Bangladesh Journal of Veterinary and Animal Sciences, Vol. 3, No. 1 & 2, July 2015

ISSN 2227-6416

Bangladesh Journal of Veterinary and Animal Sciences

Journal home page: www.cvasu.ac.bd/journal/userlist

Research article

Effect of PEAMA-g-PEG on the conformation of lysozyme by circular dichroism and florescence spectroscopy

Sumon Ganguli[1*]

[1]*Faculty of Science, Department of Applied & Environmental Chemistry, University of Chittagong, Chittagong-4331, Bangladesh.*

ARTICLE INFO

Article history :
Received 14 March 2015
Revised 22 March 2015
Accepted 30 June 2015

Keywords :
Lysozyme, PEAMA-g-PEG
Tertiary Structure
Hydrophobic Interaction

Corresponding Author :
Email: sumonganguli@yahoo.com
Tel: 01956-835822

ABSTRACT

Conjugation of biological (peptides/enzymes) and synthetic polymers has potential applications in both medicine and biotechnology. We have shown in our previous article that the enzymatic activity of lysozyme was suppressed by the complex formation with a cationic smart copolymer, poly(N,N-diethylaminoethyl methacrylate)-graft-poly(ethylene glycol) (PEAMA-g-PEG). In addition, the secondary structure of lysozyme was maintained. In the present study, tertiary structure of lysozyme were investigated using circular dichrosm (CD) and fluorescence spectroscopy. Near-UV CD spectral analysis clearly indicates that the complexation of lysozyme with PEAMA-g-PEG has almost no influence on the tertiary structure of lysozyme. Moreover, fluorescence spectrum of lysozyme and lysozyme complexed with PEAMA-g-PEG indicates the conformations of lysozyme were not disrupted.

To cite this paper : *Ganguli, S. 2015. Effect of PEAMA-g-PEG on the conformation of lysozyme by circular dichroism and florescence spectroscopy. Bangladesh Journal of Veterinary and Animal Sciences, 3 (30-34)*

INTRODUCTION

Enzymes are used in many industrial sectors such as in the production of sugar, semi-synthetic penicillin's, amino acid, cheese, fruit and wine. Enzymes can also be used in the hydrolysis of lactose, tenderization of meat and in leather industry, baking industry, brewing industry etc (Bickerstaff, 1987). Moreover, many enzymes incorporated with synthetic polymers have been approved for clinical use (Cohen and Bernstein, 1996, Solaro R. 2008). Essentially, conjugation of enzymes and synthetic polymers is an efficient means to improve control over nanoscale structure formation which can be used in many applications. Bioconjugation is kind of elegant way for developing enzyme-polymer complex for use in diversified field such as biosensor (Allard *et al.* 2004), to study enzyme inactivation (Ganguli *et al.* 2009), and for structure activity relationship (Gao *et al.* 2010), for switches (Shimboji *et al.* 2003, Tomita *et al.* 2010), for stabilization of enzyme (Okahata and Mori 1997, Ganguli *et al.* 2009, Ganguli *et al.* 2010, Venkataramani *et al.* 2013), importantly lysozyme for food processing and preservation (Proctor *et al.* 1988, Zhu *et al.* 2011, Cao *et al.* 2014), in drug delivery system (Pahwa *et al.* 2012, Lim *et al.* 2013), and so on. To note that enzymes are heterogenic, this leads to manifold types of interactions between polymers and enzymes. Thus, the complexation between polymer and enzymes in aqueous solution may be driven by hydrogen bonding (Xia *et al.* 1993), hydrophobic interaction (Gao *et al.* 2009), and electrostatics forces (Dubin *et al.* 1985). However, hydrophobic and electrostatic interactions are more common factor for complexation between enzyme-polyelectrolyte. It is well accepted that fabrication of enzyme-polymer complex involving noncovalent interactions is a simple way of mixing between them in aqueous solution. Despite the fact that the preparation method and to control the enzymatic

function, the conformational stability of the enzyme upon complexation are gained great great importance for researcher. Based on the several previous studies (Lumry and Eyring, 1954, Ganguli *et al.* 2010, Tomita *et al.* 2010, Weikl and Hemmateenejad 2013), it can be concluded that the fruitful applications of the enzymes are greatly depend on the conformational stability of the enzyme and will vastly increase upon stabilization even after any modification. Briefly, conformational stability of enzymes plays a crucial role in determining both the catalytic efficiency and the chemo-, regio-, and enantioselectivity of enzymes, thus eventually influencing their exploitability in biotechnological applications.

It was investigated that the smart cationic polymer, PEAMA-g-PEG regulate the enzymatic activity of lysozyme with maintaining the secondary structure of lysozyme (Ganguli *et al.* 2009) whereas the tertiary conformations of the lysozyme remains unclear. Moreover, qualitatively, it is understood that hydrophobic interaction directly related to the extent of hydrophobicity and complementarity of each species involved in the macromolecular complexation (Hofstee 1974; Jost *et al.* 1974). The near UV CD and spectra in the region 260-320 nm arise from the aromatic amino acids. Trp shows a peak close to 290 nm with fine structure between 290 and 305 nm; Tyr a peak between 275 and 282 nm, with a shoulder at longer wavelengths often obscured by bands due to Trp; Phe shows weaker but sharper bands with fine structure between 255 and 270 nm (Kelly *et al.* 1975). The excitation wavelength for fluorescence spectra was chosen to be 290 nm to excite selectively tryptophan (Trp) residues in lysozyme and recording the emission spectra within 300-400 nm. The near UV CD and fluorescence spectrum of an enzyme provides a valuable fingerprint of the tertiary structure of that enzyme, which can be used to compare, for example, the native enzyme with the modified one. In our present study, the used copolymer, PEAMA-g-PEG, has hydrophobic part (-diethyl-) and model enzyme, lysozyme, several amino acid residues in lysozyme which are hydrophobic in nature shows the fingerprint of the tertiary structure of lysozyme. Taken together, there is every possibility to take place hydrophobic interaction between them which may change the tertiary structural feature of lysozyme upon complexation with PEAMA-g-PEG. To address the tertiary structural feature of lysozyme upon complexation with PEAMA-g-PEG and given the versatile applications of enzyme and polymer/enzyme complex strongly related the conformational stability of enzyme, our present study focuses on the investigation of tertiary structure of lysozyme in the PEAMA-g-PEG/lysozyme complex.

MATERIALS AND METHODS

Materials

PEAMA-g-PEG was synthesized as described in our previous article (Ganguli *et al.* 2009). PEAMA-g-PEG was used before HPLC purification. Hen egg white lysozyme was obtained from Sigma Chemical Co. (St. Louis, MO, USA) was purchased from Wako (Osaka, Japan). Sodium dihydrogen phosphate dihydrate (NaH2PO4.2H2O) was obtained from Nacalai Tesque Inc. (Kyoto, Japan). All chemicals used were of high-quality analytical grade. The water used in this study was purified using the Milli-Q system (Nihon Millipore Co., Tokyo, Japan).

CD spectra measurements

Near-UV CD spectra were monitored using a spectropolarimeter (model J-720W; Jasco, Tokyo, Japan). A cuvette with 0.1-cm path length was used, and the photomultiplier voltage did not exceed 600 V during the measurements (Figure 2). The results are directly obtained from CD spectrophotometer. Aliquots of the stock solutions were mixed to prepare sample solutions containing lysozyme (0.5 mg/mL) and the polymer (10 mg/mL).

Fluorescence spectra measurements

Fluorescence spectra were obtained at 25 °C using a Hitachi F-4500 spectrophotometer. A 0.2-mm cell, with the incident light angled at 45°, was used to minimize inner filter effects. A lysozyme stock solution and a polymer stock solution of pH 7.0 were prepared separately. Aliquots of the stock solutions were mixed to prepare sample solutions containing lysozyme (0.5 mg/mL) and the polymer (10 mg/mL). Fluorescence spectra were obtained with a Hitachi F-4500 spectrophotometer. The excitation wavelength was chosen to be 290 nm to excite selectively tryptophan (Trp) residues in lysozyme and recording the emission spectra within 300-400 nm.

RESULTS AND DISCUSSION

In our previous study, we found that the enzymatic activity of lysozyme was suppressed by PEAMA-g-PEG owing to capping of the active site of lysozyme, which involved an electrostatic interaction between the negatively charged active site of lysozyme and the

positively charged amine moiety of PEAMA-g-PEG at neutral pH (Ganguli *et al.* 2009). It has to be noted that the suppressed enzymatic activity was recovered upon

the addition of poly(acrylic acid) (PAAc) to lysozyme/PEAMA-g-PEG complex (Ganguli *et al.* 2009). It was assumed that electrostatic and hydrophobic interaction plays the key role for the complexation between lysozyme and PEAMA-g-PEG. It is presumably suggested that the hydrophobic interaction between the diethyl part of the copolymers and the hydrophobic amino acid residues of lysozyme might change the tertiary structure of lysozyme. To know the effect of PEAMA-g-PEG on the tertiary structural feature of lysozyme upon complexation with PEAMA-g-PEG, in the present article, we focused on to investigate the tertiary conformational stability of lysozyme upon complexation with PEAMA-g-PEG. Normally, structural changes in enzymes caused by the complexation of polyelectrolyte are an essential part of the mechanism of action and regulation of biological activity. CD provides an experimentally very convenient means of detecting such changes which can be examined in different spectral regions. Gao and his coworkers (Gao *et al.* 2009) reported that lysozyme complexed with Sodium (Sulfamate Carboxylate) Isoprene/Ethylene Oxide (SCIEO) block copolymer influence the conformational stability of lysozyme. Briefly, lysozyme complexed with SCIEO (0.10-0.30 mg/mL) copolymers disrupted the near-UV CD spectrum of lysozyme which indicates the conformational instability of lysozyme. It worthwhile to mention that in our previous report, far-UV CD spectrum of lysozyme was almost similar with lysozyme/PEAMA-g-PEG complex which reveled that the secondary structure of the lysozyme were maintained after the complexation (Ganguli *et al.* 2009). Motivated by the above mentioned examples, one may imagine that the complexation between lysozyme and PEAMA-g-PEG leads to the disruption of the tertiary structure of the lysozyme. The near-UV CD spectrum of the enzymes generally reflects its tertiary structure (Gao *et al.* 2009). To investigate the tertiary structure of lysozyme and effect of PEAMA-g-PEG on the tertiary structure of lysozyme, near-UV CD spectra of lysozyme and lysozyme complexed with PEAMA-g-PEG were monitored. Figure 1 shows the near-UV CD spectra of lysozyme and lysozyme/ PEAMA-g-PEG complex.

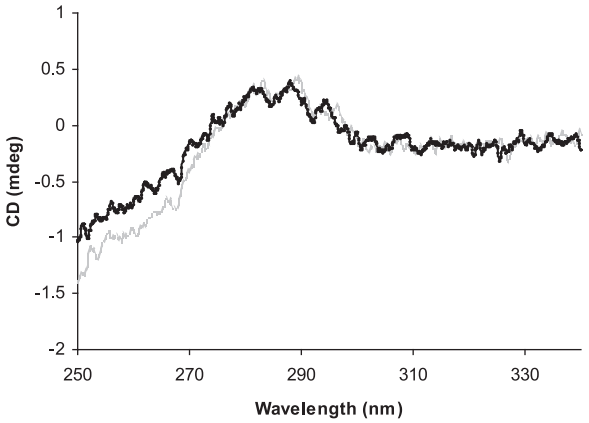

Figure 1: Near-UV CD spectrum of lysozyme (Solid line) and lysozyme/PEAMA-g-PEG complex (Dotted line). The concentration of lysozyme and PEAMA-g-PEG were 0.5 mg/mL and 10 mg/mL respectively. The measurements were carried out at 25°C and at pH 7.0.

From Figure 1, it was observed that both spectra were lies in the similar pattern which revealed that PEAMA-g-PEG have no substantial effect on the tertiary structure of the lysozyme. A very little disruption of spectra was observed at the wavelength of less than 270 nm might be due to the high concentration of PEAMA-g-PEG. It is worthwhile to mention that the concentration of lysozyme and PEAMA-g-PEG were used as 0.5 and 10 mg/mL. The reason why we used these concentrations is that lysozyme lost all of its enzymatic activity (0.12-3.9%) upon complexation with PEAMA-g-PEG (See Figure 1a, Ganguli *et al.* 2009). Figure 2 shows the changes of the photomultiplier voltage which did not exceed 600 V during the measurements.

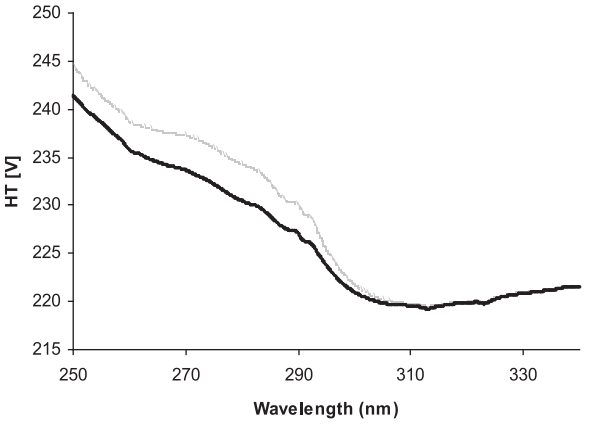

Figure 2: The changes of photomultiplier voltage during the measurements of CD spectrum of lysozyme (Solid line) and lysozyme/PEAMA-g-PEG complex (Dotted line).

To evaluate any disruption in the tertiary structure of lysozyme upon complexation with PEAMA-g-PEG again, fluorescence spectra of lysozyme and lysozyme/PEAMA-g-PEG complex were monitored. Figure 3 shows the fluorescence spectra of lysozyme and lysozyme/PEAMA-g-PEG complex. It was observed again that both spectra were same which indicates the tertiary conformation of lysozyme remains same after complexation. These results indicate that even though the complexation occurred (Ganguli *et al.* 2009), the tertiary conformation of lysozyme due to the complexation with PEAMA-g-PEG were not changed.

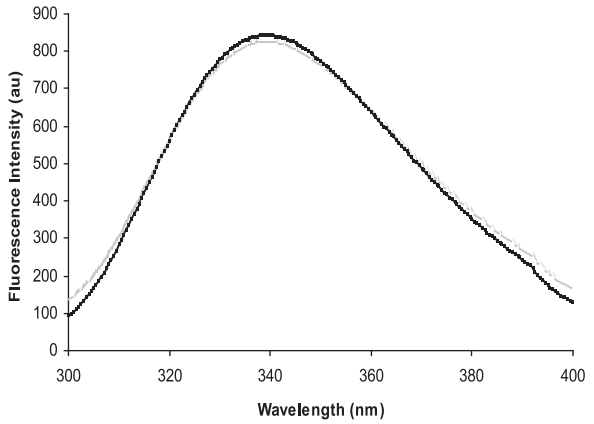

Figure 3: Fluorescence spectrum of lysozyme (Solid line) and lysozyme/PEAMA-g-PEG complex (Dotted line). The concentration of lysozyme and PEAMA-g-PEG were 0.5 mg/mL and 10 mg/mL respectively. The measurements were carried out at 25°C and at pH 7.0.

These results can be explained by the fact that here the hydrophobic interaction might not strong enough to change the tertiary structure of lysozyme. Based on versality of using enzyme and polymer modified enzyme in many biomedical and biotechnological applications, our present enzyme/polymer complex with structure maintaining effects would be useful for further study. We propose that the following two questions should be addressed during future investigations of the efficacy of PEAMA-g-PEG on enzyme inactivation and aggregation. First, can PEAMA-g-PEG form complex with other industrially important enzyme and control their enzymatic functions with conformation to lead them forward for applications? Second, to explain the mechanism of interaction between lysozyme and PEAMA-g-PEG as it is still unclear. If the above questions can be solved, this kind of strategy might be extended to regulate the enzymatic activity of other enzymes or the binding affinity of proteins to polymers/DNA/other proteins which expand the potential of enzyme in biomedical and biotechnological fields.

CONCLUSION

The complexation between lysozyme with PEAMA-g-PEG has almost no influence on the tertiary conformation of lysozyme. PEAMA-g-PEG was found to novel modifying agent for lysozyme which control the enzymatic activity of lysozyme upon complexation (Ganguli *et al.* 2009) and in conclusion our present results indicates PEAMA-g-PEG maintaining the tertiary conformation of lysozyme. So that it is expected that charged PEGylated polymers would be very useful for enzyme/polymer complex for their applications. Complexation between polymer and enzyme with the maintained structure of enzyme offers new prospects for stabilization and delivery of enzyme.

ACKNOWLEDGEMENTS

The author gratefully acknowledge the financial support of the Department of Applied and Environmental Chemistry, University of Chittagong, Chittagong 4331, Chittagong, Bangladesh.

REFERENCES

Allard, L., Cheynet V., Oriol G., Gervasi G., Imbert-Laurenceau E., Mandrand B., Delair T. and Mallet F. 2004, Antigenicity of Recombinant Proteins after Regioselective Immobilization onto Polyanhydride-Based Copolymers, Bioconju. Chem., 15: 458-466.

Bickerstaff G. F., 1987, Enzymes in Industry and Medicine, Edward Arnold (Publishers Ltd. 41 Bedford Square, London WC1B 3DQ.

Cohen S. and Bernstein H. 1996, Microperticulate System for Delivery of Proteins and Veccines, Marcel Dekker, New York, 1-49 pp.

Cao T. T., Zhou Z.Z. and Zhang Y. Q. 2014, Processing of ?-glucosidase-silk fibroin nanoparticle bioconjugates and their characteristics, Appl. Biochem. Biotechnol., 173(2): 544-551.

Dubin, P. L. Ross, T. D. Sharma, I. and Yegerlehner, B. 1987, In Ordered Media in Chemical Separations, Hinze, W. L. and Armstrong, D. W. Eds., American Chemical Society: Washington, DC, Chapter 8.

Ganguli S., Yoshimoto K., Tomita S., Sakuma H., Matsuoka T., Shiraki K. and Nagasaki Y. 2009, Regulation of Lysozyme Activity Based on Thermotolerant Protein/Smart Polymer Complex Formation, J. Am. Chem. Soc., 131 (18): 6549-6553.

Gao G., Yunfeng Y., Pispas S. and Yao O. 2010, Sustained and Extended Release with Structural and Activity Recovery of Lysozyme from Complexes with Sodium (Sulfamate Carboxylate) Isoprene/Ethylene Oxide Block Copolymer, J. of Macromol. Biosci., 10: 139-146.

Ganguli S., Yoshimoto K., Tomita S., Sakuma H., Matsuoka T., Shiraki K. and Nagasaki Y. 2010, Improving the heat resistance of ribonuclease A by the addition of poly(N,N-diethylaminoethyl methacrylate)-graft-poly(ethylene glycol) (PEAMA-g-PEG), Macromol. Biosci., 10 (8): 853-859.

Hofstee, B. H. J. 1974, Non-specific binding of proteins by substituted agaroses, Adv. Exp. Med. Biol., 42 (0): 43-59.

Jost, R., Miron, T. and Wilchek, M. 1974, The mode of adsorption of proteins to aliphatic and aromatic amines coupled to cyanogen bromide-activated agarose, Biochim. Biophys. Acta, 362 (1): 75-82.

Kelly S. M., Jess T. M. And N. C. Price N. C. 2005, How to study proteins by circular dichroism, Biochim. Biophys. Acta, 1751: 119 -139.

Lim E. K., Jang E., Lee K., Haam S. and Huh Y-M. 2013, Delivery of Cancer Therapeutics Using Nanotechnology, Pharmaceutics, 5 (2): 294-317.

Lumry R. and Eyring H. 1954, Conformation Changes of Proteins, J. Phys. Chem., 58 (2): 110-120.

Okahata Y. and Mori T. 1997, Catalytic Activity of Lipid-coated Enzymes in Organic Media, Proc. Japan Acad., 73 (B): 210-214.

Proctor V. A. and Cunningham. F. E. The chemistry of lysozyme and its use as a food preservative and a pharmaceutical, 1988, Crit. Rev. Food Sci. Nutr. 26(4), 359-95.

Pahwa R., Saini N., Kumar V. and Kohli K. 2012, Chitosan-based gastroretentive floating drug delivery technology: an updated review, Expert Opin. On Drug Deliv., 9 (5): 525-539.

Solaro R. 2008, Targeted delivery of proteins by nanosized carriers, J. Polym. Sci. Part A: Polym. Chem., 46 (1): 1-11.

Shimboji T., Larenas E., Fowler T., Hoffman A. S. and Stayton P. S. 2003, Photoswitching of Ligand Association with a Photoresponsive Polymer?Protein Conjugate, Bioconju. Chem., 13 (5): 915-919.

Secundo F. 2013, Conformational changes of enzymes upon immobilisation. Chem. Soc. Rev. 42(15): 6250-6261.

Seo J. H., Matsuno R., Lee Y., Konno T., Takai M. and Ishihara K., 2011, Effect of hydrophilic polymer conjugation on heat-induced conformational changes in a protein. Acta Biomater., 7(4): 1477-1484.

Tomita S., Ito L., Yamaguchi H., Konishi G., Nagasaki Y. And Shiraki K. Enzyme switch by complementary polymer pair system (CPPS), 2010, Soft Matter, 6 (21): 5320-5326.

Venkataramani S., Truntzer J. and Coleman DR. Thermal stability of high concentration lysozyme across varying pH: A Fourier Transform Infrared study. J. Pharm. Bioallied. Sci., 2013, 5 (2): 148-153.

Weikl T. R. and Hemmateenejad B. 2013, How conformational changes can affect catalysis, inhibition and drug resistance of enzymes with induced-fit binding mechanism such as the HIV-1 protease, Biochim Biophys Acta.,1834 (5): 867-73.

Xia, J., Dubin, P. L. and Kokufuta, E. 1993, Dynamic and electrophoretic light scattering of a water-soluble complex formed between pepsin and polyethylene glycol, Macromolecules, 26 (24): 6688-6692.

Zhu L, Hu R.P., Wang H.Y., Wang Y.J., and Zhang Y. Q. 2011, Bioconjugation of neutral protease on silk fibroin nanoparticles and application in the controllable hydrolysis of sericin. J Agric Food Chem., 59(18): 10298-10302.