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Maple syrup urine disease hair reveals the importance of 18-methyleicosanoic acid in cuticular delamination

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Abstract

18-Methyleicosanoic acid (18-MEA) is thought to be covalently bound to the outer surface of human hair and is a major component of the outer β-layer of the cuticular cell membrane complex (CCMC). Cuticular delamination, whether this occurs between the outer β- and δ-layers or within the plane of the β-layer, results in a fresh layer of 18-MEA being exposed at the newly-revealed surface. Hair from patients with Maple Syrup Urine Disease (MSUD), however, does not contain 18-MEA and here, we report on the importance this unusual fatty acid in cuticular delamination. Hair fibres were collected from 10 patients with classic (type 1A) MSUD from a Mennonite community in Pennsylvania, USA. Included amongst these were hairs from dizygotic twins (A1 and A2), one of whom had MSUD, and the other did not; it was unknown at the beginning of the study which twin had MSUD. The outer surfaces were examined using atomic force microscopy (AFM) and transverse sections imaged using transmission electron microscopy (TEM). The newly revealed intercellular surface regions from twin A2 were found to be significantly rougher than those from twin A1. TEM studies showed the trilamellar CCMC to be continuous for twin A1, but possessed discontinuities of variable length (100–1000 nm) for twin A2. In contrast with other work, TEM showed no specific defects in the outer β-layer. The outer cuticular surfaces for most MSUD patients showed a great abundance of residual endocuticle, although in other cases this was less pronounced. These differences may be explained by some residual activity of the branched-chain α-ketoacid (BCKD) dehydrogenase. Cuticular delamination in MSUD-hair probably still occurs within the general plane of the CCMC, although fracture through discontinuities of this layer results in zones of endocuticle being exposed at the new surface.

Keywords: 18-Methyleicosanoic acid (18-MEA); Atomic force microscopy (AFM); Cuticle; Maple syrup urine disease (MSUD); Hair; Transmission electron microscopy (TEM)

1. Introduction

The discovery of fatty acids found covalently-linked to the surfaces of keratin fibres (Evans et al., 1985; Wertz and Downing, 1988, 1989) has led to much renewed interest in the surface chemistry and properties of these materials (Jones and Rivett, 1997; Swift, 1999). 18-Methyleicosanoic acid (18-MEA), an unusual, branched-chain fatty acid, is a major component of the outer β-layer of the cuticular cell membrane complex (CCMC), located on the underside of the δ-layer. The layer of 18-MEA is thought to be covalently bound, probably via a thioester or ester linkage, to the underlying proteinacous epiciticle (Evans and Lanczki, 1997) (Fig. 1). Interestingly, the inner β-layer, of the CCMC, does not contain 18-MEA and is thought to be comprised mainly of the straight-chain fatty acids, palmitic (C16:0) and oleic (C18:0) acids (Jones and Rivett, 1997). The precise role of 18-MEA is uncertain (Jones and Rivett, 1997), although the large segmental volume of the anteiso-terminus is expected to exhibit considerable molecular mobility, resulting in fluid-like behaviour at cuticle surfaces (Swift, 1999) and a possible explanation for the observed differential friction effect (DFE) of hair (Swift, 1999; Adams et al., 1990). These properties have been described as being important for hair detanglement, alignment

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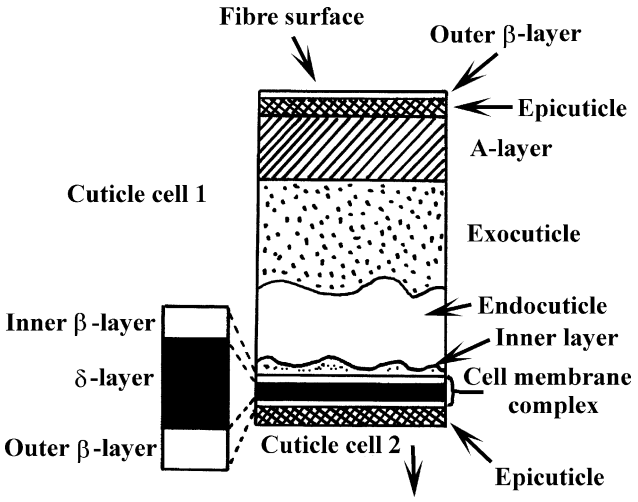

Fig. 1. Schematic diagram of the lamellar sub-structure of the human hair cuticle. (Reproduced with permission (Swift and Smith, 2001)).

and rejection of scalp detritus (Martin, 1944) and are of obvious commercial importance to the hair toiletries industry.

Whether the β-layer is of single lipid layer thickness or is present as a lipid bilayer (Swift and Holmes, 1965) is uncertain. Either (a) there is only one layer of lipid covalently attached to the adjacent cell with it is outer region abutting the δ-layer, or (b) there are two layers of intercalated lipid with one layer covalently attached to the adjacent cell and the other (covalently) attached to the δ-layer. A weak point for cuticular delamination is therefore expected at the CCMC, either at the interface between the outer β- and δ-layers (single lipid model) or within the outer β-layer (lipid bilayer model). Regardless of the cleavage pathway, 18-MEA provides at least one side of the fracture plane and it is this fatty acid that will be exposed at the newly revealed fibre surface.

The absence of 18-MEA from hairs of patients with Maple Syrup Urine Disease (MSUD) provides an interesting route to investigate the importance of this anteiso, branched-chain fatty acid (Jones et al., 1996). MSUD, first described by Menkes et al. in 1954 (Menkes et al., 1954), is an inherited metabolic and progressive neurological degenerative disorder where decarboxylation of branched-chain α-ketoacids derived from leucine, isoleucine and valine is blocked (Dancis et al., 1960). The accumulation of these branched chain fatty acids to toxic levels will result if not controlled through special dietary arrangement (Chuang, 1998). MSUD is caused through various mutations that can occur in the E1α, E1β, E2 and E3 loci of the mitochondrial branched-chain α-ketoacid (BCKD) dehydrogenase complex (Chuang, 1998). The disorder has five distinct phenotypes (Chuang and Shih, 1995), of which the classic type, first described by Menkes (Menkes et al., 1954), is the most severe with the lowest residual activity of 0–2% of normal (Chuang, 1998). Higher residual activities, 3–30%, are found for the milder intermediate and intermittent types of the disorder (Dancis et al., 1972).

Although MSUD is a rare autosomal disease (1 in 185 000) (Silao et al., 2004), in the Mennonite communities of Pennsylvania, USA, the occurrence is 1 in 176 of live consanguineous births (DiGeorge et al., 1982; Fisher et al., 1991; Matsuda et al., 1990). Mennonite MSUD is of the classic type (type 1A), where a specific mutation, a Y393N substitution, occurs in the E1α loci causing degradation of the E1β subunit (Fisher et al., 1991; Matsuda et al., 1990).

Mutations in the BCKD enzyme result in the inability of 18-MEA to be biosynthesised from its precursor, isoleucine (Jones et al., 1996). In MSUD hair, 18-MEA is replaced by straight-chain fatty acids, such as *n*-eicosanoic acid (15–19%) (Jones et al., 1996). High-resolution transmission electron microscopy (TEM) studies of transverse-sections of hair obtained from MSUD patients have show a structural defect in the outer β-layer that have led to the conclusion 18-MEA was closely associated with this layer (Jones et al., 1996).

In this study, hair fibres were collected from 10 patients with classic (type 1A) MSUD from a Mennonite community in Pennsylvania, USA. The outer surfaces were examined using atomic force microscopy (AFM) (Smith, 1998; Smith and Swift, 2002; Swift and Smith, 2000, 2001) and transverse sections imaged using TEM to investigate the importance of 18-MEA in cuticular delamination.

2. Experimental

Small tresses of scalp hairs from 10 classic-MSUD patients (neonates and adults) were obtained from the Clinic For Special Children, Strasburg, PA, USA. Included among these were hairs from dizygotic twins (A1 and A2) where one twin had MSUD and the other did not. Not until the completion of the work was it revealed which twin had MSUD. Ten hairs from each of the 10 subjects were placed in an aqueous solution of sodium dodecylsulphate (1%) and agitated in an ultrasonic bath for 2 min, rinsed extensively with double-distilled water and dried in a gentle stream of nitrogen. This procedure in known to be very effective in removing detritus from hair surfaces (Swift, 1991).

For TEM studies, hairs from the dizygotic twins (A1 and A2) were embedded without pre-treatment in size 0 gelatin capsules using Spurr's resin (Spurr, 1969). Transverse sections of ca. 60 nm thickness were cut on a diamond knife using a Reichert-Jung 'Ultracut' ultramicrotome and collected on 400-mesh gold electron microscope grids previously coated with a thin collodion supporting membrane. The grids were stained by immersing for 2 h at room temperature in a saturated solution of phosphotungstic acid (PTA) in 50% ethanol. These were rinsed for 5 s in 50% ethanol and then rapidly drained to dryness on the edge of a filter paper. The grids were examined in a ZEISS EM109 transmission electron microscope operated at 50 keV