Contribution: Poster
Email id: hrpratyush@gmail.com

Role of Ionic Liquids on Protein stability and Dynamics

Pratyush Ranjan Hota,[1] Harekrushna Sahoo[2,*]

[1,2]*Department of Chemistry, National Institute of Technology, Rourkela-769008*

Email: sahooh@nitrkl.ac.in

Environmental conditions such as pH, temperature, and the presence of chemical species are the key factors, which can affect the protein structure, conformations, and dynamics. Nowadays researchers have investigated the effect of ionic liquids (as a co-solvent) as refolding additives and as stabilizing and destabilizing agents depending on their concentrations and types of ions they constitute. By using imidazolium-based, ammonium-based, and morpholinium-based ionic liquid, the conformational stability of commercial proteins (BSA and lysozyme) has been studied. In the case of ammonium-based ionic liquids, hydrophobicity played a key role towards the thermal stability of the protein (in the case of refolding) which is confirmed from far and near-UV CD analysis. Whereas an increase in the concentration of IL shows a better stabilizing effect (without altering the native structure of Bovine Serum Albumin aka BSA), which is further confirmed by fluorescence and circular dichroism spectroscopy. In the case of triethyl octyl ammonium bromide and up to 0.02 M concentration, BSA was found in compact structures other than native one but an increase in concentration causes unfolding. In the case of different imidazolium-based ILs, the hydrophobic cationic part helps in destabilizing the thermal stability of BSA whereas the change in the anionic part also influences the stability along with the increase in the concentration of ILs. In the case of lysozyme (lyz) as a model protein, it was found that the stability and activity of lyz get increased in presence of ionic liquid as compared to the conventional buffer.

References

1. Satish, L.; Rana, S.; Arakha, M.; Rout, L.; Ekka, B.; Jha, S.; Dash, P; Sahoo, H.; S*pectroscopic Letters*, **2016**, *49 (6)*, 383–390.

Role of Ionic Liquids on Protein stability and Dynamics

Pratyush Ranjan Hota, Harekrushna Sahoo*
hrpratyush@gmail.com, sahooh@nitrkl.ac.in
Department of Chemistry, National Institute of Technology, Rourkela-769008

ABSTRACT

By using imidazolium-based, ammonium-based, and morpholinium-based ionic liquid, the conformational stability of commercial proteins (BSA and lysozyme) has been studied. In the case of ammonium-based ionic liquids, hydrophobicity played a key role towards the thermal stability of the protein (in the case of refolding) which is confirmed from far and near-UV CD analysis. Whereas an increase in the concentration of IL shows a better stabilizing effect (without altering the native structure of Bovine Serum Albumin aka BSA), which is further confirmed by circular dichroism spectroscopy. In the case of different imidazolium-based ILs, the hydrophobic cationic part helps in destabilizing the thermal stability of BSA whereas the change in the anionic part also influences the stability along with the increase in the concentration of ILs. In the case of lysozyme (lyz) as a model protein, it was found that the stability and activity of lyz get increased in presence of ionic liquid as compared to the conventional buffer.

INTRODUCTION

- The primary structure of BSA consists of 583 amino acid residues.
- The secondary structure of serum albumins is 67% helix with six turns and 17 disulfide bridges
- 19 tyrosine residues and two tryptophan (Trp132 and Trp212) residues as intrinsic fluorophores
- Mainly found in Blood plasma
- Functions as a transport protein

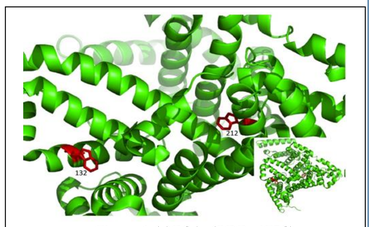
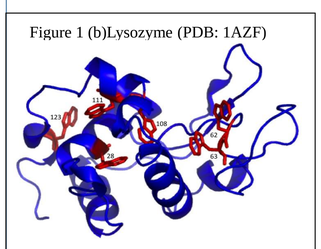


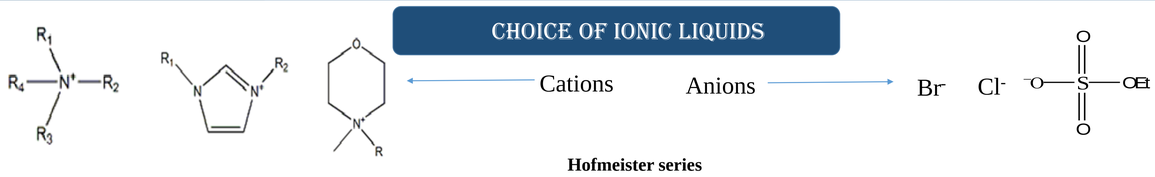
Figure 1 (a)BSA (PDB: 4F5S)

Figure 1 (b)Lysozyme (PDB: 1AZF)



- It is a small globular protein consisting of 129 amino acids.
- It has 6 tryptophans (Trp), 3 tyrosines (Tyr), and 4 disulphide bonds.
- Lyz hydrolyses the bond between N-acetylglucosamine and N-acetylmuramic acid present in the bacterial cell walls to decipher its antibacterial activity.
- It also exhibits the properties like antivirus activity and plays a major role in the transportation and deposition of fatty acids, drugs, and hormones.

CHOICE OF IONIC LIQUIDS



Hofmeister series

Anions: (kosmotropes) $PO_4^{3-} > SO_4^{2-} > EtSO_4^{3-} > OAc^- > MeSO_4^- > Cl^- > Br^- > I^- > BF_4^- > PF_6^-$ (chaotropes)

Cations: (chaotropes) $(CH_3)_4N^+ > K^+ > Na^+ > Li^+ > Ca^+ > Mg^{2+} > Al^{3+}$ (kosmotropes)

FLUORESCENCE SPECTRA OF BSA PROTEIN

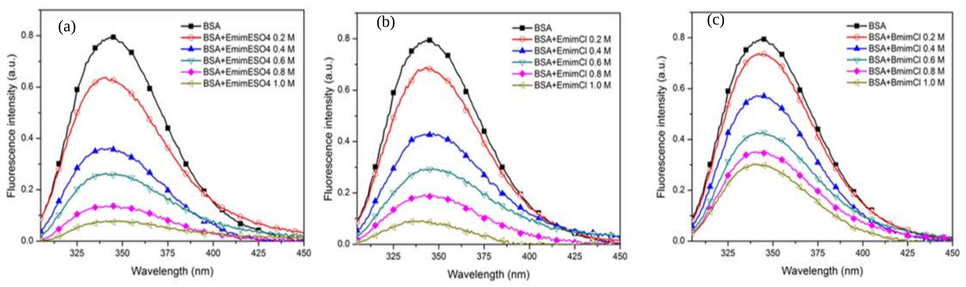


Figure 2. Emim stands for ethyl methyl imidazolium cation and spectra show the changes in fluorescence intensity with different ions with an increase in concentration from 0 to 1 M (shows quenching)

NEAR UV-CD SPECTRA OF BSA PROTEIN

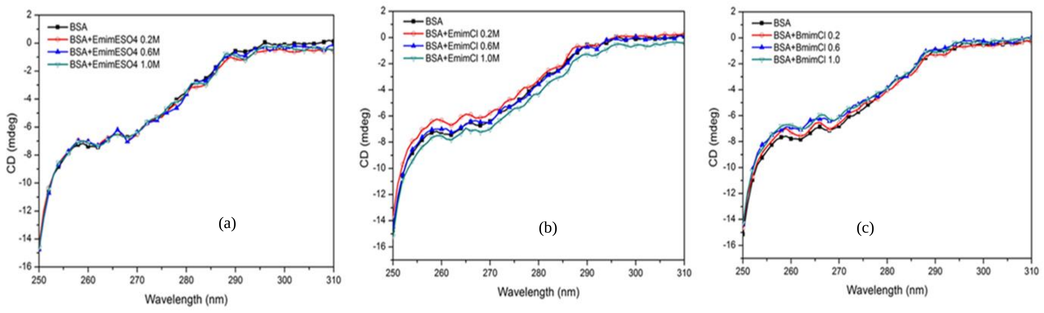


Figure 3. Near UV- CD spectra of BSA protein in the absence and presence of ILs with two characteristic minimas at 261, 268 nm correspond to disulfide bond and Phe residue and region 270-300 nm corresponds to aromatic chromophore

INTERACTIONS WITH AMMONIUM BASED IONIC LIQUIDS

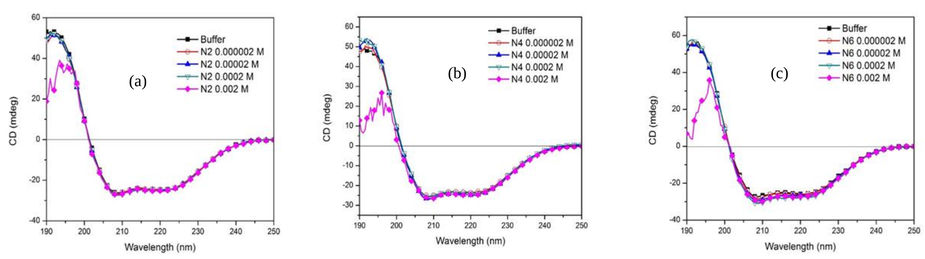


Figure 4. Far UV- CD spectra of BSA protein in the absence and presence of ILs with two characteristic minima at 208, and 225 nm which is the characteristic peak of the helical nature of the protein.

% LOSS IN HELICAL STRUCTURE OF BSA

Conc.	2×10^{-6} M				2×10^{-5} M				2×10^{-4} M				2×10^{-3} M			
IL	25^0 C	90^0C	25^0C	**% loss**	25^0 C	90^0C	25^0C	**% loss**	25^0C	90^0C	25^0C	**% loss**	25^0C	90^0C	25^0C	**% loss**
N2 (tetraethyl ammonium bromide)	64	33	53	**14**	65	34	55	**15**	65	34	55	**15**	66	33	56	**15**
N4 (triethyl butyl ammonium bromide	60	29	51	**15**	65	33	54	**17**	62	31	52	**16**	63	32	56	**11**
N6 (triethyl octyl ammonium bromide	65	35	56	**14**	68	39	61	**10**	73	40	65	**10**	68	42	64	**5**
N.B.	**In buffer loss of helical structure is 15 %**															

α- helix (%)= $(-MRE_{208} - 4000)/(33000-4000)*100$, where MRE= observed CD/10 C_p*n*l

C_p is the molar concentration of the protein, n is the number of amino acid residues of the target protein, and l is the path length in cm (0.1) 33000 corresponds to MRE value at 208 nm for pure α- helix and 4000 for random coil structure

MRE is the abbreviation of mean ellipticity residue

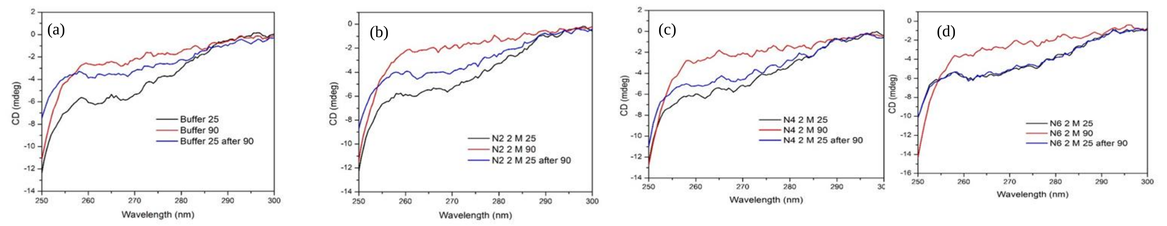


Figure 5. Near UV- CD spectra of BSA protein in the absence (a) and presence of ILs (b-d) where ILs concentration is 2 M in each case [black- native state, red- unfolded state, blue- refolded state] spectra shows with increase in hydrophobic alkyl chain, the protein will be able to regain its refolded structure more efficiently

EFFECTS OF ADDITIVES ON CONFORMATIONAL STUDY OF PROTEIN

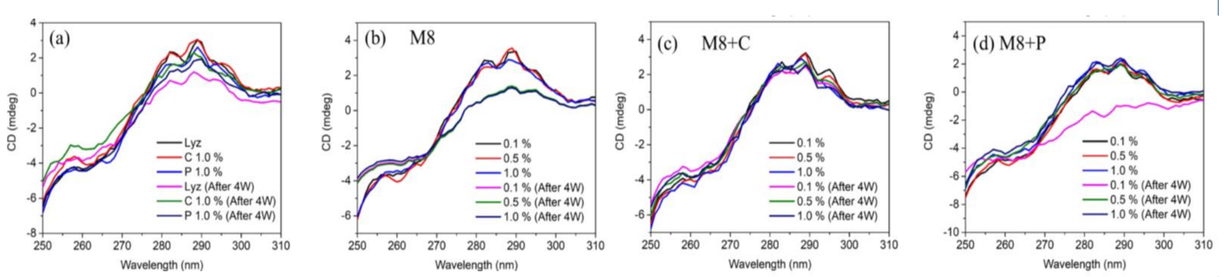


Figure 6. Near UV CD analysis of Lyz in absence of IL (a) and presence of M8 (N-octyl N-methyl morpholinium bromide) (b), M8+carboxymethylcellulose-1% (c), andM8+polyethyleneglycol-1% (d). The concentration of IL was (0.1, 0.5, and 1.0%). (4W represents 4 weeks)

CONCLUSION

- It was observed that, not only the type of ILs but also the concentrations of ILs, have an impact on the structure and stability of BSA.
- Comparing the cationic part of ILs, hydrophobicity played an important role in the destabilization of BSA
- From thermal denaturation study, IL with a higher alkyl chain i.e. higher hydrophobic exert higher stability
- IL formulations can preserve the native state of Lyz for a longer period at room temperature
- IL formulations with polymers offered more effective protection than free ILs as

REFERENCES

1. T. Vasantha, P. Attri, P. Venkatesu and R. S. R. Devi, *J. Phys. Chem. B*, 2012, **116**, 11968-11978.
2. J. V. Rodrigues, V. Prosinecki, I. Marrucho, L. P. N. Rebelo, C. M. Gomes, *Phys. Chem. Chem. Phys*. 2011, **13**, 13614-13624
3. S.S. Usmani, G. Bedi, J.S. Samuel, S. Singh, S. Kalra, P. Kumar, A.A. Ahuja, M. Sharma, A. Gautam, PLoS One, 2017, **12**, e0181748
4. U.K. Singh, M. Kumari, S.H. Khan, H.B. Bohidar, R. Patel, ACS Sustain. Chem. Eng., 2017, **6,** 803-812

ACKNOWLEDGEMENT

We are thankful to the chemistry department of NIT Rourkela for the instrument facility.