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ORIGINAL ARTICLE

Skin lipids from Saudi Arabian birds

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Abstract Skin lipids play an important role in the regulation of cutaneous water loss (CWL). Earlier studies have shown that Saudi desert birds exhibit a tendency of reduced CWL than birds from temperate environment due to adaptive changes in composition of their skin lipids. In this study, we used thin-layer chromatography (TLC) for separation and detection of non-polar and polar lipids from the skin of six bird species including sooty gull, brown booby, house sparrow, Arabian waxbill, sand partridge, and laughing dove. The lipids were separated and detected on Silica gel G coated TLC plates and quantified by using densitometric image analysis. *Rf* values of the non-polar lipids were as follows: cholesterol (0.29), free fatty acids (0.58), triacylglycerol (0.69), fatty acids methyl esters (0.84) and cholesterol ester (0.97). *Rf* values for the polar lipids were: cerebroside (0.42), ceramide (0.55) and cholesterol (0.73). The results showed the abundance of fatty acids methyl esters (47.75–60.46%) followed by triacylglycerol (12.69–24.14%). The remaining lipid compositions were as follows: cholesterol (4.09–13.18%), ceramide (2.18–13.27%), and cerebroside (2.53–12.81%). In conclusion, our findings showed that TLC is a simple and sensitive method for the separation and quantification of skin lipids. We also reported a new protocol for lipid extraction using the zirconia beads for efficient disruption of skin tissues. This study will help us better understand the role of skin lipids in adaptive physiology towards adverse climatic conditions.

1. Introduction

The outermost layer of the skin, the stratum corneum, is composed of flat, dead cells called corneocytes embedded within a lipid matrix (Bouwstra, 1997), which function collectively as the primary barrier to water vapour diffusion from the animal to the environment, a process known as cutaneous water loss (CWL) (Menon et al., 1992; Simonetti et al., 1995).

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The stratum corneum is composed of corneocytes, flattened dead cells embedded in a matrix of lipids, primarily ceramides, cholesterol and free fatty acids (Bouwstra et al., 2003) that are organized in bilayer or lamellae that minimizes water permeation through the skin (Wertz, 2000; Bouwstra et al., 2003).

It has been observed that house sparrows from the desert of Saudi Arabia exhibit 25% lower rates of CWL than temperate-zone American sparrows; this adaptive physiology has been linked to the higher percentages of both intercellular and covalently bound cerebrosides in the desert species (Gu et al., 2008; Munoz-Garcia and Williams, 2005). Due to the climate change in Saudi Arabia towards extreme temperatures, it is expected that over the long term the desert birds in this country will likely be forced to further reduce their evaporative water losses if they are to remain in their present habitat (Williams et al., 2012). How these desert birds will resolve the anticipated conflicting demands of water shortage and thermoregulation remains unknown and is a subject of further research.

Studies across a number of bird species from multiple environments may help us understand how skin lipids are organized in birds relative to CWL. Desert birds tend to have a greater proportion of more polar ceramides with longer fatty acid tails (Schaefer and Redelmeier, 1996). Haugen et al. (2003) compared intercellular lipid composition in eight species of larks across an aridity gradient however cerebrosides were not tested in that study. Champagne et al. (2012) have found that birds in arid environments have lower rates of CWL than birds from mesic environments. These differences in water loss may be explained by differences in the interactions between and among intercellular lipids, while a negative correlation between cerebrosides and CWL has been noticed (Champagne et al., 2012). Thus, considering an important role of skin lipids in climatic tolerance and adaptive physiology, it is intriguing to investigate the composition of skin lipids of Saudi Arabian birds.

Thin-layer chromatography (TLC) is a simple and rapid technique that has been used for the analysis of lipids (Macala et al., 1983; Ruiz and Ochoa, 1997; Fewster et al., 1969; Rawyler and Siegenthaler, 1980). In continuation to our previous work on thin-layer chromatographic analysis of biomolecules (Khan, 2006, 2007; Rathore and Khan, 1987, 1988; Rathore et al., 1988), we have performed TLC separation and detection of non-polar and polar lipid fractions in epidermal skin of six bird species from Saudi Arabia.

2. Materials and methods

2.1. Birds and sample collection

Sea birds (sooty gull and brown booby) were collected from an island near Jeddah, Saudi Arabia. Other four birds including house sparrow, Arabian waxbill, sand partridge and laughing dove were collected from the local market. The description of birds is given in Table 1. The birds were sacrificed and the feathers of the ventral side were removed. The skin of the ventral region (abdominal area) was thoroughly washed with antibacterial soap and specimens of 1.5–2.0 cm^2 size were cut and placed in media. The samples were stored at −80 °C until analyzed.

2.2. Extraction of skin lipids

Frozen skin samples were placed in cold phosphate buffered saline (PBS) and the subcutis portion was scratched off using a scalpel. About 30 mg of minced skin sample was placed in a 2 ml vial containing 0.5 mm zirconia beads and ice-cold 0.5 ml methanol with 0.01% butylated hydroxytoluene (BHT). The vial was placed in the slot of a minibead beater and the tissue was homogenized for 1 min at 4200 rpm. The homogenate was mixed with 1 ml chloroform and the contents were shaken overnight. Then 250 µl of 0.15 M ammonium acetate was added and the contents were re-homogenized for 1 min at 4200 rpm. The upper layer was removed and 1 ml of the lower layer was transferred to an eppendorf tube. The samples were evaporated to dryness in a rotary evaporator at 45 °C and reconstituted with 100 µl of chloroform:methanol (2:1) containing 0.01% BHT. The tubes were centrifuged at 2000 rpm for 2 min before performing the TLC.

2.3. Thin-layer chromatography

We used commercially available TLC plates (20 × 20 cm) pre-coated with 0.25 mm thick layer of silica gel G (Adsorbosil-Plus 1, Altech, Deerfield, IL, USA). The plates were preconditioned by running in chloroform:methanol (2:1) until the solution reached the top. The plates were activated by keeping them in an oven (110 °C) for 30 min. Aliquot (5 µl) of standard solution of lipids' mixture and skin sample homogenates were applied onto the plate and the plate was allowed to dry for 5 min before developing in the mobile phase.

For separation of nonpolar lipids, the TLC plate was developed in hexane:ethylether:acetic acid (80:20:2) to the top. For separation of polar lipids, the TLC plates were placed in chloroform:methanol:water (60:40:5) and run to 10 cm distance from the bottom, followed by chloroform:methanol:acetic acid (190:9:1) and run to 15 cm from the bottom, and finally placed in hexane:ethylether:acetic acid (70:30:1) and run to the top. The plate was allowed to dry for about 10 min between the successive runs and after the final run. The plate was then sprayed with cupric acetate solution (3% cupric acid + 8% phosphoric acid in distilled water) and placed in an oven at 180 °C for 30 min. The lipid bands appear grey on the white background. *Rf* values were calculated using the formula, *Rf* = distance travelled by the analyte/distance travelled by the mobile phase.

2.4. Densitometry

The TLC plate was wrapped in a transparent plastic and the coating side was scanned using a scanner. The image was saved in a computer and the intensities of lipid-specific spots were quantified using Image J software (National Institute of Health, USA).

3. Results

The images of TLC plates displaying the separations of non-polar and polar lipids are shown in Figs. 1 and 2 respectively. The *Rf* values of non-polar lipids were as follows: cholesterol (0.29), free fatty acids (0.58), triacylglycerol (0.69), fatty acids