인터페론-알파2a 단백질의 분광측정시 발생하는 광분해 반응메커니즘 및 반응속도 연구

Photobleaching Mechanism and Reaction Kinetics of Interferon-α2a Protein during Optical Measurement

김형하*, 이윤미†, 송남웅†
*한국표준과학연구원 물질량표준부 유기생물분석표준그룹, † 한국표준과학연구원
광기술표준부 나노광계측그룹
nwsong@kriss.re.kr

Optical measurement techniques such as absorption, fluorescence and circular dichroism (CD) spectroscopy are useful tools to investigate molecular properties of biological compounds as well as organic or inorganic materials. Among the various optical spectroscopy techniques, the importance of fluorescence spectroscopy as an analytical tool has continuously grown due to the increasing demand of small scale and high throughput assay techniques in the biological research field. Fluorescence measurement of proteins applied with polarized or time-resolved excitation/emission also gives structural information of the object molecules. However, some protein molecules undergo photobleaching during the observation of their optical properties. To find a way to minimize the change in protein structure, it is important to understand the photobleaching mechanism and reaction kinetics in the course of optical measurements.

In this study, the photobleaching of interferon-α2a (IFNα2a) protein during the fluorescence measurement was observed with a conventional spectrofluorometer. Interferons belong to the family of cytokines that exert antiviral, antiproliferative, and immunoregulatory activities in human body[1]. IFNα2a, one of the many subtypes of interferons, contains 165 amino acids with four cysteines and two disulfide linkages and has a molecular weight of 19.225 kDa[2]. The three dimensional solution structure of IFNα2a is an all-helical protein containing six α-helices[3].

When we compared the IFNα2a absorption spectra before and after fluorescence measurement, a distinct change in the absorption profile pattern was observed. This change indicates structural change in protein molecules [Fig. 1(a)]. Fluorescence spectra was also altered in both spectral shape and fluorescence quantum yield [Fig. 1(b)]. The CD spectral analysis results indicated that ~40 % loss of the secondary structure accompanied the ~75 % loss of the tertiary structure of IFNα2a protein during photobleaching [Fig. 2]. Fluorescence photobleaching kinetics of IFNα2a was monitored with fixed wavelength excitation (280 nm) and detection (332 nm). In pH 5.0 buffer solution, first-order reaction was the major channel for photobleaching of IFNα2a, while the second-order reaction was observed in the photobleaching kinetics of IFNα2a in pH 2.5 buffer solution. Based on the comparison of kinetics measurement results for both atmospheric and

deoxygenated conditions, the photobleaching mechanism in this case can be explained as the combination of photo-oxidation and bimolecular addition dependent on the pH values of the buffer solution.

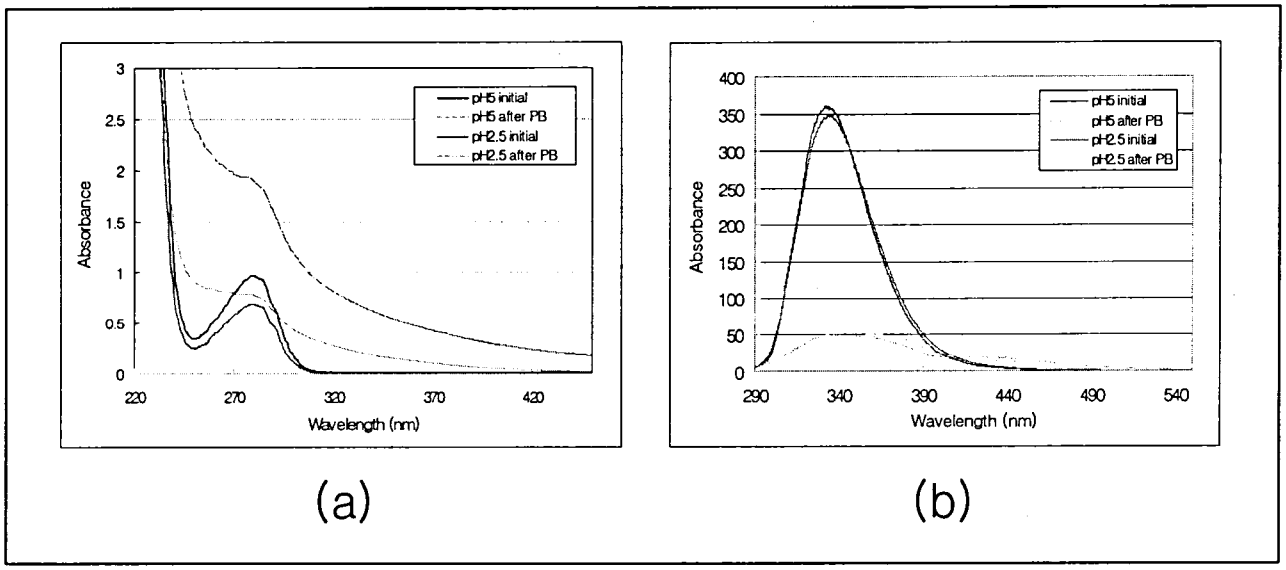

Figure 1. (a) Absorption and (b) fluorescence spectra of IFNα2a in buffer solutions of different pHs.

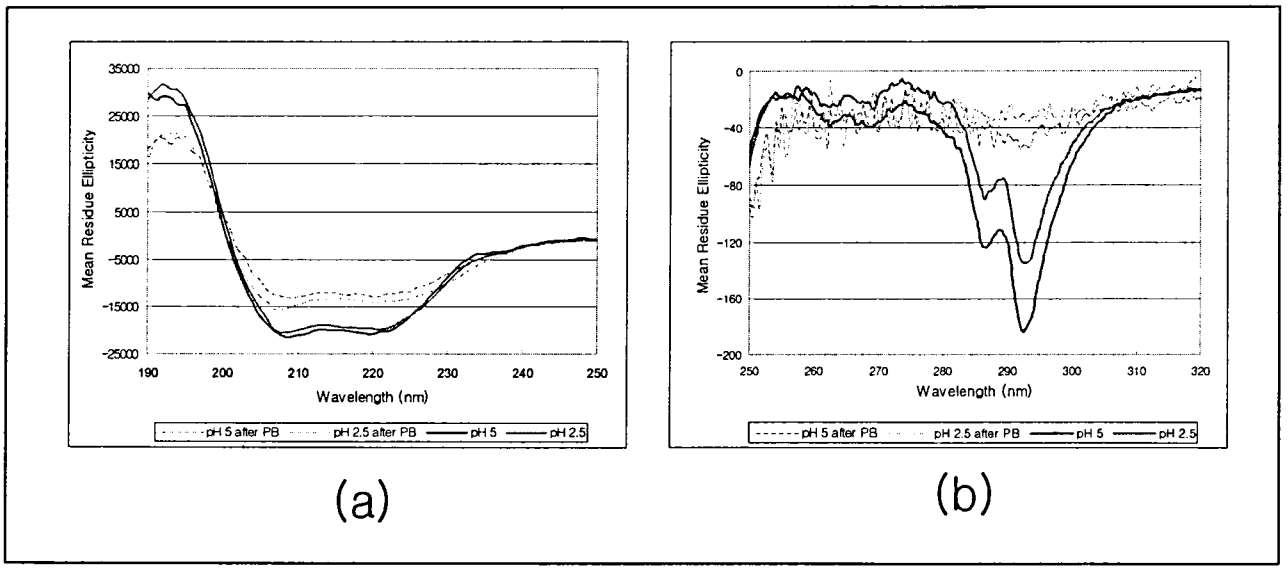

Figure 2. CD spectra of IFNα2a protein in buffer solutions with two different pHs. CD spectra obtained before and after the fluorescence measurement were compared.

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