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Effects of silica nanoparticle supported ionic liquid as additive on thermal reversibility of human carbonic anhydrase II

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ABSTRACT

Silica nanoparticle supported imidazolium ionic liquid [SNImIL] was synthesized and utilized as a biocompatible additive for studying the thermal reversibility of human carbonic anhydrase II (HCA II). For this purpose, we prepared additive by modification of nanoparticles through the grafting of ionic liquids on the surface of nanoparticles (SNImIL). The SNImIL were fully characterized by Fourier transform infrared spectroscopy, scanning electron microscopy and thermo gravimetric analysis. The characterization of HCA II was investigated by various techniques including UV–vis and ANS fluorescence spectrophotometry, differential scanning calorimetry, and docking study. SNImIL induced disaggregation, enhanced protein stability and increased thermal reversibility of HCA II by up to 42% at pH 7.75.

1. Introduction

Irreversibility causes misfolding and aggregation of proteins in cells and under in vitro conditions. Most importantly, it is involved in a wide range of diseases, including some of the most prevalent neurodegenerative disorders [1]. Human carbonic anhydrase (HCA II) is a zinc metalloenzyme which catalyzes the reversible hydration of carbon dioxide to bicarbonate and hydrogen ions [2,3]. At least 14 different CA isoforms have been isolated in higher vertebrates. These isozymes have diverse tissue distribution and subcellular localizations, and they exist in archaea, eubacteria, animals and plants [4]. Many of these isozymes are significant targets for inhibitors with clinical applications [5,6]. HCA II has a single polypeptide chain of 259 amino acid residues with a molecular mass of about 29 kDa [7]. This enzyme is known as a cytoplasmic isozyme and has a high catalytic activity with very high affinity for sulfonamides. The inhibition of CA II aggregation is important in the treatment of glaucoma, marble brain syndrome, CA II deficiency syndrome [8], altitude sickness, and obesity. More than its medical relevance, its tractability and simplicity are what make CA II particularly an attractive model enzyme [9].

Aggregation processes are important challenges while working with proteins. Biopharmaceutical products with low quality can potentially affect drug activity, immunogenicity and pharmacokinetics. All living organisms are potentially exposed to such stress conditions as non-optimal environmental temperatures and osmotic pressures, as well as the presence of toxic chemicals at certain stages of their life. These conditions pose a serious threat to the living organism often by causing the cellular proteins to unfold, which may lead to aggregation [10]. Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and prion diseases are increasingly being realized to have common cellular and molecular mechanisms including protein aggregation and inclusion body formation [1].

HCA II serves as a good model system for the study of enzymes; however, its denaturation is irreversible. Thus, any efforts to improve the thermal reversibility of HCA II could be important. Due to the aforementioned principles, several attempts to control aggregation and refolding of CA, as well as other proteins, have been made [11–22]. Some of these reports used nanoparticles of

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hydrophobized poly (vinyl alcohol), silica and hydrogel [23–25], imidazole [26], ionic liquid [27–32] or β-casien [33–40] to control aggregation and refolding of proteins. Over the past decade, nanotechnology has become one of the fastest growing research fields, mainly due to the unique large surface area, small size, physicochemical properties and successful usage of nanoparticles in different fields including imaging, diagnosis, catalysts, biocatalysis and drug delivery [41–46].

Generally, the development of silica nanoparticles as tunable supporting material in nanostructure design is of great significance for both academia and industry [47,48]. Ionic liquids, which are low temperature melting salts, have also been considered as eco-friendly alternatives to volatile organic solvents because of their negligible vapor pressure, nonflammable nature, chemical stability, relatively high ionic conductivity and wide potential window [49–51]. These properties have opened up many applications such as solvent in synthesis, catalysis, biocatalysis and in electrochemistry [52–58]. Their applications in biology such as ionic liquids (ILs) as refolding additives have been recently reported [27–32]. Although the ability of ILs in refolding has been successfully demonstrated in many fields the heterogeneous systems are still preferred, because of their easy handling and separation. Thus, supported ILs are highly desirable. Supported ILs improved the efficiency versus homogenous ones [59–61].

The combinations of silica nanoparticle and ionic liquid properties have already been probed by some researchers. Nanoparticles have significant adsorption capacities due to their relatively large surface area, therefore, they are able to bind or carry other molecules such as chemical compounds, drugs, probes and proteins attached to their surface by covalent bonds or by adsorption. Hence, the physicochemical properties of nanoparticles, such as hydrophobicity and charge, can be altered by attaching specific chemical compounds, peptides or proteins to their surface [62–65].

The efficacy of nanoparticles for any application depends on the physicochemical characteristics of both their material and surface modifiers. The composition of the nanoparticles, together with their surface properties, also determines their biocompatibility and their ability to be biodegraded. Apart from the impact on function, modifying the surface properties of nanoparticles are also used to reduce their toxicity [24].

In this report, we investigate the structural studies of HCA II reversibility assisted by silica nanoparticle supported imidazolium ionic liquids as a biocompatible and favorable additive for disaggregation and growing thermal reversibility of HCA II.

2. Materials and methods

2.1. Chemicals

All starting materials were of reagent grade. Tetraethoxysilane (TEOS) was from Fluka, chloropropyltrimethoxysilane, sodium iodide, imidazole and methanol were from Aldrich. The 8-anilino-1-naphthalene sulfonic acid (ANS) and other chemicals of the analytical grade were obtained from Sigma–Aldrich. All the solutions were prepared in doubly distilled water and all experiments were carried out in 20 mM Tris–sulfate, pH 7.75 at 27 °C, which is optimum [11].

2.2. Protein purification

HCA II isoenzyme was purified from red blood cells according to Nyman's method [66] with some minor modifications. The enzyme purity was confirmed by SDS-PAGE. The concentration of HCA II was determined by absorbance at 280 nm with an extinction coefficient (ε_{280}) $5.7 \times 10^{-4}\,M^{-1}\,cm^{-1}$ [18] or confirmed by the Lowry's method [67].

2.3. Synthesis of chloropropyltrimethoxysilane modified silica nanoparticles

In a 250 ml round bottom flask, 60 ml (10 mmol) ammonia solution (32%) and 1.98 g (110 mmol) water were added to 100 ml of absolute methanol. The solution was stirred for 5 min before adding 10.41 g (50 mmol) TEOS drop-wise. The final solution was stirred for three days at ambient temperature. Approximately, 35 ml of the prepared silica nanoparticle suspension was degassed using vacuum for several minutes to remove excessive ammonia. One gram (5 mmol) 3-chloropropyltrimethoxysilane was then added drop-wise, and the suspension was refluxed for 72 h. After cooling, the particles were collected by filtration or centrifuging, and exhaustively washed with ethanol and water, and dried under vacuum to give chloropropyl-silica nanoparticles. The synthesized silica nanoparticles (1 g) was stirred with 1-methyl imidazole (5 mmol) in toluene (30 ml) and refluxed for 24 h. After cooling, the formed silica nanoparticle supported imidazolium ionic liquid (SNImIL). The nanoparticles were then collected by centrifuging, repeatedly redispersed, washed with ethyl acetate, diethyl ether and water, and then dried under vacuum at 110 °C and isolated as SNImIL.

2.4. Aggregation study

The aggregation of thermally induced HCA II (0.1 mg/ml) was monitored at 360 nm at 60 °C for 10 min using a Cary Varian UV–vis spectrophotometer model 100 Bio. In order to reduce HCA II aggregation, 0.06 mg/ml of SNImIL was added and transferred to a 600 μl cuvette.

2.5. ANS fluorescence spectrophotometry

The ANS fluorescence of HCA II was recorded using Cary Eclipse Varian fluorescence spectrophotometry. A solution of HCA II (0.1 mg/ml in Tris–sulfate buffer at pH 7.75) in the presence of SNImIL (0.06 mg/ml) was prepared, transferred to a 0.4 ml cuvette and fluorescence intensity was measured in the presence of 5 mM ANS at λ excitation 350 nm at 58 °C with a 10 nm band width.

2.6. Calorimetric study

Calorimetric study was carried out by Nano-DSC differential scanning calorimeter (Setaram, France) equipped with a 0.348 ml cells. All DSC experiments were performed under 2 atm pressure. The concentration of SNImIL was 0.06 mg/ml and HCA II 1 mg/ml in 20 mM Tris–sulfate buffer (pH 7.75). The experiments were performed at a scan rate of 1 °C/min. To check the reversibility of denaturation, the sample was heated to 1–2 °C above the T_m, then cooled and reheated. If the original curve completely or partially reproduced, it is evident that denaturation is fully or partially reversible; otherwise it is an irreversible process. The extent of reversibility depends on a number of factors including the temperature to which the protein was heated during the first scan [68].

2.7. Docking studies

The 3D structure of HCA II, which was used for the docking studies as receptor, was obtained from Protein Data Bank (PDB) with PDB ID: 1CA2. The Discovery Studio Visualizer (version 2.5 Accelrys Software Int.) and AutoDock Tools (Version 1.5.6rc1) [69] were used to obtain the representative forms of the ligand and receptor structures, and visualization of docking results. The Kollman