Biomaterials 77 (2016) 139–148

Contents lists available at ScienceDirect

Biomaterials

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

Nano-cage-mediated refolding of insulin by PEG-PE micelle

Xiaocui Fang [a, b, 1], Tao Yang [a, c, 1], Luoyang Wang [a, c], Jibing Yu [a], Xiuli Wei [a], Yinjian Zhou [a], Chen Wang [b, *], Wei Liang [a, **]

[a] Protein & Peptide Pharmaceutical Laboratory, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Beijing 100101, China
[b] CAS Key Lab for Biological Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology, No.11 ZhongGuanCun BeiYiTiao, Beijing 100190, China
[c] University of the Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100049, China

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form
5 November 2015
Accepted 6 November 2015
Available online 10 November 2015

Keywords:
Insulin
DTT
Refolding
PEG-PE micelle
Nano-cage
Negative charge layer

ABSTRACT

Insulin aggregation has pronounced pharmaceutical implications and biological importance. Deposition of insulin aggregates is associated with type II diabetes and instability of pharmaceutical formulations. We present in this study the renaturation effect of PEG-PE micelle on dithiothreitol (DTT)-denatured insulin revealed by techniques including turbidity assay, circular dichroism (CD), thioflavinT (ThT) binding assay, bis-ANS binding assay, agarose gel electrophoresis and MALDI-TOF MS. The obtained results show that PEG-PE micelle having a hydrophilic nano-cage-like structure in which with a negative charge layer, can capture DTT-induced insulin A and B chains, and block their hydrophobic interaction, thereby preventing aggregation. The reduced insulin A and B chain in the nano-cage are capable of recognizing each other and form the native insulin with yields of ~30% as measured by hypoglycemic activity analysis in mice. The observed insulin refolding assisted by PEG-PE micelle may be applicable to other proteins.

© 2015 Published by Elsevier Ltd.

1. Introduction

Deposition of amyloidogenic proteins is the main pathological reason in number of human diseases including type II diabetes, cancer, renal, cardiovascular and neurodegenerative diseases [1]. Until 2014 about 31 extracellular amyloidogenic proteins are known in human body, out of which two proteins are iatrogenic in nature (insulin and enfurvitide) and nine have been identified in animals [2]. Molecular chaperones (important molecular machines in vivo) have an ability to control undesired protein misfolding, maintain proteome homeostasis through ATP- and cofactor-regulated binding and releasing cycles, and provide a line of defense against amyloidogenic diseases, such as heat-shock proteins (HSPs), GroEL-GroES, clusterin and apolipoprotein E (apoE) [3–5]. These amyloidogenic proteins usually adopt a β-sheet-rich structure when transformed from soluble state to insoluble amyloid fibrils. Amyloid fibrils and its precursors can impair cell function by interacting with cell membrane, increasing free calcium ions and causing oxidative stress that eventually lead to cell death [1,6,7].

Literature survey revealed that amyloid formation is influenced by those factors that can affect the intermolecular interaction between fibrils. These factors include ionic strength, pH, temperature, hydrogen and disulfide bonding, among these disulfide bonding plays a critical role in fibril formation [8,9]. Disulfide bonds, that exist in 65% of secreted proteins and in 15% of the human proteome, play critical role in protein folding, stability, oligomerization, and amyloidogenecity [10,11]. Recent evidence indicates that formation of disulfide bonds is associated with more than 50% of amyloidogenic proteins, such as prion [11], lysozyme [12], superoxide dismutase 1 (SOD1) [13], insulin [14] and islet amyloid polypeptide (IAPP, amylin) [15].

Insulin is a small hormone crucial for glucose metabolism, contains one intrachain (A6-A11) and two interchain (A7-B7, A19-

Abbreviations: DTT, dithiothreitol; CD, circular dichroism; ThT, thioflavinT; HSPs, heat-shock proteins; apoE, apolipoprotein E; SOD1, superoxide dismutase 1; IAPP, islet amyloid polypeptide; PDI, protein disulphide-isomerase; PEG-PE, poly(-ethylene glycol)-phosphatidylethanolamine; CMC, critical micelle concentration; bis-ANS, 4,4′-Dianilino-1,1′-binaphthyl-5,5′-disulfonic acid dipotassium salt; FITC, fuorescein isothiocyanate; TRITC, tetraethyl rhodamine isothiocyanate; AFM, Atomic Force Microscopy; PEG, poly(ethylene glycol); SDS, surfactant sodium-dodecyl sulfate; ER, endoplasmic reticulum.

* Corresponding author.
** Corresponding author.
E-mail addresses: wangch@nanoctr.cn (C. Wang), weixx@sun5.ibp.ac.cn (W. Liang).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2015.11.007
0142-9612/© 2015 Published by Elsevier Ltd.

B20) disulfide bonds [16,17]. All these three disulfide bonds are critical for the structural stability of insulin [18,19]. Dithiothreitol (DTT)-induced reduction of disulfide bonds results in the dissociation of insulin into two separate chains, A chain and B chain. These unfolded chains expose their hydrophobic surfaces and bind together through hydrophobic interactions, and eventually form large, insoluble aggregates [18]. Thus, DTT-induced aggregation of insulin in vitro is a representative model aggregation assay for quantification of anti-aggregation activity of chaperones [19–21]. Insulin is the most important pharmaceutical peptide for diabetes treatment, but aggregation of insulin occurs readily in its biopharmaceutical process, which affect its bio-production, long-term storage, delivery and bioavailability [22]. Local insulin amyloid deposition can also take place in vivo near the site of repeated insulin injections in diabetes patients, exhibiting an increased immunogenicity and a reduced biologic activity [18,23]. In vitro studies have shown that protein disulphide-isomerase (PDI) [19,20], ethanol [24], metalloporphyrins [25,26], targeted peptides [27], α-crystallin [28], α-casein [29], and heparin [30] have inhibitory effects on insulin aggregation. Most of these additives are thought to act as artificial chaperones to prevent aggregation-prone species from sticking together during insulin refolding. Although various strategies have been developed to inhibit insulin aggregation and enhance its refolding yield, the efficiency of various additives is usually different from case to case and is condition-dependent, thereby limiting their general applicability. Thus, the development of a new approach is still a challenge in protecting insulin from aggregation and functional incapacitation.

Herein, we developed a strategy based on self-assembly method to prepare poly(ethylene glycol)-phosphatidylethanolamine (PEG-PE) micelle at critical micelle concentration (CMC) of 10 μM [31], which exhibits a 9 nm radius sphere with 2 nm radius of DSPE core, and the phosphorus atoms distribute within the interval of 2.5–3.5 nm radius and possess negative charges [32]. PEG-PE is an FDA-approved diblock copolymer with good biocompatibility and safety. Our previous studies have demonstrated that PEG-PE micelle provides rich structural information and physicochemical properties [33–35]. It is widely accepted that GroEL-GroES chaperonin system of *Escherichia coli* form a chaperonin nano-cage for a single protein molecule to fold in isolation [36–39]. A notable three-dimensional structure characteristic shared by GroEL and PEG-PE micelle is that they have a hydrophilic nano-cage with a negative charge layer. Based on the perspective that similar structural characteristics may share general effects, PEG-PE micelle, like GroEL-GroES, may assist non-native protein refolding and avoid protein aggregation. In this context, we present a detailed mechanistic study of PEG-PE micelle-assisted insulin refolding from DTT-denatured state. In order to clarify the refolding property that is applicable to the preformed protein aggregates, we studied two different scenarios: simultaneous effect of PEG-PE micelle and DTT over insulin aggregation and effect of PEG-PE micelle over DTT-induced insulin aggregates. This work is the first report about PEG-PE micelle being an artificial chaperone for protein renaturation.

2. Materials and methods

2.1. Materials

Lyophilized porcine insulin, PEG 2000-DSPE (PEG-PE) and PEG 2000-PLA 2000 (PEG-PLA) were purchased from Wan ban jing qiao Pharmaceuticals Limited Co. (Xuzhou, China), Avanti Polar Lipids and Bank Valley Co. (Suzhou, China), respectively. 4,4′-Dianilino-1,1′-binaphthyl-5,5'-disulfonic acid dipotassium salt (bis-ANS), sodium-dodecyl sulfate (SDS), PEG 2000, thioflavinT (ThT), DTT, fluorescein isothiocyanate (FITC), and tetraethyl rhodamine isothiocyanate (TRITC) were purchased from Sigma–Aldrich. Blood glucose monitoring system and Blood glucose test strip were purchased from Changsha Sinocare, Inc.

2.2. Animals

BALB/c male mice (6–8 weeks old) were provided by Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All animal procedures were performed following the protocol approved by the Institutional Animal Care and Use Committee at the Institute of Biophysics, University of Chinese Academy of Sciences.

2.3. Preparation of insulin aggregates

Insulin aggregates were prepared by adding DTT in insulin solution at 37 °Caccording to the reported literature [19–21]. PEG-PE, PEG 2000, SDS, and PEG-PLA were added in insulin solution in the presence of DTT in two ways. First way was simultaneous addition of each reagent and DTT into insulin solution at 37 °C while in second way each reagent was added after insulin aggregation in the presence of DTT at 37 °C.

2.4. Turbidity assay

Turbidity measurement of insulin at 360 nm is a general aggregation assay [40]. Samples were withdrawn at predefined time intervals in a 96-well micro plate and shaken for 30 s to evenly resuspend the samples.

2.5. ThT binding assay

Extent of insulin aggregation was determined using ThT, a fluorescent dye, which specifically binds with the β-sheet of amyloid structures [41]. Samples were shaken for 30 s prior to each measurement and relative fluorescence intensities were measured at an excitation wavelength of 450 nm and an emission of 482 nm.

2.6. Atomic force microscopy (AFM)

Morphology of the insulin aggregates on mica surfaces were characterized by AFM. AFM samples were applied to freshly cleaved mica and dried in flow of N_2. AFM experiments were performed under ambient condition using silicon cantilevers in tapping mode on a Nanoscope IIIA system (VEECO, USA).

2.7. Bis-ANS binding assay

Bis-ANS is naphthalene based fluorescent dye, which can bind with exposed hydrophobic clusters on the surface of non-native protein species. This binding leads to large change in fluorescence emission spectra [41,42]. Change in fluorescence intensity of bis-ANS fluorescence hydrophobic probe upon binding to hydrophobic residues of insulin was measured at excitation wavelength of 387 nm and emission was scanned between 400 and 600 nm on a F3 system (Thermo, USA).

2.8. Circular dichroism (CD) spectroscopy

Secondary structure of insulin was determined by CD spectroscopy, and samples were scanned at room temperature in a 1-mm path length fused quartz cuvette using a spectropolarimeter (Applied Photophysics Ltd, UK). Spectra were obtained from 200 to 260 nm at 1-nm bandwidth, 5-nm step and 1s response time averaged over 6 runs.

Download English Version:

https://daneshyari.com/en/article/6485244

Download Persian Version:

https://daneshyari.com/article/6485244

Daneshyari.com