingly, identical PER were needed for the copolymer to solubilize the different enzymes in chloroform and toluene which differed significantly in hydrophobicity and solvent characteristics. Experiments were also conducted to extract solubilized PEC in organic solvents into water, and the results revealed that protein extraction efficiency was lower for PEC based on PEtOx and P(EtOx-co-HepOx) compared to PMOx. Comparison of enzyme activity after noncovalent conjugation with polymers revealed a slight decrease in activity compared to native enzymes in water for HRP, laccase and CT. Except for HRP, both laccase and CT exhibited significant loss of enzymatic activity after extraction of solubilized PEC in organic solvent into water. Finally, solubilized PEC of laccase, HRP and CT exhibited large enhancement in enzymatic activities compared to native enzymes in chloroform and toluene.

One contribution of the paper is the demonstration of the role played by IDA groups in poly(2-oxazoline)s in binding to enzymes and enabling their greater dissolution in organic solvents. This builds on earlier work where PECs were constructed based on covalent linkage of enzyme to polymer, but the authors failed to fully articulate the benefits of the noncovalent

conjugation approach whether through theoretical discussion or experimental work. Secondly, the paper demonstrated that noncovalent PEC could enhance the activities of different enzymes in chloroform and toluene, which are industrially relevant organic solvents not miscible with water. The extent of activity enhancement observed was also relatively high compared to other systems for enhancing enzyme solubility in organic solvents.[2] Other strengths of the paper include functional tests conducted to assess whether noncovalent conjugation with polymer led to reduced enzymatic activity using an activity assay in water. More importantly, similar assays were also performed to determine possible denaturation of enzyme in noncovalent PEC after solubilization in organic solvents. One advantage of such functional assays is that they provide direct evidence of enzyme activity compared to structural analysis of the PEC, and they also validated that functional enzymes were obtained after noncovalent conjugation with polymers.

Weaknesses of the paper include deficiencies in experiment design and writing. One example concerns the extraction of PEC from organic solvents into water. While useful for understanding the thermodynamic partitioning of PEC between organic and aqueous phase, the shaking time of 10 seconds and contact time of 3 minutes was insufficient for thermodynamic equilibrium to be achieved. More importantly, different copolymerisation ratio of P(EtOx-co-HepOx)-IDA should have been explored as polymer-enzyme conjugates for enhancing the solubility and activity of enzymes in the important nonpolar solvent toluene. In discussion of experimental results, attempt was not made to correlate enzyme structure and activity loss after extraction of PEC from organic solvents into water. Future work may seek to expand the types of enzymes and organic solvents tested. In addition, MALDI-MS could be used to determine the stoichiometry of binding between polymer and enzyme. Determination of the molecular structures of the PEC conjugates in organic solvents by circular dichroism or fluorescence spectroscopy would also offer clues to the enhanced enzyme activities observed. Given that small amount of water in anhydrous organic solvents have been reported to confer higher activity to enzymes,[3] future experiments may examine how water content of organic solvent influences activity of PEC.

Collectively, the article demonstrated the utility of noncovalent PEC in enhancing the solubilities and activities of different enzymes in industrially relevant organic solvents; thereby, providing a useful tool for biocatalysis. But the approach could have been extended to more polymer types and enzyme classes, and would benefit from an investigation of the mechanisms underlying observed improvement in enzyme activity of PEC in organic solvents.

References

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Conflicts of interest

The author declares no conflicts of interest.

Funding

No funding was used in this work.