International Journal of Biological Macromolecules 104 (2017) 89–96

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Salt-regulated reversible fibrillation of *Mycobacterium tuberculosis* isocitrate lyase: Concurrent restoration of structure and activity

Harish Shukla[a], Ranjeet Kumar[b], Amit Sonkar[a], Kalyan Mitra[b], Md. Sohail Akhtar[b,*], Timir Tripathi[a,*]

[a] *Molecular and Structural Biophysics Laboratory, Department of Biochemistry, North-Eastern Hill University, Shillong 793022, India*
[b] *Molecular and Structural Biology Division, CSIR-Central Drug Research Institute, Jankipuram Extension, Lucknow 226031, India*

ARTICLE INFO

Article history:
Received 14 May 2017
Received in revised form 30 May 2017
Accepted 2 June 2017
Available online 4 June 2017

Keywords:
Mycobacterium tuberculosis
Fibril
Fluorescence
Fluorescence anisotropy
Fibrillation

ABSTRACT

Protein fibrillation is associated with a number of neurodegenerative diseases. Nevertheless, several proteins not related to disease can also form fibrils *in vitro* under specific conditions. In the present study, we demonstrate the reversible fibrillation of a globular protein that is modulated by salt under physiological pH. *Mycobacterium tuberculosis* Isocitrate lyase (MtbICL) is a crucial enzyme involved in the glyoxylate shunt and a potential drug target against *M. tuberculosis* infection. Under physiological pH, the enzyme self-assembles into a fibrillar structure in the absence of salt *in vitro*. The mature fibrillar structure of MtbICL is dynamic and restores its tetrameric structure as well as activity with the addition of salt. The kinetics of fibril formation was investigated spectroscopically using 8-Anilinonaphthalene-1-sulfonic acid (ANS). Further, Transmission electron microscopy (TEM) and Atomic force microscopy (AFM) imaging also confirmed the formation of elongated fibrils in the absence of salt. The results indicate the balance between stabilizing forces and the localized electrostatic repulsions destabilizing the tetrameric MtbICL is adjusted via ion shielding. Our result is in congruence of the hypothesis that amyloid formation is an intrinsic property of most, if not all natural proteins under an appropriate set of conditions.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Fibril formation by proteins in neuronal cells is recognized as the molecular hallmark of several human neurodegenerative disorders [1–3]. Assembly of proteins into fibrils is a multistep process initiated by conformational changes, during which certain intermediate aggregation states such as oligomers, protofibrils, and filaments are sampled. Because of its relevance to mechanism of disease, the paths traversed during fibrillogenesis, the kinetics of the process, and the solution variables affecting the fibrillation are of considerable interest [4–12]. Structurally, the fibrils are made up of cross-β sheet structure in which the polypeptide chains form β-strands oriented perpendicular to their axis, resulting in β-sheets propagating in the direction of the fibril [13,14]. This general structure is conserved in most amyloid fibrils despite low sequence similarities among these proteins. The conformational rearrangements leading to the cross-β-structure cannot take place in a packed folded conformation and thus somewhat unfolded conformational state, in which some of the side chain amide and carboxyl groups and few hydrophobic moieties become accessible, trigger specific intermolecular interactions that are necessary for fibrillation [15,16]. The mature fibrils are generally inactive [17,18], though certain functionally active amyloids like RNase has been reported [19]. This strengthens the view that fibrillar structures could rather be in a selected protein fold as a functional protein structures [19,20]. These findings raises question that whether native-like structural domains undergo conformational modification or they simply refold upon fibril formation. Though several diseases are linked with protein aggregation and amyloid fibrillation, fibrillation *par se* is an intrinsic property of polypeptides as the formation of catalytic globular proteins comes at the expense of an inherent propensity of proteins to aggregate [5,14,21–24]. Several globular proteins can access aggregation-prone states from native-like conformations by crossing the energy barrier required for unfolding. Therefore, understanding the mechanism of self-assembly of proteins into fibrils and the biophysical basis of amyloid formation are

Abbreviations: MtbICL, *Mycobacterium tuberculosis* isocitrate lyase; SEC, size exclusion chromatography; ANS, 8-Anilinonaphthalene-1-sulfonic acid; ThT, Thioflavin T.

* Corresponding authors.
E-mail addresses: sohail@cdri.res.in (Md.S. Akhtar), timir.tripathi@gmail.com, ttripathi@nehu.ac.in (T. Tripathi).

http://dx.doi.org/10.1016/j.ijbiomac.2017.06.008
0141-8130/© 2017 Elsevier B.V. All rights reserved.

important in developing inhibitors that can preferentially bind to regions that harbour amyloidogenic tendencies [25–27].

Isocitrate lyase (ICL), one of the key enzymes of the glyoxylate shunt catalyses the first step in the conversion of isocitrate to succinate and glyoxylate [28]. This enzyme, in combination with malate synthase, catalyzes the conversion of isocitrate to malate, a reaction that allows maintenance of the TCA cycle and assimilation of carbon by gluconeogenesis during growth on C2 substrates. Beta-oxidation, gluconeogenesis and glyoxylate shunt are important for the survival of *Mycobacterium tuberculosis* inside the phagosomes of macrophages during persistence, which are glucose deficient, but fatty acid replete [29]. The glyoxylate shunt is absent in vertebrates, but are widespread among prokaryotes, lower eukaryotes and plants [30]. The *M. tuberculosis* ICL (MtbICL) is considered as one of the potential and attractive drug targets against persistent infection [31]. Structurally, MtbICL is a tetramer with each subunit consisting of two domains. Each monomer is further composed of a large core domain having eight α-helices and eight β-strands forming an unusual α/β barrel and a small β-domain containing active site regions that lies at top of the α/β core barrel [32]. The subunits are interlinked by the exchange of the C-terminal region. A Mg^{2+} ion binds to the α/β barrel-domain of the enzyme and is essential for the functional activity of the enzyme [33].

In the present study, we have examined the fibril forming properties of MtbICL and characterized the kinetics of fibril formation. The results indicate that salt increases the tetramer stabilization and reduces protein fibrillation *in vitro*. Also, we show the reversible nature of fibril formation with respect to both structure and activity.

2. Methods

2.1. Preparation of protein and fibril formation

The overexpression, purification and enzymatic assays of MtbICL were performed as described earlier [12,33–35]. Following purification, the protein was dialysed in 1 mM phosphate buffer pH 7.5 containing 20 mM NaCl. For fibrillation studies, MtbICL (10 mg/mL) was dialyzed in 1 mM phosphate buffer pH 7.5, with or without salt at 4 °C for 24 h. Aliquots were taken at desired time intervals to monitor the kinetics of fibril formation.

2.2. Size exclusion chromatography (SEC)

SEC experiments were carried out on a Superdex™ 200 HR 10/300 column (manufacturer's exclusion limit 600 kDa) with ÄKTA-FPLC (GE healthcare Biosciences, USA). The column was equilibrated with buffer with or without salt, before running the test samples. 500 μL samples were loaded on the column and run at 25 °C at a flow rate of 0.3 mL/min, and the protein was detected at 280 nm.

2.3. Circular dichroism (CD) spectroscopy

Far-UV CD measurements were made on JASCO J810 spectropolarimeter calibrated with ammonium (+)-10-camphorsulfonate. 3 μM MtbICL was used in 1 mM phosphate buffer, pH 7.5 with or without salt with a 1 mm path length cell at 25 °C. Spectra were collected at a scan speed of 20 nm/min, a response time of 1 s and a bandwidth of 2 nm. The spectra were averaged over five scans to eliminate signal noise. All scans were made in the far UV region between 200 and 250 nm [36,37].

2.4. Fluorescence measurements

Fluorescence spectra were recorded with a PerkinElmer Life Sciences LS55 B spectrofluorometer in 5 mm path length quartz cell at 25 °C. 3 μM of MtbICL was used in 1 mM phosphate buffer, pH 7.5 with or without salt for the studies. The samples were excited at 290 nm and the emission spectra were recorded in the wavelength range of 300 and 400 nm keeping the excitation and emission slits as 6 and 4 nm, respectively [38].

2.5. Thioflavin T (ThT) binding

Free ThT has excitation and emission maxima at 350 and 450 nm, respectively. However, upon binding to fibrils the excitation and emission λ_{max} changed to 450 and 480 nm, respectively. Fresh 1 mM stock solution of ThT was prepared in 1 mM phosphate buffer (pH 7.5) with or without salt. ThT was added to protein samples of both the conformational forms and excited at a wavelength of 450 nm, with emission measured at 482 nm.

2.6. 8-Anilinonaphthalene-1-sulfonic acid (ANS) binding

For ANS binding studies, excitation wavelength was 350 nm, and the emission spectra were recorded between 400 and 500 nm. The final concentration of ANS used for the experiments was 10 μM. 3 μM aliquots of the protein samples were added to a solution containing ANS in 1 mM phosphate buffer (pH 7.5) with or without salt and mixed for 2 min before measuring the fluorescence emission. Background absorption of the buffer for the native MtbICL was subtracted from each reading. All the readings were taken in triplicate.

2.7. Acrylamide quenching studies

Quenching of the intrinsic protein fluorescence was studied using acrylamide-based fluorescence quenching assays. To a fixed amount of protein (3 μM) pre-equilibrated with desired buffer, increasing amounts of acrylamide (0–1 M) was added and the samples were incubated for one hour at 25 °C before the measurements were made. The excitation wavelength used was 280 nm. The emitted light intensity was integrated over the period of 1 s and detected between 300 and 400 nm. The results were analysed by Stern–Volmer and modified Stern–Volmer plots using the following equations [39–41]:

$$F_0/F = 1 + K_{SV}[Q]$$

$$F_0/(F_0 - F) = 1/f_a K_c[Q] + 1/f_a$$

Where F_0 and F are the fluorescence intensities at aspecific wavelength in the absence and presence of quencher, respectively, K_{sv} is the Stern–Volmer constant, f_a is the fraction of the fluorophores accessible to the quencher, K_c is the collisional quenching constant, and Q is the concentration of the quencher.

2.8. Transmission electron microscopy (TEM)

Around 7 μL of protein sample was deposited on freshly glow discharged carbon coated copper grids and allowed to adsorb. Excess solution was blotted off using a filter paper and the deposit was negatively stained with 1% uranyl acetate (pH 4.2). The grids were air dried and observed under the TEM (FEI Tecnai Twin) at 80 kV after complete gun alignment and astigmatism correction. Images were acquired using a MegaView II CCD camera. Images were analyzed using analysis software.

Download English Version:

https://daneshyari.com/en/article/5511627

Download Persian Version:

https://daneshyari.com/article/5511627

Daneshyari.com