Supramolecular Interaction of Molecular Cage and β-Galactosidase: Application in Enzymatic Inhibition, Drug Delivery and Antimicrobial Activity

Avijit Mondal,[a] Imtiyaz Ahmad Bhat,[b] Subbaraj Karunakaran,[a] and Mrinmoy De*[a]

Enzyme inhibitors play a crucial role in diagnosis of a wide spectrum of diseases related to bacterial infections. We report here the effect of a water-soluble self-assembled Pd^{II}_8 molecular cage towards β-galactosidase enzyme activity. The molecular cage is composed of a tetrapyridyl donor (**L**) and *cis*-[(en)$Pd(NO_3)_2$] (en = ethane-1,2-diamine) acceptor and it has a hydrophobic internal cavity. We have observed that the acceptor moiety mainly possesses the ability to inactivate the β-galactosidase enzyme activity. Kinetic investigation revealed the mixed mode of inhibition. This inhibition strategy was extended to control the growth of methicillin-resistant *Staphylococcus aureus*. The internalization of the Pd(II) cage inside the bacteria was confirmed when bacterial solutions were incubated with curcumin loaded cage. The intrinsic green fluorescence of curcumin made the bacteria glow when put under an optical microscope. Furthermore, this curcumin loaded molecular cage shows an enhanced antibacterial activity. Thus, Pd^{II}_8 molecular cage is quite attractive due to its dual role as enzyme inhibitor and drug carrier.

Introduction

Biomolecules are natural targets for the development of many inorganic drugs. Several small molecules,[1] nanomaterials and macromolecules[2] have been developed to target various biomolecules. Metal based supramolecular coordination architectures have been exploited extensively in catalysis, sensing, host-guest study, cavity induced unusual organic transformations, antimicrobial activity etc.[3] Similarly, their biomolecular interaction also opened up new avenues for biomedical research which include, sensing of biomolecules,[4] recognition of proteins,[5] recognition of nucleotide base,[6] anticancer therapy,[7] drug delivery[8] etc. Among them, recognition of protein is key to control a range of cellular processes, such as cellular signal transduction, protein antigen/antibody interaction and DNA transcription.[9] However, the effect of supramolecular coordination assemblies on protein is not well studied. Kamiya *et al.* have shown the interaction of saccharide coated $M_{12}L_{24}$ molecular spheres with protein concanavalin-A and peanut agglutinin.[5] The polysaccharides present on the outer surface of the sphere help to recognize the protein surface of concanavalin A. Though $M_{12}L_{24}$ sphere can recognize protein surface but the mode of interaction of the metallacage with protein and their kinetic behaviour have not been explored so far. To get insight about the interaction of supramolecular coordination architecture with protein, we have chosen β-galactosidase (β-Gal) as a model protein and a water-soluble $[Pd_8L_4]^{16+}$ (**1**) molecular cage as metallasupramolecular architecture.

β-Gal is a therapeutically relevant enzyme which catalyses the hydrolysis of β-D-galactoside to galactose and alcohol, which are the key sources to produce energy. It is a vital enzyme for the human body; the deficiency of which can cause galactosialidosis or Morquio B syndrome.[10] β-Gal is an essential enzyme for various microorganisms as well. Enhanced proliferation rate of various pathogenic bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (E. coli) rely on galacto-oligosaccharides which is formed through several enzymatic reactions catalysed by β-Gal.[11] Although there is a little difference exists between human β-Gal and bacterial β-Gal in their oligomerization state and domain organization, but the mode of action is nearly same. The loop region of β-domain 2 (residues 482–491) in human β-Gal execute the same role as the complementation loop of E. coli β-Gal. Moreover, the active site of human β-Gal is easily approachable from the bulk solvent whereas the active site of E. coli β-Gal is not easily accessible from the bulk solvent.[12] Hence, developing β-Gal inhibitor from E. coli benefits to tune the activity for various relevant application. In literature various small molecules have been reported as β-Gal inhibitor, such as L-ribose, D-galactose, D-galactonolactone etc.[13] Not only small molecules but also several nanomaterials have been developed to alter the enzymatic activity of β-Gal. Surface engineered gold nanoparticles,[14] MoS_2 nanosheets,[15] ZnO nanoparticles[16] and positively charged graphene oxide (GO)[17] have been used to tune the activity of β-Gal. By controlling the activity of β-Gal, bacterial growth can also be controlled as reported by Kotov *et al.*[16] However, to the best of our knowledge, metal based supramolecular coordination architectures have not yet been

[a] *A. Mondal, S. Karunakaran, Dr. M. De*
Department of Organic Chemistry, Indian Institute of Science
Bangalore 560012 (India)
E-mail: md@iisc.ac.in
[b] *I. A. Bhat*
Department of Inorganic and Physical Chemistry
Indian Institute of Science, Bangalore 560012 (India)

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