Journal of Chromatography A, 1511 (2017) 25–36

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Full length article

Molecular interaction mechanisms in reverse micellar extraction of microbial transglutaminase

Tingting Yu, Mingxiang Lin, Junfen Wan*, Xuejun Cao*

State Key Laboratory of Bioreactor Engineering, Department of Bioengineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

ARTICLE INFO

Article history:
Received 21 March 2017
Received in revised form 23 June 2017
Accepted 3 July 2017
Available online 5 July 2017

Keywords:
Reverse micellar extraction
Microbial transglutaminase
Dissipative particle dynamics
Molecular interactions
Size exclusion

ABSTRACT

Reverse micellar extraction is an efficient and economical alternative for protein purification. In this study, microbial transglutaminase (MTGase) from crude materials was purified using reverse micellar extraction, and the molecular interaction mechanism in reverse micellar extraction of MTGase was explored. By using a molecular simulation study, the interaction mechanism of forward extraction was investigated. The molecular simulation results reveal the interaction of MTGase-water-surfactant is the major driving force for the forward extraction. Further, the effect of ionic strength on molecular interactions in backward extraction was investigated using 1H low-field nuclear magnetic resonance (LF-NMR) and circular dichroism (CD) spectra. In backward extraction, the interactions between water and the other two molecules (MTGase and surfactant molecules) are enhanced while the interactions between target molecules (MTGase) and the other two molecules (water and surfactant molecules) are weakened as the ionic strength increases. Moreover, the effect of size exclusion on backward extraction was also investigated. The results demonstrate size exclusion has limit effect at high ionic strength, and the weakened interaction of MTGase-water-surfactant is the main reason causing the release of the target molecules in backward extraction. This work might provide valuable reference to the MTGase purification and downstream processing.

1. Introduction

Reverse micelles (RMs) are self-assembly aggregates formed by surfactants in an organic solvent [1]. The nanometer-sized water pools formed in the polar cores of RMs can host various hydrophilic solutes, and provide a mild medium for bioseparations [2]. A typical reverse micellar extraction includes two steps: forward and backward extraction [3]. Highly efficient purification of the target molecules using reverse micellar extraction can be achieved by varying parameters (such as pH, ionic strength and the concentration of the surfactant) in both the organic phase and the aqueous phase [4,5]. Therefore, reverse micellar extraction has a high potential for downstream processing.

Cetyltrimethyl ammonium bromide (CTAB) is a cationic surfactant and has been widely employed in reverse micellar extraction of proteins such as bromelain [6], tannase [7]. Meanwhile MTGase isolated from microbiological sources is one group of transglutaminase (EC 2.3.2.13) [8]. MTGase exhibits wide substrate specificity over a wide range of temperature and pH values and has been widely used from food to pharmaceutical industry in recent years [9]. The production and purification of MTGase has received extensive attention [10]. To our knowledge, there are few published reports on reverse micellar extraction and purification of MTGase. In this study, MTGase from crude materials was purified by reverse micellar extraction using a CTAB/octane/*n*-hexanol system.

It is well known that the major factor determining protein solubilisation in reverse micellar systems are the aggregation properties of the surfactant and the electrostatic interactions among the solutes and the charged surfactant heads [11,12]. The solubilisation process can therefore be controlled by the pH, ionic strength and type of ions present in the aqueous protein solution [13]. For a reverse micellar system with definite surfactant, the interactions between reverse micelles and proteins control the forward extraction of proteins [14]. The charge interactions between the protein and the surfactant molecules produce a significant deformation of the interface leading to protein envelopment by the interface [15]. Those papers focused on the study of electrostatic interactions in the forward extraction. However studies on the molecular interaction mechanism in the backward extraction are rarely reported. Moreover, the detailed information about the influence of the pH and ionic strength on the molecular interaction

* Corresponding authors.
E-mail addresses: wanjunfen@ecust.edu.cn (J. Wan), caoxj@ecust.edu.cn (X. Cao).

http://dx.doi.org/10.1016/j.chroma.2017.07.006
0021-9673/

mechanism is still not clear. What's more, the molecular interaction mechanism in reverse micellar extraction is the key issues in the application of reverse micellar extraction in industry. For reverse micellar extraction in purification of proteins, the molecular interaction mechanisms in reverse micellar extraction need to be thoroughly studied.

In forward extraction of MTGase, it is impossible to study the molecular interactions during the self-assembly process of reverse micelles in detail experimentally. Dissipative particle dynamics simulations (DPD) can indicate the entire process of self-assembly in micelles conformation [16,17], and have been used in predicting the reverse micellar extraction of papain [18]. Therefore, the DPD simulations are considered as powerful tools that could provide evidences to the effect of the molecular interaction during the formation of reverse micelles in the forward extraction. So DPD simulations were used to simulate the self-assembly process and investigate the molecular interaction mechanism in the forward extraction of MTGase.

In backward extraction, the effect of ionic strength on molecular interaction was investigated extensively. The mobility of water molecules in RMs has a close relationship to interactions between water and other two molecules (CTAB and MTGase molecules). So to investigate the change of interactions between water and other two molecules (CTAB and MTGase molecules), the mobility of water in RMs after backward extraction was measured using 1H low-field nuclear magnetic resonance (LF-NMR). Moreover, it has been reported that size exclusion can make the release of the target molecules when the size of RMs is reduced [19]. And the size of RMs usually reduces when the water content decreases, otherwise the opposite [20]. Therefore the water content of the reverse micellar solution was measured by Karl Fischer method. The conformational change of the target molecules can be caused by the electrostatic interactions between the target molecules and surfactant molecules [14]. So the conformational change of the target molecules can reflect the change of interactions between the target molecules and surfactant molecules. Therefore the secondary structures of MTGase in aqueous phase after backward extraction were measured using circular dichroism (CD) spectra. In order to investigate the effect of size exclusion on backward extraction, the size of RMs was measured by dynamic light scattering (DLS). Moreover transmission electron microscopy (TEM) measurements were used as verifications and supplements of the DLS method.

The objective of this study is to thoroughly study the molecular interaction mechanisms in reverse micellar extraction of MTGase. Meanwhile, an optimization methodology of reverse micellar extraction with MTGase from crude materials is proposed. The reported results might provide valuable reference for downstream processing.

2. Experimental

2.1. Materials

Crude MTGase and commercial MTGase (MW = 38 kDa, pI = 7.3) both from *Streptomyces Hygrodcpicus* were purchased from Yixing Company (Shanghai, China). *N*-carboxybenzoyl-L-glutaminylglycine (N-CBZ-Gln-Gly) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., Ltd. D_2O, Karl-Fischer reagent (without pyridine) and other chemicals were from Guoyao Com. (Shanghai, China). All chemicals were of analytical grade.

2.2. Reverse micellar extraction

The organic phase was prepared by adding 0.728 g CTAB (MW = 364) to 100 mL organic solution (the volume ratio of hexanol and octane was 1:5). The enzyme solution was prepared by adding 40 mg crude materials of MTGase in 10 mL sodium phosphate buffer solution (pH 9.0, 10 mM), and dissolved by magnetic stirrer for 30 min (100 rpm), then the supernatant collected by centrifuge was used as aqueous phase of forward extraction. At last 0.04 M urea was added in the enzyme solution. The stripping solution of backward extraction was prepared with dissolving 5.59 g KBr (MW = 119) in 100 mL sodium phosphate buffer solution (pH = 5.0, 10 mM).

Forward extraction: 1.0 mL of the aqueous phase, 2.375 mL of the organic phase and 0.125 mL of ethanol were mixed for 3 min, and settled for 4 h to achieve phase separation [20]. After phase separation, the activity of MTGase and protein concentration in the aqueous phase was measured. Forward extraction recovery was calculated according to Eq. (1). The upper phase containing MTGase was transferred into another tube for backward extraction.

Backward extraction: 1 mL of the stripping solution, 2.25 mL of the upper phase containing MTGase and 0.25 mL of ethanol were mixed for 3 min, and settled for 2 h to achieve phase separation [20]. After phase separation, the activity of MTGase and protein concentration in the stripping solution was measured. Backward extraction recovery was calculated according to Eq. (2), total activity recovery was calculated according to Eq. (3) and the purification factor (F) was calculated according to Eq. (4).

Reverse micellar solutions after backward extraction with different concentration of KBr (0–1.0 M) in the stripping solution were detected by CD spectra, LF-NMR and DLS. Samples of reverse micellar solutions contained D_2O (replaced H_2O) and commercial MTGase (replaced crude materials of MTGase) were prepared in the same way with the reverse micellar solutions after backward extraction respectively.

The activity of MTGase in the aqueous phase was measured with the colorimetric hydroxamate procedure using N-CBZ-Gln-Gly [21]. The protein content in the aqueous phase was measured by the Bradford method using BSA [22]. Sodium dodecyl sulphate (SDS)-polyacryl amide gel electrophoresis (PAGE) was performed using an electrophoresis apparatus (VE-180, Tanon, Shanghai, China) with a 5.0% (w/v) stacking gel and 12.0% (w/v) separation gel [20].

2.3. Calculation

The forward extraction recovery (E_f), backward extraction recovery (E_b), total activity recovery (R) and purification factor (F) were calculated using the equations given below:

The recovery of forward extraction (E_f):

$$E_f(\%) = \frac{U_{rm,f} \times V_{rm,f}}{U_{aq,0} \times V_{aq,0}} \times 100 \quad (1)$$

The recovery of backward extraction (E_b):

$$E_b(\%) = \frac{U_{aq,b} \times V_{aq,b}}{U_{rm,f} \times V_{rm,f}} \times 100 \quad (2)$$

Total activity recovery (R):

$$R(\%) = \frac{U_{aq,b} \times V_{aq,b}}{U_{aq,0} \times V_{aq,0}} \times 100 \quad (3)$$

Purification factor (F):

$$F = \frac{S_{aq,b}}{S_{aq,0}} \quad (4)$$

V is the volume of the solution (mL), U is the activity of MTGase ($U\,mL^{-1}$), S is the specific activity of MTGase ($U\,mg^{-1}$); aq is the aqueous phase, rm is the organic phase, 0 is the initial phase, f is forward extraction, and b is backward extraction.