Journal of Chromatography A, xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Short communication

Assessment study of ion-exchange chromatography combined with solution X-ray scattering measurement for protein characterization

Yasushi Watanabe

Food Research Institute, National Agriculture and Food Research Organization, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

ARTICLE INFO

Article history:
Received 31 October 2017
Received in revised form 19 January 2018
Accepted 21 January 2018
Available online xxx

Keywords:
Ion-exchange chromatography
Small-angle X-ray scattering
Real-time X-ray transmittance monitor
Protein solution property

ABSTRACT

The performance of ion-exchange chromatography combined with small-angle X-ray scattering measurement was evaluated by characterization of the hen egg white lysozyme as a model protein. The X-ray transmittance was estimated using a micro-ionization chamber equipped with a sample cell holder for the real-time monitoring of the X-ray beam strength through the salt gradient elution. The radius of gyration of the eluted protein was estimated to be 1.50 ± 0.06 (n = 3) nm and 1.4 ± 0.1 nm as the value at the zero protein concentration. By using the X-ray transmittance values for the scattering intensity correction, the molecular weight of the eluted protein was estimated to be $15{,}200 \pm 500$ (n = 3) and $14{,}400 \pm 200$ as the value at the zero protein concentration. These values are close to those of the monomer of this protein. The ion-exchange chromatography combined with the small-angle X-ray scattering measurement system equipped with the X-ray transmittance monitor is a reliable method for protein characterization in solution.

1. Introduction

Size-exclusion chromatography combined with small-angle X-ray scattering (SEC-SAXS) is a reliable method for the determination of the radius of gyration (*Rg*) value, molecular weight and structural properties of biopolymers with dimensions of 1–10 nm [1–6]. The SEC-SAXS has been investigated through the structural characterization based on the sizes [2–6], molecular weights and molecular structures of several proteins [1,3–6] and polysaccharide [5]. The SEC-SAXS is usually applied for biopolymer characterization in solution at the synchrotron stations [1–6]. The field-flow fractionation coupled with SAXS has also been proposed for the detection of maghemite nanoparticles [7]. These studies showed the advantage of the combination of the separation methods and SAXS measurement although the measuring limit depends on the SAXS instrumentation.

On the other hand, an alternative chromatography mode is ion-exchange chromatography (IC). Investigations dealing with the IC combined with SAXS (IC-SAXS) are extremely limited. In a recent report about IC-SAXS [8], the background subtraction for continuous gradients by shifting a reference measurement was shown for characterization of proteins such as bovine serum albumin, while the molecular weight was estimated using the higher angle data by the Porod volume approach in which the used parameters are both contrast and concentration independent. Salt gradients are the commonest means of eluting proteins from ion-exchangers, except for the isocratic elution. Either potassium or sodium chloride is usually used to generate the gradient. In a previous study [9], we showed that the real-time monitoring of the X-ray transmittance using a micro-ionization chamber for the static SAXS measurements was useful for the characterization of proteins at various salt concentrations. In the present study, therefore, since the X-ray transmittance depends on the salt concentration in the eluent [9], the assessment study of the IC-SAXS method equipped with the X-ray transmittance monitor was carried out. Furthermore, since the scattering data were scattered in the higher-angle regions, the volume approach was not suitable for the molecular weight estimation in this study. Therefore, the molecular weight of the eluted protein was estimated using the scattering intensities corrected by the X-ray transmittance. The obtained values of the *Rg* and molecular weight were reasonable for the hen egg white lysozyme monomer as a model protein.

2. Materials and methods

2.1. Materials

Hen egg white lysozyme (6 times crystallized sample) and bovine serum albumin monomer were purchased from the

E-mail address: yasuw@affrc.go.jp

https://doi.org/10.1016/j.chroma.2018.01.046
0021-9673/

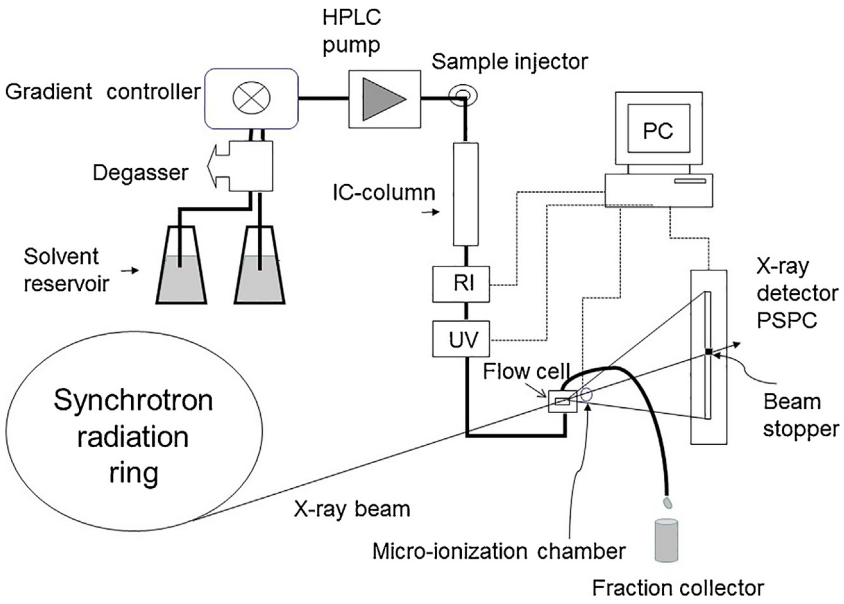

Fig. 1. Schematic drawing of the IC-SAXS measurement system.

Seikagaku Corporation, Tokyo, Japan and Sigma-Aldrich, St. Louis, MO, USA, respectively. All other chemicals were of analytical grade.

2.2. IC system

The high-performance liquid-chromatography (HPLC) system was equipped with a sample loop having an internal volume of 500 μL, an HPLC-type cation-exchange column (TSKgel BioAssist S, 50 × 4.6 mm, Tosoh, Tokyo, Japan) and two sequential detectors, a differential refractometer (RI-2031, JASCO, Tokyo, Japan) and an ultraviolet spectrophotometer (AC-5100S, ATTO, Tokyo, Japan) at ~22 °C. This column was composed of a polymer containing sulfopropyl groups as the ion-exchanger. The refractometer was used for estimation of the gradient patterns of the NaCl concentrations. While the ultraviolet spectrophotometer can monitor the protein elution, the high optical density was not quantitatively estimated. Therefore, the eluted lysozyme concentration was spectrophotometrically estimated for 0.6-mL aliquots of the eluted solutions assuming that the absorbance value of a 1 % solution at 280 nm was 26.9 in a 1-cm light-path cuvette [10].

The cation-exchange column was equilibrated with the starting buffer, 20 mM sodium phosphate buffer, pH 6.7, at the flow rate of 0.6 mL/min. A 500-μL volume of the lysozyme sample solution (8.7 mg/mL) in the starting buffer was applied to the column from a sample loop of the same internal volume. This injection was repeated three times at the intervals of 5 min. After passage of the starting buffer for 4 min beyond the last injection, the NaCl concentration was linearly increased from 0 M to 0.4 M in the starting buffer during 1 min and then from 0.4 M to 0.55 M in the starting buffer during 40 min at the flow rate of 0.6 mL/min. The solvent delivery system to the column consisted of the following equipment connected in the order mentioned: an AG-34 degasser (Flom, Tokyo), a LPG-1000 low pressure gradient controller (Tokyo Rikakikai Co., Ltd, Tokyo) and an AI-12 HPLC-pump (Flom, Tokyo).

2.3. IC-SAXS measurement system

The SEC-SAXS measurement system has been previously described [4–6]. In this study, the IC system mentioned above was used instead of the SEC system. Therefore, the IC system was combined with synchrotron radiation SAXS measurement equipment after the refractometer and the ultraviolet spectrophotometer as shown in Fig. 1. The synchrotron radiation SAXS measurements were performed using an optics system at the beamline BL-10C station in the Photon Factory of the High Energy Accelerator Research Organization as previously described [3–6,9]. The wavelength (λ) of 0.1488 nm was used. The temperature of the flow cell with a 1-mm light path and a pair of 20-μm-quartz windows was maintained constant at 22.0 ± 0.1 °C using the metallic cell holder through which constant temperature water was circulated. Data were collected for 3 min by a position sensitive proportional counter at the sample-to-detector distance of 1.98 m. The obtained signals were corrected for solvent scattering which was obtained by the same operation without the sample, then normalized to the beam intensity to yield the net scattering intensity $I(q)$, where q (= $(4\pi/\lambda)\sin\theta$, λ is the X-ray wavelength, 2θ is the scattering angle) is the modulus of the scattering vector. Moreover, a micro-ionization chamber (20 mm along the beam direction and 30 mm perpendicular to it, Repic Corporation, Tokyo) was placed on the back (the X-ray detector's side) of the X-ray flow cell holder for the real-time monitoring of the X-ray transmittance as previously described [9]. Therefore, the final scattering intensity was corrected using the X-ray transmittance values. The q-value was calibrated using a diffraction pattern of dried chicken collagen. The characteristic of the standard dried collagen sample was evaluated using a laboratory-type small-angle X-ray scattering measurement apparatus (M18X, MAC Science, Yokohama, Japan). The concentration of bovine serum albumin, which was used as the standard protein for molecular weight estimations, was spectrophotometrically estimated assuming that the absorbance value of a 1 % solution at 280 nm was 6.78 in a 1-cm light path cuvette [10].

3. Results and discussion

3.1. X-ray transmittance estimation

Lysozyme is a model enzyme that plays an important role in protein chemistry, enzymology, crystallography and molecular biology. In particular, hen egg white lysozyme has been used as the standard protein for small-angle X-ray scattering measurements [4,11,12]. Therefore, the hen egg white lysozyme is suitable for the assessment study of the IC-SAXS method. Fig. 2 shows the

Please cite this article in press as: Y. Watanabe, Assessment study of ion-exchange chromatography combined with solution X-ray scattering measurement for protein characterization, J. Chromatogr. A (2018), https://doi.org/10.1016/j.chroma.2018.01.046