

Biotechnol Bioeng. 2013 Sep;110(9):2352-60.

doi: 10.1002/bit.24910. Epub 2013 Apr 22.

## Stabilization of enzymes in ionic liquids via modification of enzyme charge

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- PMID: 23532939 DOI: [10.1002/bit.24910](https://doi.org/10.1002/bit.24910)

### Abstract

Due to the propensity of ionic liquids (ILs) to inactivate enzymes, the development of strategies to improve enzyme utility in these solvents is critical to fully exploit ILs for biocatalysis. We have developed a strategy to broadly improve enzyme utility in ILs based on elucidating the effect of charge modifications on the function of enzymes in IL environments. Results of stability studies in aqueous-IL mixtures indicated a clear connection between the ratio of enzyme-containing positive-to-negative sites and enzyme stability in ILs. Stability studies of the effect of [BMIM][Cl] and [EMIM][EtSO<sub>4</sub>] on chymotrypsin specifically found an optimum ratio of positively-charged amine-to-negatively-charged acid groups (0.39). At this ratio, the half-life of chymotrypsin was increased 1.6- and 4.3-fold relative to wild-type chymotrypsin in [BMIM][Cl] and [EMIM][EtSO<sub>4</sub>], respectively. The half-lives of lipase and papain were similarly increased as much as 4.0 and 2.4-fold, respectively, in [BMIM][Cl] by modifying the ratio of positive-to-negative sites of each enzyme. More generally, the results of stability studies found that modifications that reduce the ratio of enzyme-containing positive-to-negative sites improve enzyme stability in ILs. Understanding the impact of charge modification on enzyme stability in ILs may ultimately be exploited to rationally engineer enzymes for improved function in IL environments.

**Keywords:** biocatalysis; enzyme engineering; enzyme modification; ionic liquids.

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## Methods for stabilizing and activating enzymes in ionic liquids — A review

- July 2010 [Journal of Chemical Technology & Biotechnology](#) 85(7):891-907

DOI: [10.1002/jctb.2375](https://doi.org/10.1002/jctb.2375)

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## Abstract

Ionic liquids (ILs) have evolved as a new type of non-aqueous solvents for biocatalysis, mainly due to their unique and tunable physical properties. A number of recent review papers have described a variety of enzymatic reactions conducted in IL solutions; on the other hand, it is important to systematically analyze methods that have been developed for stabilizing and activating enzymes in ILs. This review discusses the biocatalysis in ILs from two unique aspects (1) factors that impact the enzyme's activity and stability, (2) methods that have been adopted or developed to activate and/or stabilize enzymes in ionic media. Factors that may influence the catalytic performance of enzymes include IL polarity, hydrogen-bond basicity/anion nucleophilicity, IL network, ion kosmotropicity, viscosity, hydrophobicity, the enzyme dissolution, and surfactant effect. To improve the enzyme's activity and stability in ILs, major methods being explored include the enzyme immobilization (on solid support, sol-gel, or CLEA), physical or covalent attachment to PEG, rinsing with n-propanol methods (PREP and EPRP), water-in-IL microemulsions, IL coating, and the design of enzyme-compatible ionic solvents. It is exciting to notice that new ILs are being synthesized to be more compatible with enzymes. To utilize the full potential of ILs, it is necessary to further improve these methods for better enzyme compatibility. This is what has been accomplished in the field of biocatalysis in conventional organic solvents. Copyright © 2010 Society of Chemical Industry

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Chapter

**First Online:** 01 October 2013

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## Abstract

The potential of ionic liquids as a green alternative to environmentally harmful volatile organic solvents has been well recognized. Being considered as “designer solvents”, ionic liquids have been used extensively in a wide range of applications including biotransformations. As compared to those in traditional organic solvents, enzyme performance in ionic liquids is showed enhance in their activity, enantioselectivity, stability, as well as their recoverability and recyclability. This chapter will cover the biocompatibility issue of ionic liquids with enzymes. The effects of ionic liquid properties on the enzymatic reactions and conformation of enzyme as well as methods for activation and stabilization of enzymes in ionic liquids will be described. In addition, the current attempts for rational design of biocompatible ionic liquids will be also discussed.

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