Protein Expression and Purification 120 (2016) 72–86

Contents lists available at ScienceDirect

Protein Expression and Purification

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

Intermolecular interactions at early stage of protein/detergent particle association induced by salt/polyethylene glycol mixtures

Takayuki Odahara [a, *], Koji Odahara [b]

[a] National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central–6, 1–1 Higashi, Tsukuba, Ibaraki, 305–8566, Japan
[b] Fukuoka Agriculture and Forestry Research Center, Chikusino, Fukuoka, 818–8549, Japan

ARTICLE INFO

Article history:
Received 5 November 2015
Received in revised form
7 December 2015
Accepted 11 December 2015
Available online 17 December 2015

Keywords:
Detergent
Integral membrane protein
Polyethylene glycol
Precipitation curve
Salt

ABSTRACT

Mixtures of neutral salts and polyethylene glycol are used for various purposes in biological studies. Although the effects of each component of the mixtures are theoretically well investigated, comprehension of their integrated effects remains insufficient. In this work, their roles and effects as a precipitant were clarified by studying dependence of precipitation curves on salt concentration for integral membrane protein/detergent particles of different physicochemical properties. The dependence of precipitation curves was reasonably related to intermolecular interactions among relevant molecules such as protein, detergent and polyethylene glycol by considering their physicochemical properties. The obtained relationships are useful as basic information to learn the early stage of biological macromolecular associations.

1. Introduction

Mixtures of salts and polyethylene glycol (PEG) are widely used as a precipitant in biological studies, for instance, for the purpose of fractionation [1–3], concentration, and crystallization [4–6] of macromolecules such as proteins, and as a substance to produce an environment similar to physiological fluid [7–9]. The salts are known to exert two main effects in an aqueous solution. The first effect, by ions dissociated from the salts, is to screen electric fields from charges and dipoles on macromolecular surfaces [10,11]. The second effect is to increase surface tension of water to decrease the whole interfacial area between hydrophobic parts of macromolecular surface and an aqueous solvent [10–12].

PEG is thought to exert two effects as a driving force of macromolecular aggregation. First, PEG reduces the relative dielectric constant of an aqueous solution, which increases electrostatic free energy of the macromolecular surface based on the electric charges and dipoles. This effect strengthens not only intermacromolecular electrostatic interactions but also a tendency that PEG and macromolecules mutually exclude from their surface region [3,13–18]. Second, when PEG added into a macromolecular solution exceeds a certain concentration, PEG molecules tend to leave an intermacromolecular space for bulk solvent so as to increase their own motional freedom (translational entropy). Then, an attractive force is generated to get the macromolecules closer below the diameter of a PEG molecule [19,20], and an osmotic pressure by PEG, higher in the bulk than in the intermacromolecular space, operates on the macromolecular association region as an external force [19,20]. Thus, the effects of each component of salt/PEG mixtures are theoretically well analyzed and modeled [3,13–20].

However, appropriate use of the mixtures depending on an individual purpose necessitates further comprehension about roles, integrated effects and influence of salts and PEG on intermolecular interactions contributing to the macromolecular association. It is thought that these intermolecular interactions are reflected by precipitation curves of the macromolecules [18]. Integral membrane proteins, especially pigment–containing proteins from photosynthetic bacteria, are excellent targets for studying intermacromolecular interactions, for the following reasons. Different

Abbreviations: BisTris, Bis(2–hydroxyethyl) iminotris–(hydroxymethyl) methane; $C_{12}E_8$, *N*–dodecyl–octaoxyethylene; LDAO, *N,N*–dimethyldodecylamine–*N*–oxide; LM, *N*–dodecyl–β–D–maltopyranoside; MEGA9, nonanoyl–*N*–methylglucamide; NaN_3, sodium azide; NTM, *N*–nonyl–β–*D*–thiomaltoside; OG, *N*–octyl–β–*D*–glucoside; OTG, *N*–octyl–β–*D*–thioglucoside; SML, β–D–fructopyranosyl–α–D–glucopyranoside monododecanoate; TX100, *T*–octylphenoxypolethoxyethanol; Tris, Tris(hydroxymethyl)aminomethane hydrochloride.

* Corresponding author.
E-mail address: odahara-takayuki@aist.go.jp (T. Odahara).

http://dx.doi.org/10.1016/j.pep.2015.12.009
1046-5928/

detergents alter the property of a protein/detergent particle [21], that is, the interactions among relevant molecules such as protein, detergent and PEG. Additionally, absorption spectra of the pigment–containing proteins reflect not only the protein concentration but also the protein structures [21–24]. These features of the proteins enable us to simultaneously measure the supernatant protein concentration and the protein stability [21]. To study the intermacromolecular interactions induced by salt/PEG mixtures, dependence of a precipitation curve on salt concentration was investigated for several protein/detergent particles with different properties. On the basis of the result, we clarified the roles of the mixtures in the particle association on the levels of functional groups.

2. Materials and methods

2.1. Preparation of protein/detergent complexes

Rhodobacter (*Rb.*) *capsulatus* light–harvesting protein–pigment complex 2 (LH2), *Rb sphaeroides* LH2 and photoreaction center (RC), *Rhodopseudomonas* (*Rp.*) *viridis* RC, and RC with light–harvesting protein-pigment complex 1 (PRU) were purified according to the published methods [21,25–30]. The final protein solutions were prepared according to the reported methods [21].

2.2. Protein properties

The Stokes radii of the five proteins in *N*–dodecyl–β–D–maltopyranoside (LM) micelle were evaluated from the retention times on HiLoad 26/60 Superdex™ 200 prep grade (Amersham Biosciences) molecular sieve chromatography and/or from the measurement of scattered light of 830 nm wavelength with a DynaPro molecular sizing instrument (Protein Solution Inc.).

The experimental isoelectric points (pI) were determined for the proteins in LM micelle using isoelectric focusing (5% (wt./vol.)) acrylamide, 1.3% (wt./vol.) *N*,*N*′–methylene–bisacrylamide, 1.5% (wt./vol.) ammonium persulfate, 0.005% (wt./vol.) *N*,*N*,*N*′,*N*′–tetra–methylene–ethylenediamine, 10%(wt./vol.) SERVALYT@3–10 (Feinbiochemica GmbH&Co.) and (5 mg/mL LM) and anion exchange chromatography (DEAE Sepharose™ (Amersham Biosciences); 30 mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris)–HCl (pH 8.5–7.0), 30 mM Bis(2–hydroxyethyl) imino-tris–(hydroxymethyl) methane (BisTris)–HCl (pH 7.0–5.0) and 30 mM glycine–HCl (pH 4.0–2.0)). The theoretical pI values were calculated from the amino-acid sequences [31–37].

The sizes of the extramembranous protein regions were compared on the basis of their electron density maps or tertiary structures [38–44]. With *Rb. sphaeroides* and *Rb. capsulatus* LH2s whose tertiary structures have not been experimentally determined, the extramembranous sizes were evaluated from the amino acid sequences [36,37] and the structural models [45]. These protein properties are listed in Table 1. Besides, the ratio of micelle surface area to particle surface area was evaluated from the structures [38–44] and structural models [45]; in the increasing order, *Rp. viridis* PRU (55%) < *Rp. viridis* RC (65%) < *Rb. sphaeroides* RC (70%) < *Rb. capsulatus* LH2 = *Rb. sphaeroides* LH2 (85%).

2.3. Determination of protein concentrations in supernatant

Concentrated solutions of protein (50 mg/mL), salt (1–8 M), PEG 4000 (625 mg/mL), buffer (0.5 M), and detergent (200 mg/mL) were put into a small test tube (0.5 mL), and were vigorously mixed on a vortex mixer. The total protein concentration was adjusted to be 20 mg/mL in all the final sample solutions of 0.05 mL. For efficient pipetting of viscous protein and PEG solutions, micromans (GILSON) were used. After amorphous precipitates of the protein/detergent particles were removed from the mixed solutions by centrifugation at 12,000 rpm (Hitachi T15AP21) for 6 min, the protein concentration in the supernatant was photometrically evaluated from the absorbance characteristics of the relevant protein. Those operations were done at 21–23 °C. It was difficult to determine precise concentrations of protein/detergent particles due to the adhering detergents, but they were thought to be directly proportional to the concentrations of corresponding proteins. Hence, we used the protein concentration instead of the concentration of protein/detergent particles. The concentrations of *Rb. sphaeroides* LH2, *Rb. capsulatus* LH2, *Rb. sphaeroides* RC, *Rp. viridis* RC, and *Rp. viridis* PRU were based on the relationship that one absorbance unit at 800, 830, 800, 800, and 1020 nm corresponds to 0.33, 0.45, 0.083, 0.083, and 0.086 mg/mL of these proteins, respectively [21]. To compare precisely the effect on particle–particle and particle–PEG interactions of each factor such as types and concentrations of salts, and properties of detergent and protein, the same solutions of the proteins and chemicals were employed in each of the following experiments.

3. Results and discussion

The aim of this study is to comprehend roles of salt/polyethylene glycol (PEG) mixtures as a precipitant and to obtain relationships for practical use. The content of Results and Discussion is constituted of eight sections as follows. In Section 3.1, a precipitation curve (PC), that reflects intermolecular interactions contributing to the early stage of assembly of proteins (protein/detergent particles, in this case) in the native states, is defined on the basis of kinetics and reproducibility of protein concentration in a supernatant. In Section 3.2, it is shown that electrical screening effectiveness of various salts reflects the charge distribution and hydration of ions. In Section 3.3, salt concentrations required to electrostatically balance particles are related to the charge distribution on the particle surface. In Section 3.4, the roles and influence of entropically unfavorable polar–nonpolar contact between polar species and PEG molecules in particle assembly are deduced from peculiar variation of PC at high concentrations of multivalent anions. In Sections 3.5 and 3.6, contributions of short–range particle–particle and particle–PEG interactions to particle association are evaluated by comparing PCs reflecting these interactions with the properties of various particles. In Section 3.7, other effects of salts and PEG are evaluated from the horizontal positions of PCs: contributions of interparticle attractive interactions induced by augmentation in motional freedom of PEG molecule to particle aggregation, effects of divalent cations to strengthen attractive interaction among particles within a precipitate, and roles of interfacial tension of interparticle solvent within the particle association region depending on ionic species. In Section 3.8, examples about appropriate usage of salt/PEG mixtures depending on individual purposes are shown.

3.1. Kinetics and reproducibility of PCs

First, kinetics and reproducibility of precipitation curves (PCs) were examined for several protein/detergent particles because PCs are based on protein concentration in a non–equilibrium supernatant. Protein concentration was measured for supernatant at several time points after the addition of NaCl/PEG mixture, by which the presence of PCs of the five proteins was confirmed in the previous study [21]. All the protein/detergent particles exhibited an approximately linear relationship between the logarithm of supernatant protein concentration $[D]_{ppt}$ and PEG concentration [P] at