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NMR studies on binding sites and aggregation–disassociation of fluorinated surfactant sodium perfluorooctanoate on protein ubiquitin

Run-Chao Lu [a], Xian-Rong Guo [a,b], Changwen Jin [a,b], Jin-Xin Xiao [a,c,*]

[a] Beijing National Laboratory for Molecular Sciences, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
[b] Beijing Nuclear Magnetic Resonance Center, Beijing 100871, China
[c] Beijing FLUOBON Surfactant Institute, Beijing 100080, China

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ABSTRACT

The fluorinated surfactant sodium perfluorooctanoate (SPFO) could bind onto ubiquitin (UBQ) and induce the unfolding of UBQ. By using ^{15}N-edited heteronuclear single-quantum coherence (HSQC) NMR and ^{19}F NMR to monitor ^{15}N-labeled UBQ and SPFO, respectively, the binding sites and the aggregation process of SPFO on UBQ at various SPFO concentrations were observed. A detailed process from specific binding to cooperative binding of SPFO on UBQ, and a detailed structure change of UBQ upon the increase of SPFO concentration were obtained. The refolding of UBQ in UBQ–SPFO complex was carried out by adding cationic surfactant. It was shown that added cationic surfactants formed mixed micelles with SPFO and resulted in the dissociation of the UBQ–SPFO complex, and consequently, most ubiquitin could be refolded to its native state.

1. Introduction

A central goal of the studies on protein folding is to obtain information on how the binding of different ligands can affect protein structure and function and how the disordered polypeptide chain of a denatured protein folds into the native structure. Surfactants, a group of amphipathic substance with hydrophilic groups at one end and hydrophobic groups at the other, are important protein ligands. They can induce the unfolding of proteins, and in some special cases, stabilize proteins at a very low concentration [1]. They are also solubilizing agents for membrane proteins [2]. Organisms contain a lot of amphipathic substances, and many biological and medical products as well as food contain both proteins and surfactants [2]. There are some reports studying protein-surfactant interaction by such as surface-tension, viscosity, dialysis, and dynamic light scattering, and speculating the binding mechanism of surfactant to protein [2]. However, few works study how surfactant binding affects protein structure and function at the molecular level. Otzen studied the effect of sodium dodecyl sulfate micelle structure, ionic strength, pH, and temperature on the unfolding of protein S6 [3]. Sun et al. studied the interaction of BSA with cetylpyridinium bromide and found that cetylpyridinium bromide at low and high concentrations could induce the unfolding and refolding of BSA, respectively [4]. The existence of specific binding and cooperative binding in the binding process of surfactants to protein is now widely accepted [2], but detailed mechanisms at the molecular level, such as the surfactant binding sites and the binding process, remains elusive. Our approach to this problem makes use of NMR spectroscopy to observe the detailed structural changes of protein when interacting with surfactants.

The fluorinated surfactant sodium perfluorooctanoate (SPFO) was a protein denaturant [5]. In our previous work [6], we found that fluorinated surfactants exhibited stronger interactions with proteins than hydrogenated ones with similar critical micelle concentrations (cmc). In order to study the detailed mechanisms of surfactant–protein interaction at the molecular level, especially the binding sites and the binding process of surfactants on proteins, in this study SPFO and ^{15}N-labeled ubiquitin (UBQ) were used. As SPFO contains no H and N atoms, and UBQ contains no F atoms, we used ^{15}N-edited heteronuclear single-quantum coherence (HSQC) NMR and ^{19}F NMR to monitor ^{15}N-labeled UBQ and SPFO, respectively. First of all, the binding sites and aggregating process of SPFO on UBQ were determined. Subsequently, we tried to refold UBQ by adding a cationic surfactant.

2. Materials and methods

Sodium perfluorooctanoate ($C_7F_{15}COONa$, SPFO) was prepared by the procedure described in our previous article [7]. Dodecyltrimethylammonium chloride ($C_{12}H_{25}N(CH_3)_3Cl$, DTAC) and cetyltrimethylammonium chloride ($C_{16}H_{33}N(CH_3)_3Cl$, CTAC) were from Alfa Aesar. All

* Corresponding author.
E-mail address: xiaojinxin@pku.edu.cn (J.-X. Xiao).

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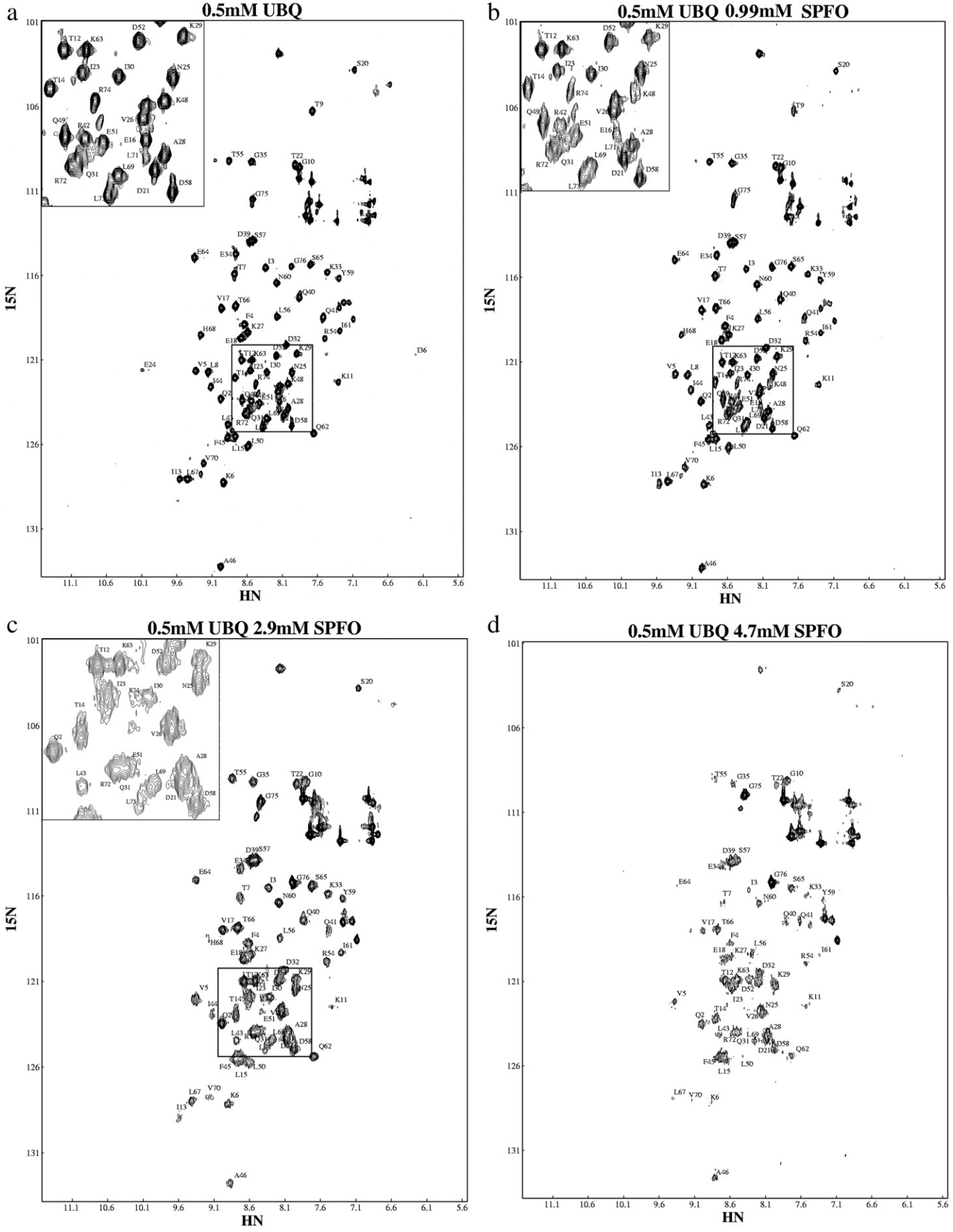

Fig. 1. The HSQC spectra of ubiquitin (UBQ) in the presence of SPFO at 298 K.