Biochemical Engineering Journal 86 (2014) 41–48

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Short communication

Thermal-aggregation suppression of proteins by a structured PEG analogue: Importance of denaturation temperature for effective aggregation suppression

Takahiro Muraoka, Nabanita Sadhukhan, Mihoko Ui, Shunichi Kawasaki, Enrikko Hazemi, Kota Adachi, Kazushi Kinbara *

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan

ARTICLE INFO

Article history:
Received 29 August 2013
Received in revised form 17 February 2014
Accepted 4 March 2014
Available online 12 March 2014

Keywords:
Poly(ethylene glycol)
Aggregation
Circular dichroism spectroscopy
High-tension voltage analysis
Protein denaturation
Refolding

ABSTRACT

Development of protein stabilizing reagents, that suppress aggregation and assist refolding, is an important issue in biochemical technology related with the synthesis and preservation of therapeutic or other functional proteins. In the precedent research, we have developed a structured poly(ethylene glycol) (PEG) analogue with triangular geometry, which turns into a dehydrated state above ca. 60 °C. Focusing on this rather lower dehydration temperature than that of conventional linear PEGs, a capability of the triangle-PEG to stabilize proteins under thermal stimuli was studied for citrate synthase, carbonic anhydrase, lysozyme and phospholipase. Variable temperature high-tension voltage and circular dichroism spectroscopic studies on the mixtures of these proteins and the triangle-PEG showed that the triangle-PEG stabilizes carbonic anhydrase, lysozyme and phospholipase that exhibit denaturation temperatures higher than 60 °C, while substantially no stabilization was observed for citrate synthase that denatures below 60 °C. Hence, the dehydrated triangle-PEG likely interacts with partially unfolded proteins through the hydrophobic interaction to suppress protein aggregation.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

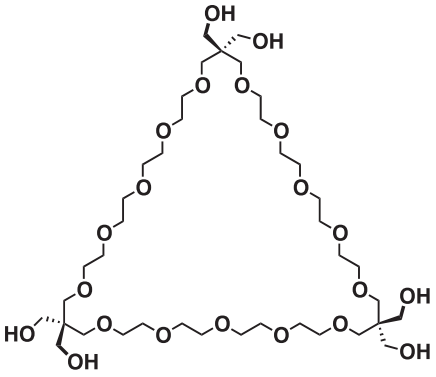
Functions of proteins are developed by folding of linear peptide chains into programmed ternary conformations. Since the folded conformation is constructed not only by covalent bonds such as disulfide bonds but also by non-covalent interactions including hydrogen bonding and hydrophobic interactions, protein higher-order structures are perturbed by a change of temperature, pressure and pH and contamination of a denaturation agent. Once the folded structure is collapsed, the hydrophobic amino acid residues are exposed to the aqueous environment and readily interact with other hydrophobic parts similarly appeared by unfolding. In consequence, the hydrophobic interaction leads to protein aggregations, which are inherently irreversible process, to give insoluble precipitates. Thus, development of protein stabilizing reagents to suppress aggregation and/or assist refolding is an important subject in biochemical technology. So far, stabilizing reagents, including water-soluble polymers, sugars, salts and amino acids, that suppress protein aggregation, have been developed [1–11]. Possible roles of these reagents in aggregation suppression are stabilization of the native conformation, destabilization of incorrectly folded conformations, and solubilization of correctly folded proteins or folding intermediates [1,12–14].

Recently we have developed a structured poly(ethylene glycol) (PEG) analogue with a triangle structure (triangle-PEG), which undergoes a thermo-responsive conformational change from *gauche* to *anti* forms at the ethylene oxide units in response to a temperature increment [15]. As is known for conventional PEGs, the polarity of the ethylene oxide units decreases by the *gauche*-to-*anti* conformational change due to the large difference in dipole moment between *gauche*- and *anti*-ethylene oxide units [16]. In fact, ^{1}H NMR study visualized that triangle-PEG is dehydrated at ca. 60 °C, at the temperature lower than that for conventional linear PEGs. This property is expected to give a driving force to interact with the exposed hydrophobic domains of an unfolded protein through hydrophobic interactions and prevent them from thermal aggregation. Actually, we found that triangle-PEG efficiently suppresses thermal aggregation of lysozyme [15], where a spectroscopic study revealed that triangle-PEG stabilizes the partially folded structure of lysozyme at high temperatures. In this article, we report the thermal stabilization capability of triangle-PEG for

* Corresponding author. Tel.: +81 22 217 5612; fax: +81 22 217 5612.
E-mail address: kinbara@tagen.tohoku.ac.jp (K. Kinbara).

http://dx.doi.org/10.1016/j.bej.2014.03.001
1369-703X/© 2014 Elsevier B.V. All rights reserved.

common proteins, citrate synthase, carbonic anhydrase, lysozyme and phospholipase based on HT voltage analysis and circular dichroism (CD) spectroscopy with a comparison of the effect of a linear PEG. We found that triangle-PEG has a stabilization effect for the proteins that denature at temperatures higher than the dehydration temperature of triangle-PEG.



Triangle-PEG

2. Materials and methods

2.1. Materials

Triangle-PEG was obtained according to the procedure described in our previous literature [15]. Citrate synthase from porcine heart, lysozyme from chicken egg white, and phospholipase A2 from honeybee venom were purchased from Sigma–Aldrich (St. Louis, MO, USA). Carbonic anhydrase II from bovine erythrocytes was purchased from SERVA Electrophoresis (Heidelberg, Germany). PEG-1000 was purchased from Tokyo Chemical Industry (Tokyo, Japan). These proteins and reagents were used without further purification.

2.2. Methods

CD spectra and high-tension voltage data were recorded on JASCO J-820 spectropolarimeter attached with a temperature controller, where a screw-capped 1-mm thick quartz cell was used for the measurements. The sample was annealed for 10 min at a specified temperature before starting the measurement.

3. Results and discussion

The aggregation suppression capability of triangle-PEG was studied for four proteins, citrate synthase from porcine heart (CS, 85.0 kDa, T_m = 53 °C [17]), carbonic anhydrase II from bovine erythrocytes (CAB, 29.0 kDa, T_m = 64 °C [18]), lysozyme from chicken egg white (14.3 kDa, T_m = 74 °C [19]; re-investigated for the present study [15]), and phospholipase A2 from honey bee venom (PLA, 14.5 kDa, T_m > 90 °C). These proteins were dissolved in pH 7.2–8.0 buffers, where they can exhibit intrinsic enzymatic activity. The unfolding and aggregation formation of the proteins were monitored by CD spectroscopy and high-tension (HT) voltage analysis, respectively.

3.1. HT voltage analysis on protein aggregation upon heating

Changes in HT voltage are known to arise from the change in the light scattering, due to the changes in the particle size and/or refractive index. Namely, the aggregation of the thermally unfolded or misfolded protein molecules is considered to cause the increase in the HT voltage [20]. Thus, HT voltage change of the aqueous buffer solution of proteins (0.035 mM) at 280 nm upon elevation of the temperature was investigated in the absence or presence (28 mM) of triangle-PEG (Fig. 1) to monitor the effect of triangle-PEG in the thermal aggregation events.

At first, CS, in the absence of triangle-PEG (red filled circles in Fig. 1a), showed a constant HT voltage from 20 to 50 °C. However, at 60 °C, the HT voltage sharply increased, indicating the progress of thermal aggregation of CS. Further heating up to 70 °C resulted in decrease in the HT voltage, which is likely due to the precipitation of CS aggregates to give a virtually transparent supernatant solution. As for the mixture of CS and triangle-PEG, a sharp enhancement of the HT voltage was also observed at 60 °C (blue filled circles in Fig. 1a), accompanied by the formation of precipitates. Obviously, triangle-PEG hardly stabilizes CS against the thermal stimuli.

CAB (red filled circles in Fig. 1b) also showed a constant HT voltage from 20 to 50 °C followed by a gradual increment at 60 and 70 °C. Above 80 °C, the HT voltage decreased similarly to CS. In addition, after cooling from 90 to 20 °C, substantially no recovery of the HT voltage was observed. On the other hand, the mixture of CAB and triangle-PEG showed a slightly different profile (blue filled circles in Fig. 1b), where no increment of the HT voltage was observed up to 60 °C. From 60 to 70 °C, the HT voltage increased sharply, followed by a sharp decrease from 70 to 80 °C. In this case, triangle-PEG likely protects CAB from aggregation to some extent, at the initial stage of thermal denaturation occurring between 50 and 60 °C.

In sharp contrast to the cases of CAB and CS, no HT voltage increment was observed during the heating and the subsequent cooling process in the case of lysozyme and triangle-PEG mixture (blue filled circles in Fig. 1c), although lysozyme alone shows HT voltage increment between 80 and 90 °C due to the aggregation (red filled circles in Fig. 1c). Thus, as is reported in our previous paper [15], HT voltage analyses also displayed that triangle-PEG effectively prevents lysozyme from thermal aggregation.

In the case of PLA, which is intrinsically stable upon thermal stimuli, no significant increase of the HT voltage was observed during the heating up to 98 °C, both in the absence and presence of triangle-PEG (red and blue filled circles in Fig. 1d, respectively).

In order to demonstrate the importance of the geometry of triangle-PEG, we investigated the protein-stabilization capability of the corresponding linear PEG. PEG-1000 (M_w = 993), which has a similar molecular weight with triangle-PEG (M_w = 883), was chosen as a reference. The HT voltage of CS and PEG-1000 (28 mM) mixture remained almost constant from 20 to 50 °C followed by a sharp increment within 50–60 °C (orange filled circles in Fig. 1a), which is similar to the change of CS in the absence of any additives. CAB and PEG-1000 mixture also showed a similar HT voltage change to CAB alone, but larger increment of HT voltage was observed from 50 to 60 °C in the presence PEG-1000 (orange filled circles in Fig. 1b). In the case of lysozyme and PEG-1000 mixture, HT voltage increased from 70 to 80 °C, even though lysozyme alone showed no HT voltage increase up to 80 °C (orange filled circles in Fig. 1c). PLA and PEG-1000 mixture displayed quite similar HT voltage change to PLA alone (orange filled circles in Fig. 1d). These results suggest that PEG-1000 hardly shows protein aggregation suppression effect, or rather it causes destabilization of proteins, as observed for CAB and lysozyme. Hence, it is clearly demonstrated that triangle-PEG and PEG-1000 show contrasting effects with respect to the suppression of protein aggregation.

It should be noted here that, in the HT voltage analysis, all the aqueous buffer solution of the mixtures of protein and triangle-PEG showed higher values than the corresponding aqueous buffer solution of protein without triangle-PEG. This is likely due to the larger refractive index caused by the presence of triangle-PEG, since aqueous solutions of triangle-PEG showed higher refractive index values than water. The HT voltage of the mixture of lysozyme and triangle-PEG was lower than the ones of other proteins in the

Download English Version:

https://daneshyari.com/en/article/3180

Download Persian Version:

https://daneshyari.com/article/3180

Daneshyari.com