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Topics for Teaching: Chemistry in Nature

Horns, Scales, Beaks: The Versatility of Keratin

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Abstract: The structures of α- and β-keratins and their roles in nature are introduced, along with how demand for keratin-based products impacts on pangolin and rhinoceros conservation.

Keywords: Chemical education · Keratin · Wildlife conservation

In earlier columns in this series, we introduced protein structure[1] and the role that β-keratin plays in the setae of geckos.[2] Keratins are the most common structural proteins found in epithelial cells, *i.e.* the cells that constitute the protective animal tissue known as the epithelium. Keratins are insoluble, fibrous proteins with a high sulfur content – recall that disulfide bridges are important motifs in protein chemistry.[2] The classes of α- and β-keratins (defined on the basis of their X-ray diffraction patterns) differ in their secondary protein structure which is described by the presence of α-helices (Fig. 1a/b), β-sheets (Fig. 1c), turns and coils. Hydrogen bonding is critical to both the helical and sheet motifs shown in Fig. 1. In α-keratin, α-helix chains twist around one another to give double helices and these assemble together to form a protofilament. The formation of disulfide bridges is fundamental to this assembly process. Four protofilaments are then aligned along their long axes to produce an intermediate filament which is based on a coiled-coil structure similar to that found in mantis egg cases.[1] In β-keratin, β-sheets are the dominant secondary protein structure. Each polypeptide chain folds to give four β-strands which pack side by side with C=O...H–N hydrogen bonds stabilizing the assembly (Fig. 1c); an antiparallel arrangement of β-strands gives rise to the most stable assembly.[3] The β-sheet has a pleated (non-planar) profile, and two sheets associate together to form a filament of β-keratin.

Both α- and β-keratins exhibit a nanoscale structure. The β-keratin intermediate filaments possess a 7 nm diameter, while the β-keratin filaments composed of β-pleated sheets have a 3 nm diameter. The different structures and properties of β- and β-keratins lead to different functions in nature. These are summarized in Fig. 2.

The structure of keratin can be considered as a polymer/polymer composite made up of crystalline filaments embedded in an amorphous matrix.[3] Keratins represent some of the toughest biological materials, and Figs. 3 and 4 illustrate three of nature's applications of these proteins. Pangolin scales (Fig. 3) contain both α- and β-keratins, while the beak of a hornbill and the horn of the rhinoceros (Fig. 4) are comprised of β- and α-keratin, respectively. Pangolins are unique in possessing defensive scales composed of keratin; in contrast, the protective plates of South American armadillos are composed of bone, covered with a layer

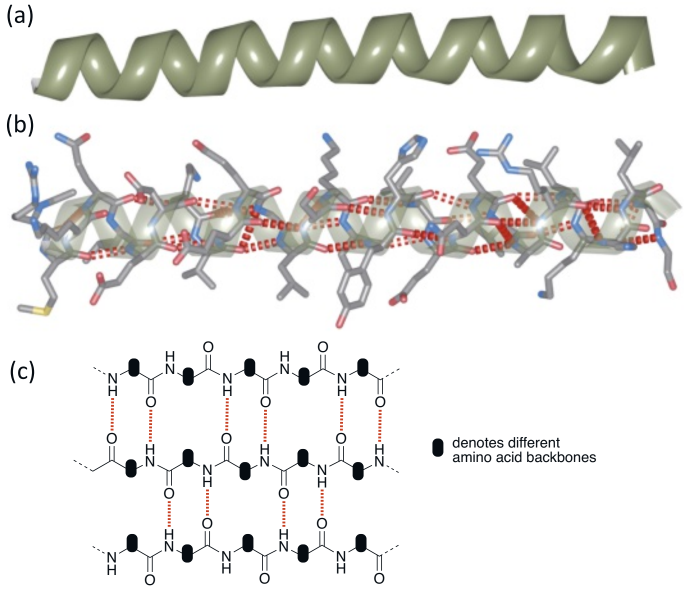

Fig. 1. (a) Representation of the α-helical secondary structure of a protein. (b) The α-helix is supported by C=O...H–N hydrogen bonds (red hashed lines) between peptide residues. (c) Part of a b-sheet showing hydrogen bonding between anti-parallel b-strands of polypeptide chains.

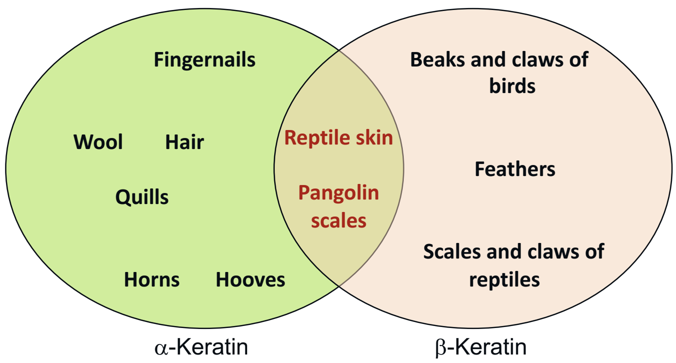

Fig. 2. Functional roles of α- and β-keratins. Reptile skin and pangolin scales contains both α- and β-keratins. Reptile skin and pangolin scales combine both types of keratin structure.

of keratin. Pangolin scales overlap with one another to give an efficient body armour, especially when the pangolin curls up in a defensive position (inset in Fig. 3), *e.g.* when attacked by a predator. The scales exhibit transverse isotropic properties in the plane of the scale surface and this is associated with a crossed protein fibre structure. The mechanical properties of pangolin scales have been discussed in detail,[4] but are beyond the scope of this article.

Fig. 3. A ground pangolin (*Smutsia temminckii*); the inset (top left) shows a pangolin rolled up with its defensive scales protecting its body. Photo credit: Ruan Roos, Jabulani Safari.

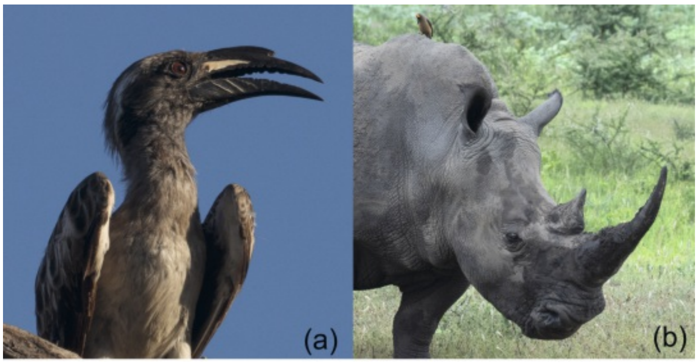

Fig. 4. (a) An African grey hornbill (*Tockus nasutus*) and (b) a white rhinoceros (*Ceratotherium simum*). Photo credit: Edwin Constable.

Fig. 4a shows an African grey hornbill and the large size and appearance of its beak are typical of all hornbills. Despite the size of the beak, its construction and the biomaterials from which it is composed render it lightweight but strong. The structure and material properties of the beak of a Wreathed Hornbill (*Rhyticeros undulatus*) have been studied in detail.[5] The beak consists of a fibrous network of cells composed of calcium-rich proteins with an external layer of β-keratin comprising hexagonal scales. The overall density of the beak material is 0.3–0.4 g cm^{-3}.[4] The horn of a rhinoceros (Fig. 4b) comprises fibres of α-keratin which form tubular structures embedded in a matrix of dead skin keratinocyte cells, *i.e.* the horn is completely composed of keratin. Each fibre within the horn is around 200 μm (200,000 nm) in diameter.[6] Compare this with the 7 nm diameter of a single α-keratin intermediate filament.

The survival of all five species of rhinoceros (Sumatran, Javan and Indian rhinoceros and the African black and white rhinoceros) is of critical concern because of illegal trade in rhinoceros horn. This is driven both by its use in Asian medicine and as a raw material for carved art pieces. Traditional Chinese medicine is also linked to a demand for pangolin scales, and during the period 2010 to 2015, over 55,000 kg of scales were confiscated from international illegal trading; this corresponds to *ca.* 100,000 pangolins.[7] Like the rhinoceros, all species of pangolin (eight worldwide) are threatened with extinction if illegal trading continues. A recent investigation concluded that raising awareness among practitioners of traditional Chinese medicine of the illegal sourcing of pangolin scales and encouraging the use of alternative substitutes could be highly effective in pangolin conservation efforts.[7] Where can chemists and materials scientists help? Among the different approaches possible, we highlight two. The first involves development of substitute products to intercept the illegal market, and the second is the use of a specialist mass spectrometric technique to detect different keratin types.

In 2019, Vollrath and coworkers described the use of hair from the tail of a horse to mimic the fibres in rhinoceros horn. Horsetail hair was chosen because of its keratin composition, similar mechanical properties to rhinoceros horn fibres, and comparable dimensions and density. One significant difference in the fibres was an outer scaly layer on horsehair and this was removed by immersing the hair in aqueous LiBr solution. The horsehairs were then combined using a natural protein (regenerated silk fibroin produced by silkworms) as a matrix to produce a solid composite biomaterial in the form of horn-shaped pieces. After drying, the synthetic 'horns' possessed both an external appearance and an internal micro-structure that mimicked rhinoceros horn extremely well.[6] This investigation is proof-of-concept of the production of a synthetic material that could, in principle, be used to confuse the market of illegally traded rhinoceros horn.

In order to enforce the regulations surrounding the trade of rhinoceros horn and pangolins, it is essential to correctly identify pristine material. This can be problematic because of trade in imitation products as detailed above. Trials with the specialized technique of direct analysis in real time (DART) ionization paired with time-of-flight mass spectrometry (DART-TOFMS) have shown that the mass spectra of keratins for rhinoceros horn keratin, bovid (cow) horn keratin, domestic horse hoof keratin and pangolin scale keratin are characteristic, enabling the types to be distinguished. Advantages of the technique are that solid samples can be used directly without particular sample preparation, analysis is fast with a result in less than a minute, and the cost of sample analysis is relatively low.[8]

In this Education Column, we have extended our earlier descriptions of protein structure[1,2] to introduce the structure and versatility of α- and β-keratins. This links directly into the conservation of endanered species, with a particular emphasis on the rhinoceros and pangolin.

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This column is one of a series designed to attract teachers to topics that link chemistry to Nature and stimulate students by seeing real-life applications of the subject.