Biochemical and Biophysical Research Communications 440 (2013) 56–61

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

The effect of exposing a critical hydrophobic patch on amyloidogenicity and fibril structure of insulin

Yang Li [a], Lianqi Huang [a], Xin Yang [a], Chen Wang [a], Yue Sun [b], Hao Gong [a], Yang Liu [a], Ling Zheng [b], Kun Huang [a,c,*]

[a] Tongji School of Pharmacy, Huazhong University of Science & Technology, Wuhan, Hubei 430030, PR China
[b] College of Life Sciences, Wuhan University, Wuhan, Hubei 430072, PR China
[c] Centre for Biomedicine Research, Wuhan Institute of Biotechnology, Wuhan, Hubei 430075, PR China

ARTICLE INFO

Article history:
Received 29 August 2013
Available online 13 September 2013

Keywords:
Porcine insulin
Desoctapeptide-(B23–B30) insulin (DOI)
Amyloidogenicity
Fibril structure
Membrane damage
Hemolysis

ABSTRACT

It is widely accepted that the formation of amyloid fibrils is one of the natural properties of proteins. The amyloid formation process is associated with a variety of factors, among which the hydrophobic residues play a critical role. In this study, insulin was used as a model to investigate the effect of exposing a critical hydrophobic patch on amyloidogenicity and fibril structure of insulin. Porcine insulin was digested with trypsin to obtain desoctapeptide-(B23–B30) insulin (DOI), whose hydrophilic C-terminal of B-chain was removed and hydrophobic core was exposed. The results showed that DOI, of which the ordered structure (predominantly α-helix) was markedly decreased, was more prone to aggregate than intact insulin. As to the secondary structure of amyloid fibrils, DOI fibrils were similar to insulin fibrils formed under acidic condition, whereas under neutral condition, insulin formed less polymerized aggregates by showing decreased β-sheet contents in fibrils. Further investigation on membrane damage and hemolysis showed that DOI fibrils induced significantly less membrane damage and less hemolysis of erythrocytes compared with those of insulin fibrils. In conclusion, exposing the hydrophobic core of insulin can induce the increase of amyloidogenicity and formation of higher-order polymerized fibrils, which is less toxic to membranes.

1. Introduction

The deposition of proteins has been found to be associated with over twenty diseases, such as Alzheimer's and Prion diseases [1,2]. To discover pathological mechanism and new therapeutic approaches of these diseases, a lot of work has been done to understand the progress of amyloid fibril formation and many intrinsic factors have been found to be related to amyloid fibril formation, including amino acid sequence, disulfide bond and so on [3,4]. Among these factors, the hydrophobic residues play an important role [5,6]. In aqueous solution, minimizing the number of hydrophobic side chains exposed to water is the principal driving force for hydrophobic interaction [7], which drives hydorphobic residues interacting with each other and leads to the formation of oligomers, protofibrils and amyloid fibrils [8]. On the other hand, the process of amyloid fibril formation can be inhibited by masking the hydrophobic residues [9].

Here, we chose insulin as a model to investigate the effect of exposing a critical hydrophobic patch on amyloid fibril formation of insulin. The native structure of insulin under physiological condition is mainly helical, with two chains linked by two inter-chain disulfide bonds. We have previously demonstrated that certain hydrophobic residues residing the hydrophobic core, like Val^{A3} and Val^{B12}, are essential for receptor binding and activity [10,11]. During amyloid formation, the initial step is related to the displacement of the B-chain C-terminal region that exposes the hydrophobic core [12]. Our previous study also showed that foreshortening the connecting peptide of proinsulin markedly retards the fibrillation [13], possibly by stabling conformation and avoiding exposure of hydrophobic residues [14].

However, the effect of exposing the critical hydrophobic core on amyloid formation remained unclear. In this study, a critical hydrophobic patch exposed insulin analog, desoctapeptide-(B23–30) insulin (DOI, Fig. 1A and B), was obtained by removing the C-terminal of B-chain; and this analog was used to investigate the effect of exposing the hydrophobic patch on amyloid formation

Abbreviations: DOI, desoctapeptide-(B23–B30) insulin; RP-HPLC, reversed phase high performance liquid chromatography; CD, circular dichroism; ThT, thioflavin-T; TEM, transmission electron-microscopy; POPG, 2-oleoyl-1-palmitoyl-sn-glycerol-3-phospho-rac(1-glycerol) sodium salt; POPC, 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine.

* Corresponding author at: Tongji School of Pharmacy, Huazhong University of Science & Technology, Wuhan, Hubei 430030, PR China.
E-mail address: kunhuang2008@hotmail.com (K. Huang).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.032

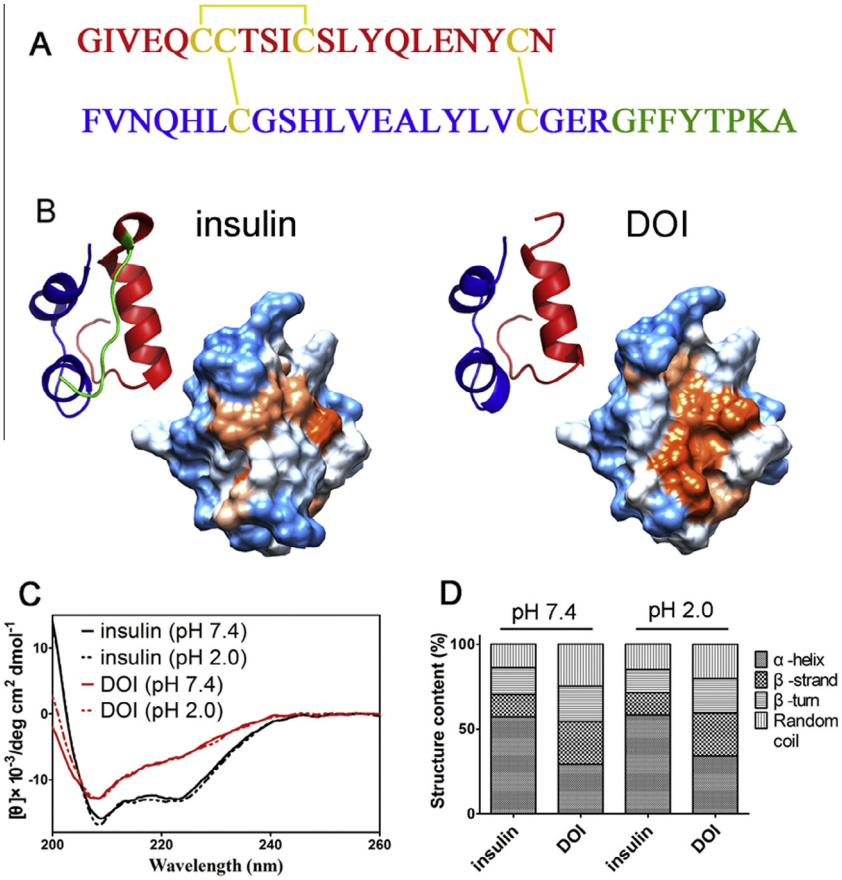

Fig. 1. (A) Primary structure of porcine insulin and DOI. (B) Surface model of porcine insulin and DOI. The hydrophilic residues are shown in blue and the hydrophobic residues are shown in orange (PDB accession number 2EFA). (C) Far-UV circular dichroism spectra of porcine insulin and DOI dissolved in phosphate-buffered saline (PBS, 50 mM phosphate, 100 mM NaCl, pH 7.4) and 0.01 N HCl (0.01 N HCl, 150 mM NaCl, pH 2.0). (D) Deconvolution results of CD spectra. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by comparing amyloidogenicity and fibril structure with insulin. A previous study has indicated that DOI forms amyloid more rapidly than intact insulin under neutral condition [12]. In this study, the amyloidogenecity of DOI was measured under both acidic and neutral conditions, and the secondary structures of fibrils were also investigated. Comparative toxicity of insulin and DOI fibrils were also investigated *via* dye leakage and hemolysis assays.

2. Materials and methods

2.1. Materials

Porcine insulin (PI) was obtained from Wanbang Biopharmaceuticals (Xuzhou, China). Trypsin, Thioflavin-T (ThT), 2-oleoyl-1-palmitoyl-sn-glycerol-3-phospho-rac(1-glycerol) sodium salt (POPG) and 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC) were purchased from Sigma–Aldrich (St. Louis, USA). Fresh blood was drawn from healthy volunteers using heparin as anticoagulant. All other chemicals were of the highest grade available.

2.2. Preparation of samples

The stock solution of trypsin (5 mg/mL) was prepared in 1 mM HCl and PI was dissolved in 50 mM PBS (pH 7.6) to make a final concentration (1.0 mg/mL) for digestion. Then trypsin was added into PI solution to reach a concentration of 40 μg/mL (molar ratio of PI to trypsin was 25:1). After incubated at 37 °C for 12 h, the solution was filtered through a 0.22 μm Millipore filter, and then injected into a Hitachi 2000 HPLC system (Tokyo, Japan). A Kromasil C8 semi-preparative reverse-phase column (NY, USA) was used for separation. Solvents for the mobile phase were water (A) and acetonitrile (B). The gradient elution was a linear gradient 25–40% B for 20 min. The peaks were detected at 280 nm and the molecular weights of each analyte were confirmed by matrix-assisted laser desorption ionization mass (MALDI-TOF).

2.3. Far-UV circular dichroism (CD) and data analysis

CD spectra were performed on JASCO-810 spectropolarimeter (JASCO, Tokyo, Japan) and the data were recorded at 25 °C under a constant flow of N_2 from 260 to 200 nm with a 1 mm pathlength. Porcine insulin and DOI were dissolved to a final concentration of 30 μM in two solutions: (i) phosphate-buffered saline (PBS, 50 mM phosphate, 100 mM NaCl, pH 7.4), (ii) 0.01 N HCl (0.01 N HCl, 150 mM NaCl, pH 2.0). The spectra were recorded at time intervals indicated, with a scanning speed of 50 nm/min, a response time of 1 s and a bandwidth of 2 nm. Each result was given as the average of three measurements. The data were converted to mean residue ellipticity [θ] and were further analyzed using the software package CDPro as described [15].

2.4. Amyloid formation and Thioflavin-T (ThT) fluorescence assays

Insulin and DOI were dissolved in dimethyl sulfoxide (DMSO) and sonicated for 2 min for homogenization. Proteins were made 60 μM in each of the following conditions as we previously