Journal of Colloid and Interface Science 484 (2016) 125–134

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Regular Article

Surface active complexes formed between keratin polypeptides and ionic surfactants

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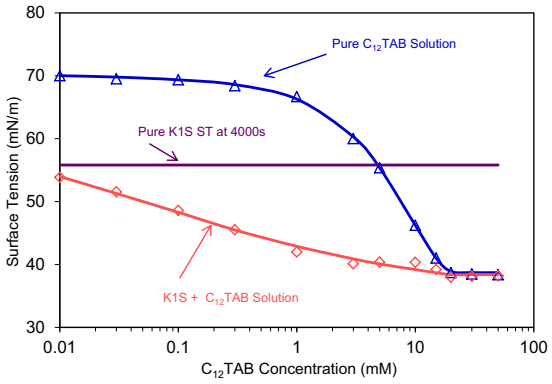
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 June 2016
Revised 30 August 2016
Accepted 31 August 2016
Available online 1 September 2016

Keywords:
Surface tension
Protein adsorption
Surface activity
Protein-surfactant complexes
Neutron reflection
Keratin polypeptides

ABSTRACT

Keratins are a group of important proteins in skin and hair and as biomaterials they can provide desirable properties such as strength, biocompatibility, and moisture regaining and retaining. The aim of this work is to develop water-soluble keratin polypeptides from sheep wool and then explore how their surface adsorption behaves with and without surfactants. Successful preparation of keratin samples was demonstrated by identification of the key components from gel electrophoresis and the reproducible production of gram scale samples with and without SDS (sodium dodecylsulphate) during wool fibre dissolution. SDS micelles could reduce the formation of disulphide bonds between keratins during extraction, reducing inter-molecular crosslinking and improving keratin polypeptide solubility. However, Zeta potential measurements of the two polypeptide batches demonstrated almost identical pH dependent surface charge distributions with isoelectric points around pH 3.5, showing complete removal of SDS during purification by dialysis. In spite of different solubility from the two batches of keratin samples prepared, very similar adsorption and aggregation behavior was revealed from surface tension measurements and dynamic light scattering. Mixing of keratin polypeptides with SDS and C_{12}TAB (dodecyltrimethylammonium bromide) led to the formation of keratin-surfactant complexes that were substantially more effective at reducing surface tension than the polypeptides alone, showing great promise in the delivery of keratin polypeptides via the surface active complexes. Neutron reflection measurements revealed the

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http://dx.doi.org/10.1016/j.jcis.2016.08.082

coexistence of surfactant and keratin polypeptides at the interface, thus providing the structural support to the observed surface tension changes associated with the formation of the surface active complexes.

1. Introduction

Keratins are important structural proteins and are the main constituting components in skin, hair, wool, feathers, nails, horns and connecting tissues [1]. Their physical and biological studies have direct relevance to health and disease and also bear important implications to hair and skin care. In the past few decades, keratin-based research has been undertaken in a diverse range of subjects from the perspective of biomaterials including wound healing, tissue engineering and regenerative medicine, drug delivery, orthopaedic medical devices [2–14] and cosmetics [15,16] to the biomedical care of skin and organ [17–19] (e.g., ageing, health, cancers). Hair fibres have also been widely studied using a wide range of techniques such as in-situ skin Raman and fluorescence imaging, X-ray diffraction, solid state NMR [20] and ion beam analysis [21].

In contrast, direct studies of keratin molecules from the physiochemical perspective of keratin based aqueous solutions are lacking, although this work is fundamental for developing keratin polypeptide biomaterials and technologies. Keratins are mostly produced by recombinant expressions for biological and biomedical research where micro- to milli-gram levels of samples are often sufficient. Larger scale keratin production from recombinant expressions currently suffers from difficulties in separation and purification. Alternative approaches are to dissolve hair/wool fibres and convert them into water-soluble or water-dispersible form, which is what we have done in this work.

Hair and wool are both characterized by two hard keratin groups, type Ia being approximately 40–48 kDa and type IIa being approximately 59–62 kDa [22]. Apart from different physical appearances, human hair and wool share over 90% similarity in amino acid sequence and composition [22]. In the work so far, we have produced stable aqueous solutions of keratin polypeptides from wool with and without SDS, and the schematic diagram of keratin extraction from wool is shown in Fig. SI1.

As water-soluble keratin polypeptides can be regenerated from renewable sources and the process can help transform low grade of wool into biocompatible biomaterials, one of the main drives has been to use them to replace some of the ingredients in personal care products. Current skin and hair care products are however formulated from surfactants and polymers that are predominantly derived from petrochemicals. It is crucial to understand how the polypeptides would interact with other ingredients and skin physically and biologically. It can be envisaged that major barriers associated with the development of products incorporating skin friendly polypeptides are based around the methods and approaches that could provide reliable manipulation of their interactions with the formulation ingredients and skin. Small molecules such as amino acids and surfactants can bind to keratin polypeptides and the outcome could alter how the key ingredients in the newly formulated products interact with hair/skin, resulting in new hair/skin properties and appearance.

Hair and skin are also made up of lipids and other proteins and it is also useful to understand how fibrous proteins such as keratins interact with model lipids and surfactants. In spite of extensive research so far, there remains a lack of reliable information concerning the nature of interactions involved with respect to skin care and the consequential effects on the proteins concerned. This situation largely arises from the lack of adequate experimental approaches to gain reliable data at the molecular level.

The dissolution of keratins into aqueous solution would enable a systematic study to be made using techniques such as light and neutron scattering to characterize bulk solution behavior and surface tension, ellipsometry and neutron reflection for surface and interfacial adsorption and film properties. Upon successful production of keratin polypeptide solutions, the aim of this work was to provide a basic characterization of their behavior at surface and in solution incorporating the assessment of their interactions with model ionic surfactants such as SDS (sodium dodecylsulphate) and C_{12}TAB (dodecyltrimethylammonium bromide). This work from the model keratin system will thus form a useful basis for further studies into the roles of surfactants on hair and skin concerning their interactions with keratins.

Although this work represents one of the first studies of surface adsorption of the keratin polypeptides and their interaction with surfactants, the behavior of mixed surfactant-protein systems has been extensively studied [23]. However, model proteins such as lysozyme, BSA and HSA have been largely used so far due to the availability of data concerning their sequences and basic physical properties. Surfactants interact with proteins and peptides through a combined action of electrostatic and hydrophobic forces, and the complexes formed may differ depending on the exact protein and surfactant involved. As already demonstrated by Miller et al., surfactant-protein complexes often show surface activities that are different from the protein or surfactant alone and complex activities also vary with environmental conditions, as in the case of surface adsorption from the binary mixtures of surfactant-β-lactoglobulin systems involving surfactants such as cationic C_{12}TAB, anionic SDS and non-ionic Tween 20 [23]. Surface tension measurement is one of the most widely used techniques for characterizing these systems, but it remains difficult to understand surface and interfacial molecular processes from surface tension changes alone. Neutron reflection studies can provide useful information about the thickness and composition of the surface and interfacial systems which help understand how surface activities respond to structural changes in response to different solution conditions [24,25]. The combined studies of surface tension and neutron reflection shall help us to understand how keratin polypeptides interact with the two ionic surfactants and if their interactions can be categorised into the general behavior of the model protein-surfactant systems.

2. Experimental section

2.1. Materials and chemicals

Raw sheep wools (black and white) were supplied from a local farmer in Halifax, UK. The white wool was used as the source for keratin extraction in this work. Chemicals including urea, sodium dodecylsulfate (SDS), sodium metabisulfite ($Na_2S_2O_5$), DL-dithiothreitol (DTT), Coomassie brilliant blue G-250, hexane, dichloromethane and ethanol were all analytical reagents from Sigma-Aldrich. Tris base and Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) were from BDH and sodium hydrogen carbonate ($NaHCO_3$) was from Fisher. Dialysis tubing cellulose membrane was also from Sigma-Aldrich and Protein Marker was from Biolabs.