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Keratin-associated protein micromaterials for medical and cosmetic applications

RAPID COMMUNICATION

Summary — Procedure of preparation of keratin associated protein micromaterials from hair, wool and bristle like natural sources has been developed. Procedure involves a combination of chemical activation and enzymatic digestion of natural substrates. Keratin associated proteins could be applied as microscaffolds in medicine and cosmetics.
Key words: keratin associated protein micromaterials, sheep wool, pig bristle, human hair.

MIKROMATERIAŁY BIAŁEK WSPÓŁWYSTĘPUJĄCYCH Z KERATYNĄ DO ZASTOSOWAŃ MEDYCZNYCH I KOSMETYCZNYCH
Streszczenie — Opracowano sposób otrzymywania mikromateriału białek współwystępujących z keratyną z naturalnego surowca włosów, wełny i szczeciny. Sposób otrzymywania polega na połączeniu chemicznej aktywacji i enzymatycznego trawienia naturalnych substratów. Otrzymane preparaty białek współwystępujących z keratyną mogą znaleźć zastosowanie jako mikroszkieletowy materiał w medycynie i kosmetyce.
Słowa kluczowe: mikromateriały białek współwystępujących z keratyną, wełna owcza, szczecina świni, włos ludzki.

In hair cortex, keratin intermediate filaments are embedded in an interfilamentous matrix, consisting of hair keratin associated proteins (KAP). They are essential for the formation of a rigid and resistant hair shaft through their extensive disulfide bond cross-linking between abundant cysteine residues of hair keratins. The matrix proteins include high-sulfur and high-glycine-tyrosine keratins [1, 2].

Human hair is structurally similar to wool or bristle. Its structure is composed of an external cuticular sheath, an inner cortex and a central medulla. Keratin proteins that belong to large keratin multigene family form central compartments. In the cortex region, keratins gradually aggregate and these aggregates interact with proteins present in an amorphous space called the matrix. The KAPs are the major component of the matrix. KAPs are essential for the formation of a rigid and resistant shaft through multi-disulfide bond cross-linking with abundant cysteine of KAPs. Various components of KAPs have been classified according to their sulfur and amino acids compositions as high-sulfur (16—30 mol. % of cysteine), ultra high-sulfur (>30 mol. % of cysteine) and high-glycine-tyrosine contaminations [3, 4].

Multiple disulphide bonds of KAPs make hair, wool and bristle exceptionally resistant to biological and chemical degradations. Apart of textile industry, KAPs containing natural substrates have been used as natural source for isolation of cysteine for cosmetic and chemical applications. However, often hair, bristle or low quality wool are classified as a waste. We elaborated a new technology of KAPs separation through isolation from enzymatically digested keratin proteins. The micromaterial KAPs form microscaffolds with three-dimensional structure dependent on the parent substrates. The obtained microscaffolds could be applied for a number of applications in medicine and cosmetics, including 3D tissue harvesting or as the carriers of biological by active substances in medicine or cosmetology [5—7].

Usually, application of proteins as structural biopolymers requires chemical modification resulting in increased stability of their 3D structures [8—10]. On the contrary, keratin associated proteins that contain multiple disulfide bridges have determined the structures that are extremely resistant to physicochemical and biochemical modifications. In the living organisms these structures are filled with other biological substances, including keratin proteins. These proteins can be removed by enzymatic digestion. Residual skeletal proteins might be applied for a number of applications in medicine and cosmetics.

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General procedure presented here for wool preparation could be adopted for other natural KAPs containing products.

EXPERIMENTAL

Materials

New Zealand sheep wool has been purchased from distributor in Poland. Pig bristles have been obtained from local slaughter-house. Not chemically treated hair has been donated by white male. Pepsin and other chemicals have been purchased from Sigma-Aldrich.

Preparation of keratin associated protein micromaterial

100 g of sheep wool was suspended in 3 % solution of NaOH for 1 hour at room temperature (21 ^{o}C). Next, wool was filtered off and washed twice with water. Then, wool was suspended in water which was acidified to pH = 2.1. 1.8 g of pepsin was added to the suspension, and reaction was continued at 37 ^{o}C with shaking for 24 hours. Residue was filtered off and dried. Dry material was ground to the small fragments (<0.2 mm). Then, solid material was digested again 24 hours with pepsin (1.5 g), washed with water and dried. The final product was filtered off, washed with water and dried. This product was defined as sheep keratin associated protein preparation (S-KAP).

The same general procedure was applied for preparation of keratin associated protein micromaterials from pig bristle (P-KAP) and from human hair (H-KAP).

Methods of testing

Scanning electron microscope (SEM) type JEOL JSM-6490 LV (Japan) has been used to examine the shape and surface morphology of the substrates and products. The transmission electron microscope (TEM) type JEOL-1200 EX (Japan) has been applied for the morphological ultrastructural analysis of the producs [11].

RESULTS AND DISCUSSIONS

The keratin associated protein micromaterials from wool, bristle and hair were prepared in two-stage gene-

T a b l e 1. The yields of the processes of preparation of keratin associated protein micromaterials

Kind of natural product	Yield of process, %		
	first step	second step	total
Sheep wool	44	77	34
Pig bristle	82	78	64
Human hair	83	92	76

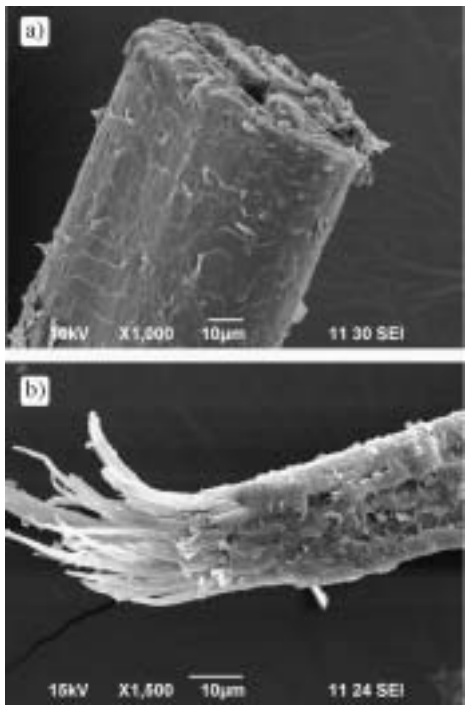

Fig. 1. SEM images of human hair before (a) and after (b) preparation

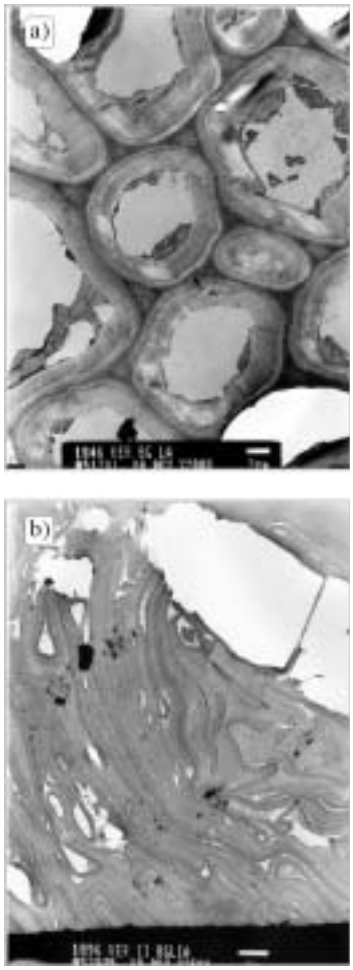

Fig. 2. TEM images of KAPs from sheep wool (a) and pig bristle (b)

ral procedure. First step was a chemical activation of natural substrates. In the second step water unsoluble intermediates were digested. The yields of particular steps of the process and total yields obtained for three kinds of used natural materials are listed in Table 1.

Introductory chemical activation of hair, wool or bristle makes them accessible for enzymatic digestion with proteases of keratin proteins. The permeability of enzyme into digested structures is limited. The grinding of intermediate material after first digestion allows to remove digestible proteins more effectively. The residual unsoluble materials of keratin associated proteins form three-dimensional stable structures. Figure 1 shows SEM micrographs of human hair and H-KAP obtained from it. The TEM micrographs of S-KAP and P-KAP are presented in Figure 2. Keratin associated protein materials obtained could be applied as scaffolds for various medical and cosmetic applications.

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REFERENCES

1. Elleman T. C., Dopheide T. A.: *J. Biol. Chem.* 1972, **247**, 3900.
2. Fratini A., Powell B. C., Rogers G. E.: *J. Biol. Chem.* 1993, **268**, 4511.
3. Powell B. C., Rogers G. E.: in: "Formation and Structure of Human Hair", (Eds: Jolles P., Zahn H., Hocker H.), Basel: Birkh 1997, pp 59—148.
4. Rogers M. A., Langbein L., Praetzel-Wunder S., Winter H., Schweizer J.: *Int. Rev. Cytol.* 2006, **251**, 209.
5. Jurga M., Lipkowski A. W., Lukomska B., Buzanska L., Kurzepa K., Sobanski T., Habich A., Coecke S., Gajkowska B., Domanska-Janik K.: *Tissue Eng*, in press.
6. Polish Pat. Appl. P381103 (2006).
7. Polish Pat. Appl. P380011(2006).
8. Tylingo R., Mazur-Sandomierska A., Sadowska M.: *Polimery* 2008, **53**, 576.
9. Sztuka K., Kołodziejska I.: *Polimery* 2008, **53**, 627.
10. Sztuka K., Kołodziejska I.: *Polimery* 2008, **53**, 725.
11. Gajewska M., Gajkowska B., Motyl T.: *J. Physiol. Pharmacol*. 2005, **56**, 143.

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