Research Letter

Characterizing Wool Keratin

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Keratin from wool is a reactive, biocompatible, and biodegradable material. As the biological structural component of skin (soft keratins) and of nails, claws, hair, horn, feathers, and scales (hard keratins) pure keratin comprises up to 90% by weight of wool. Wool was treated in alkaline solutions to extract from 68% to 82% keratin within 2 to 5 hours of exposure at 65°C. The keratin products were water-soluble and were confirmed to contain intermediate filament and microfibrillar component-proteins of fractured, residual cuticle, and cortical cells. Oxidation of wool by peroxycarboximide acid in alkaline hydrogen peroxide produced keratin products with distinct microcrystalline structures: descaled fibers, fibrous matrices, and lyophilized powders. Morphology and confirmation of peptide functionality were documented by SEM, Amino Acid Analysis, SDS-PAGE gel electrophoresis, MALDI-TOF/TOF, and FTIR analyses. The reactivity of keratin from wool models the reactivity of keratin from low-value sources such as cattle hair.

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1. Introduction

Wool with up to 95% keratin by weight is a rich and pure source of intermediate filament proteins (IFPs) which can be used in a wide range of biomaterials applications. As a polymeric polyamide, keratin exhibits a high degree of chemical functionality. It has high potential for bio-based niche market applications in sponges, films, matrices for agent retention and transport [1–4]. Functionalities and end-uses are determined by hydrolysis conditions of oxidation and reduction to break or restore disulfide linkages. Sites of reactivity include amide, carboxyl, sulfoxide, sulfide, and thiosulfide. Solubilized wool fiber with the transformed morphologies of keratin powders (the lyophilized products of solubilized wool) exhibits the unique characteristics of natural keratin and upon characterization can be feedstock for developing novel products and applications. Wool was hydrolyzed under severe alkaline hydrolysis at elevated temperature in various modified systems to obtain from 68% to 80% recovery of pure keratin in the form of IFPs and constituent microfibrillar and matrix proteins. Various stages of chemical degradation of cuticle and cortical cells to protein residues were recorded by scanning electron

microscopy (SEM). Four keratin preparations were produced from alkaline oxidation and reduction methods. The keratin products were recovered in powder-form for analysis by gel electrophoresis leading to MALDI-TOF identification and characterization of keratin Type II cortical fragments and their associated matrix proteins, each having identified protein profiles. These profiles were documented as peptide mass spectral fingerprints of known IFP theoretical sequences found in web-based databases [5–7].

2. Experimental Procedures

Scoured, domestic wool in Figure 1(a) was immersed in four NaOH solution systems at pH 12 to 13. These systems were designed to produce a variety of physical products in Figures 1(b), 1(c), and 1(d) for modification to higher-value-added products. Each treatment dissolved the fibers within 2 to 5 hours at 65°C with keratin yielding from 68 to 82%. The keratin hydrolysates were filtered and centrifuged, dialyzed, and lyophilized to powders.

Alkaline hydrolysis of wool gave products with different morphologies shown in Figures 1(b), 1(c), and 1(d). Samples

FIGURE 1: Untreated wool and keratin products from keratin preparations from alkaline hydrolysis: (a) wool fiber; (b) 8 M urea, 1 N NaOH; (c) descaled, smoothed fibers after exposure to 0.5 N NaOH charged with NaOH/H₂O₂; (d) lyophilized powder of keratin hydrolysate product from apparent fractured cuticle and cortical cells.

1 and 2 were pretreated with 8 M urea with additions of 0.1 N and 1 N NaOH, respectively. 0.1 M NaBH₄ was coadded to sample 2. Treatments for samples 1 and 2 were not as severe as those used to produce samples 3 and 4. Sample 3 was treated with 0.5 N NaOH with the addition of a charge after 1 hour with 2% by weight of fiber NaOH dissolved in 10 mL 50% H₂O₂. Immediate evolution of oxygen completely dissolved the wool within 30 minutes to form keratin powder. In sample 4, wool was treated with peroxy-carboximidic acid, highly active bleach at room temperature, and novel to the ARS process for whitening, biopolishing, and shrinkproofing wool [8]. Subsequent addition of 0.1 N NaOH in 50% H₂O₂ produced an exothermic reaction which stripped the wool fibers of surface scales and recovered fiber mass with fiber integrity (57% of original weight) in Figure 1(c). The dialyzed supernatant solution was lyophilized to keratin powder (30% by original wool) in Figure 1(d).

3. Results and Discussion

Amino acid analysis confirmed cystine oxidation to cysteic acid with high cysteic acid content in samples 3 and 4, loss of serine in sample 1 and threonine in samples 1 and 2, and the conversion of cystine to cystine-S-dioxide. These alkaline oxidation/reduction methods converted keratin amides and disulfides to the corresponding acids with degradation to smaller protein fragments composed of Type II keratin intermediate filament and keratin with microfibrillar structure, revealed by MALDI-TOF/TOF in Table 1.

SEM was used to document the various morphologies of keratin products from wool hydrolysis. Lyophilized keratin powder was attached to aluminum specimen stubs with

TABLE 1: Keratin-associated proteins identified by database search in NCBIInr (<http://www.ncbi.nlm.nih.gov/>) after trypsin digestion.

Protein	Peptide sequence
Type II keratin intermediate filament protein	HGETLRR; LEEEEEIR;
	FAAFIDKVR; RLYEEEEIR;
	AKQDM(Ox)ACLLK;
	DVDC*AYLRK; RYEEEEVALR;
	TKEEINELNR; SRAEAESWYR;
	LGLDIEIATYRR;
	VIQRLTAIVENAK;
	FLEQQNKLETK;
	SDLEANVEALIQETDFLR;
	KSDLEANVEALTQEIDFLR;
Type II keratin micro-fibrillar, component 7c protein	LYEEIEIRVLQAHISDTSVIVK
	HGETLRR;
	LLEGEEQR;
	FAAFIDKVR;
	KDVDC*AYVR;
	DVDC*AYVRK;
	AKQDM(Ox)ACLLK;
	TKEEINELNR;
	LGLDIEIATYRR;
	VLQANISDTSVIVKM(Ox)DNSR
Type II keratin micro-fibrillar, low-sulfur protein	KSDLEANVEALTQEIDFLR;
	SDLEANSEALIQEIDFLRR;
	LEAAVTQAEQQGEVALNDARC*K
	LVVQIDNAK;
	DVEEWYIR;
	QLVEADLNLRR;
	AQYEALVETNRR;
	QNQEYQVLLDVR;
	TVNALEVELQAQHNLR;
	DLERQNQEYQVLLDVR;
	LPCNPC*ATTNTC*GKPIGPC*
	ISNPC*VSRTR

M(Ox) corresponds to oxidized methionine and C* corresponds to cysteine carbamidomethyl derivative.

double-sided scanning electron microscopy (SEM) tape (Electron Microscopy Sciences, Ft. Washington, PA) and sputter-coated with a thin layer of gold. Imaging was performed with a model JSM840A SEM microscope (JOEL USA, Peabody, mass, USA) operating at 10 kV in the secondary electron imaging mode and coupled to an Imix-I digital image workstation (Princeton Gamma-tech, Princeton, NJ, USA).

Molecular weights (MWs) of the keratin preparations of samples 1–4 from SDS-PAGE were estimated as 6500–18,000 Da. The bands were excised and analyzed by MALDI-TOF (mass range, 800 to 4000 Da) for protein analysis. Amino acids, Asn, and Gln, were present in Type II keratin microfibrillar, component 7c, Type II keratin intermediate filament, and Type II keratin microfibrillar;

FIGURE 2: Infrared spectra (a) and (b) Samples 1–4, relative absorptions for amide and sulfoxide regions, respectively; (c) and (d) wool keratin pulverized fiber, 1700–1400 cm⁻¹ and 1400–1000 cm⁻¹ regions, respectively; (e) and (f) Sample 4, 1700–1400 cm⁻¹ and 1400–1000 cm⁻¹ regions, respectively.

low-sulfur protein and the identified sequences corresponding to these proteins covered between 20% to 25% of the sequence of matched protein in the data base. These sequences confirmed certain amino acid groups, for example, keratin preparations, samples 1, 2, 3, and 4, corresponding to keratin Type II intermediate filament and microfibrillar protein contained glutamine (Q) and lysine (K). The validation of the presence of these amino acids suggests the potential for enzymatic self-crosslinking utilizing enzyme-mediated transglutaminase transamidation to obtain higher molecular weight protein [9]. The sequences of the tryptic-digested proteins identify cysteine residues, free N-terminal amino functions, and the location of amide side chains for subsequent reactivity. Knowing these protein profiles, higher MW proteins can be produced from the identified protein fragments using self-condensation reactions.

FTIR spectra of keratin products which were formed into KBr pellets were collected in the transmission mode using a Nicolet Magna System 560 spectrometer equipped with an MCT/A detector to record the amide and sulfoxide absorptions in Figures 2 and 3. Notably samples 3 and 4 produced more cysteic acid (1045 cm⁻¹) with cleavage of disulfide linkages. Second derivative spectra were not used to identify discrete peak absorptions, rather those reported for amine and sulfoxide in Figures 3(a) and 3(b) were arbitrarily assigned by peak resolve software. Note that the resolved bands in Figure 2 are broad, and the wavelengths reported in Figure 3 are within the absorption ranges of the relevant, resolved peaks.

From peak resolution of the sulfoxide region, the main oxidation products were cystein-S-dioxide in samples 1 and 2 and cysteic acid in samples 3 and 4. The presence of the oxide forms of sulfur is significant for determining the extent

FIGURE 3: Peak area, %, of resolved amide and sulfoxide bands in keratin preparations: samples 1 to 4, (a) amide region: Amide I (1680 and 1645 cm^{-1}), Amide II (1550 and 1515 cm^{-1}), and Amide III (1435 cm^{-1}); (b) sulfoxide regions: Bunte-salt, Cysteine-S-sulfonate; Cy-S-SO₃⁻ (1012 cm^{-1}), Cysteic acid, CySO₃H (1045 cm^{-1}), Cystine-Smonoxide, Cy-SO-S-Cy (1080 cm^{-1}), and Cystine-Sdioxide, CySO₂-S-Cy (1137 cm^{-1}).

of oxidation, assuming that oxidation of the disulfide bond occurs by way of monoxide-to-dioxide, to full oxidation with the formation of cysteic acid. All keratin preparations show increases in the amide regions, and this is consistent with the fragmentation of wool keratin into low MW protein with increased amide functionality [10–12].

4. Conclusion

Wool fiber was hydrolyzed using severe alkaline hydrolysis to obtain high yields of keratin powder after short exposure times. The water soluble keratin powders contained protein fractions of apparent fractured, residual cuticle, and cortical cells. SEM, amino acid analysis, SDS-PAGE gel electrophoresis, MALDI-TOF/TOF, and FTIR analyses of the powders from the keratin preparations showed different morphologies, confirmations of peptide chains, and functions available for further reactivity. Alkaline nucleophilic treatment with NaOH altered the chemical properties of keratin by increasing amide and sulfoxide contents to suggest discrete pathways for subsequent keratin modifications. Treating wool keratin with urea is generally known to swell the wool fiber to allow permeation of treatment solution beyond the surface cuticle. In this case, the fiber is predisposed to alkaline attack of

its structure-forming cystine cross-links, thereby resulting in the endpoint formation of cysteic acid from sulfoxide precursors.

Each of the alkaline hydrolysis methods produced reproducible protein compositions. The amino acids comprising the keratin protein, as revealed by MALDI-TOF/TOF, indicate possibilities for subsequent reactivity. The keratins in wool were distinguished by basic/neutral (Type II) constituents which formed the IFPs cytoskeletal network of the epithelial cells. IFPs possess a central α -helical rod domain of secondary structure that forms the basis of keratin morphology as IFPs assemble by association to form coiled molecular structure. The protein compositions in Table 1 show the individual building blocks found in the individual keratin powders. It can be inferred that the solid form of wool fiber is formed by multiple cross-links of keratin intermediate filaments with multiple keratin associated proteins. This information can inform decisions regarding the use of these keratin preparations to identify pathways for the addition of subsequent amino acids in a stepwise fashion to build keratin proteins of higher molecular weight with discrete functionalities. Samples 2 and 4 showed the absence of S-sulfonated forms while samples 3 and 4 showed the possibility of reactivity through cysteic acid functions. These wool keratin products produced from alkaline peroxide and their protein profiles point the way to subsequent reactivity for higher value-added products. The ability to produce these biological starting materials relatively quickly, at high yield, with processing ease from simple alkaline systems, while maintaining the basic structural integrity of keratin protein will confer new market potentials to wool.

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