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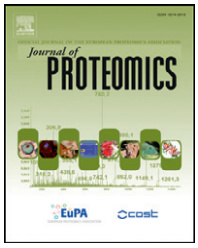

Review

Unravelling the proteome of wool: Towards markers of wool quality traits☆

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ABSTRACT

With ongoing efforts to make wool more competitive alongside other fibres, notably synthetics, there is a need to obtain a better understanding of the relationship between protein composition and characteristic wool properties to assist sheep breeding programmes. Before this can be achieved, the wool proteome needs to be mapped, by gel and non-gel techniques, and methods developed to reliably quantitate protein expression. Nevertheless, in setting out to achieve this, there are numerous challenges to be faced in the application of proteomics to wool, including the relative lack of wool protein sequence information in the publically accessible databases, the wide variety of proteins in the wool fibre, the high homology within the Type I and Type II keratins, the high degree of homology and polymorphism within individual keratin associated protein families, the dominance of the keratin proteins over others in wool and the peculiar chemistries found in keratins and their associated proteins. This review will discuss the various strategies that have been developed to both identify these proteins in the wool protein map and quantify them with the view to their application to the identification of markers for wool quality traits. This article is part of a Special Issue entitled: (SI: Farm animal proteomics).

Contents

Abbreviations: HGTP, high glycine–tyrosine protein; HSP, high sulphur protein; ICPL, isotope-coded protein label; KAP, keratin associated protein; UHSP, ultra-high sulphur protein.

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1. Introduction

There are a number of wool quality traits that are of particular interest to the wool processing and manufacturing industries, in particular staple tenacity (longitudinal strength) and fibre curvature. Staple strength, a measure of how much force is required to break a staple of wool, influences the efficiency with which wool is combed and also the amount of fibre breakage and wastage through this process. Hence it is one of the major determinants for the spinning of wool and ultimately its value. Curvature is important for bulk, which is a measure of compressibility of a mass of unprocessed wool fibres. Wool bulk is related to the dimensional measurements of fibre diameter and curvature and is an important parameter in the production of carpets.

Characterisation of the proteins in wool is a necessary prerequisite for identifying potential relationships between protein expression and wool quality traits. For instance studies of the effect of over-expression of KAP5.1 in wool on fibre found that fibres of lower intrinsic strength were produced, while over-expression of KAP6.1 reduced both the load-bearing capacity and extensibility of the fibres [1]. Wool is notable for being primarily composed of proteins, which make up 90–95% of the fibre. Two major types are present, the intermediate filament-forming keratin proteins and the keratin associated proteins (KAPs) [2]. The keratins are further divided into two types, the acidic Type Is and the neutral-basic Type IIs [3–6]. The KAPs have been divided into three classes: high sulphur proteins (HSPs), ultra-high sulphur proteins (UHSPs) and high glycine–tyrosine proteins (HGTPs) [2], though from studies of human hair some new KAPs have been identified that do not fit into any of these categories [7].

This is only part of the story, as considerable diversity has also been observed among the keratins and KAPs. A total of 25 families have been reported for human hair, these together containing a total of 113 proteins [5,8,9], while 19 families have so far been found in wool, with a total of 70 proteins among them (Yu et al., unpublished data). Of these, a total of 11 Type I and 6 Type II keratins have been reported in human hair and 10 Type Is and 7 Type IIs in wool. Consequently there are many more KAPs than keratins and there is quite a variation in the number of proteins in each KAP family, some having only one member, while one sheep UHSP family has been found to have 27 proteins, with the equivalent hair family having only 15 members.

When proteomic techniques are applied to the search for markers for wool quality traits, the issues with either a gel or non-gel approach are the same. Both for the keratins and KAPs, one of the main problems is the high degree of homology (based on % sequence identity) found within each family, which can be as high as 92% between some members of the Type I keratins and 85% among the Type II keratins. Similar degrees of homology have been also observed for the KAPs and here the situation is further complicated by the existence of polymorphism in some families, notably KAP1.1 [10], KAP1.2 [11], KAP1.3 [12], KAP5.4 [13], KAP7.1, KAP8.1 [14] and KAP11.1 [15]. Nevertheless, studies have provided evidence of distinctive variation of some of these proteins both within and between breeds [16]. Hence both this and the polymorphism observed [10,12] offer some promise in the search for gene/protein markers for specific wool quality traits, efforts towards which will be reviewed in this paper along with current work in the area.

2. Mapping the proteome of wool

2.1. *Identification of proteins on 2-DE gels*

Initial studies into mapping the wool proteome were centred around gel-based techniques, particularly 2-DE as this technique resolved the proteins best. For consistency wool was sourced from the mid-side region of the animal. Preparation prior to extraction involved scouring the wool using the commercial detergent Teric GN9, with the laboratory modification of two washes with dichloromethane to remove additional lipids that were found to affect the extractability of proteins from the fibre [17,18]. Separation over the pH range 4–7 in addition to pH 3–11 was found to be necessary because it was possible to resolve the Type I keratins, in particular, into individual spots using the narrower pH range.

Separation of wool keratins by 2-DE over the range of pH 4–7 results in a characteristic pattern of spots on the resulting map with the Type I keratins appearing as a tight cluster of four chains around pH 5 and 45 kDa, while the Type II keratins form a long train of spots running from pH 5.5 to 6.5 at 60 kDa (Fig. 1) [17]. Quite why these proteins form these long strings is unclear. An early study suggested they were due to phosphorylations [19] but more recent work has demonstrated that no phosphorylations or glycosylations are present in them [20]. However, identification of the protein in each spot used to be limited by the relative lack of sequences in the international databases such as Swiss-Prot and NCBInr, [21]. Only two known Type I and two Type II keratin sequences were available when this research was initially carried out, meaning that only two Type I and two Type II keratins were identified on the 2-DE map, though there were indications of at least four Type Is and as many Type IIs [22]. In fact, recent studies of human keratins have shown as many as 11 Type Is and six Type II keratins [3–5]. Recent work on sequencing the sheep genome within this organisation has revealed a similar picture, with full or partial sequences for 10 Type Is and seven Type IIs now available [6].

With this new sequence information, a re-examination of the keratin region of the 2-DE maps was possible. As the focus of this study was on the keratins, the wool protein