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The bisretinoids of retinal pigment epithelium

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ABSTRACT

The retina exhibits an inherent autofluorescence that is imaged ophthalmoscopically as fundus autofluorescence. In clinical settings, fundus autofluorescence examination aids in the diagnosis and follow-up of many retinal disorders. Fundus autofluorescence originates from the complex mixture of bisretinoid fluorophores that are amassed by retinal pigment epithelial (RPE) cells as lipofuscin. Unlike the lipofuscin found in other cell-types, this material does not form as a result of oxidative stress. Rather, the formation is attributable to non-enzymatic reactions of vitamin A aldehyde in photoreceptor cells; transfer to RPE occurs upon phagocytosis of photoreceptor outer segments. These fluorescent pigments accumulate even in healthy photoreceptor cells and are generated as a consequence of the light capturing function of the cells. Nevertheless, the formation of this material is accelerated in some retinal disorders including recessive Stargardt disease and ELOVL4-related retinal degeneration. As such, these bisretinoid side-products are implicated in the disease processes that threaten vision. In this article, we review our current understanding of the composition of RPE lipofuscin, the structural characteristics of the various bisretinoids, their related spectroscopic features and the biosynthetic pathways by which they form. We will revisit factors known to influence the extent of the accumulation and therapeutic strategies being used to limit bisretinoid formation. Given their origin from vitamin A aldehyde, an isomer of the visual pigment chromophore, it is not surprising that the bisretinoids of retina are light sensitive molecules. Accordingly, we will discuss recent findings that implicate the photodegradation of bisretinoid in the etiology of age-related macular degeneration.

Contents

Abbreviations: ABCA4, ATP-binding cassette, sub-family A, member 4; DHA, docosahexaenoic acid; ESI-MS, electrospray ionization mass spectrometry; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSH, glutathione; mSOD2, mitochondrial superoxide dismutase-2; NRPE, *N*-retinylidene-phosphatidylethanolamine; PE, phosphatidylethanolamine; RDH, retinol dehydrogenase; RPE, retinal pigment epithelium.

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1. The origin of RPE lipofuscin

The lipofuscin of retinal pigment epithelial (RPE) cells is amassed with age in organelles of the lysosomal compartment of the cells, in both healthy and diseased retina. It is generally agreed that this material originates, for the most part, from ingestion of shed photoreceptor outer segment membrane. Historically, however, opinions as to the molecular composition of RPE lipofuscin, have differed (Chio et al., 1969; Chio and Tappel, 1969; Chowdhury et al., 2004; Eldred and Katz, 1991, 1989), with one notion being that it consists of oxidatively modified lipid. Carboxyethyl pyrrole protein (CEP)-adducts, modifications that are generated from the oxidation of docosahexaenoate-containing lipids in photoreceptor cells have been detected in RPE lipofuscin (Ng et al., 2008). Presumably, the photooxidative process responsible for generating these carboxyethyl pyrrole-protein adducts could occur in photoreceptor cells before RPE phagocytosis of outer segment membrane or could occur within the lysosomal bodies in which RPE lipofuscin is stored, or both. The notion that oxidation processes are otherwise responsible for the formation of RPE lipofuscin, is not consistent with what is known regarding the composition of this material. For instance, the spectral properties of the blue–green emitting fluorescent products of lipid oxidation are substantially different (excitation maxima ~350, emission maxima ~435 nm) (Rein and Tappel, 1998) than spectra generated from RPE lipofuscin (Eldred and Katz, 1991; Eldred et al., 1982). Specifically, spectra obtained with intact RPE cells, RPE extracts (Eldred and Katz, 1991; Eldred et al., 1982), explants of adult human eyes (Delori et al., 1995) and suspensions of lipofuscin storage bodies (Boulton et al., 1990; Feeney-Burns and Eldred, 1983), have all demonstrated that RPE lipofuscin emits with an emission maximum of approximately 590–600 nm (yellow–orange) when excited by light in the 'blue' region of the spectrum. It has also been assumed that RPE lipofuscin consists of cross-linked oxidatively modified proteins (Brunk and Terman, 2002) derived from phagocytosed photoreceptor outer segments. However, Feeney-Burns and colleagues (Eldred and Katz, 1991; Eldred et al., 1982) and Boulton and colleagues (Boulton, 2009) have pointed out that the presence of photoreceptor proteins in preparations enriched in lipofuscin granules (lysosomal organelles) (Schutt et al., 2002; Warburton et al., 2005), is attributable to contamination with phagosomes. Moreover, a proteomic study of purified lipofuscin granules revealed that the amino acid content was only 2% (w/w) (Ng et al., 2008). It is also worth noting that the view that RPE lipofuscin accumulates because of inhibition of lysosomal enzymes, cannot be reconciled with the accumulation of this material in all healthy eyes even at young ages. Instead, it is likely that RPE lysosomal enzymes that would otherwise degrade the bisretinoid, do not recognize the unprecedented structures that constitute this material.

2. The composition of RPE lipofuscin

2.1. Known bisretinoids of retina

Considerable evidence has accumulated that, unlike the lipofuscin that accumulates in other non-dividing cells (Brunk and Terman, 2002), the lipofuscin pigments in RPE originate in photoreceptor cell outer segments (Katz et al., 1986) from random non-enzymatic reactions of retinaldehyde (Katz et al., 1987) (Fig. 1). Indeed, all of the constituents of RPE lipofuscin that have been isolated and characterized have been shown to form in this manner (Sparrow, 2007b; Sparrow et al., 2010a). The transfer from photoreceptor cell to RPE occurs with phagocytosis of shed outer segment membrane. Currently at least 25 bisretinoid pigments can be identified chromatographically and by mass spectrometry; these compounds can be grouped within 4 families: 1) A2E, the first RPE lipofuscin constituent to be described; 2) A2-glycerophosphoethanolamine (A2-GPE) a recently characterized pigment (Yamamoto et al., 2011); 3) A2-dihydropyridine-phosphatidylethanolamine (A2-DHP-PE); 4) all-*trans*-retinal dimer, all-*trans*-retinal dimer-phosphatidylethanolamine and all-*trans*-retinal dimer-ethanolamine ADD SAKAI (Ben-Shabat et al., 2002b; Fishkin et al., 2005; Kim et al., 2007a, 2007b; Liu et al., 2000; Parish et al., 1998; Wu et al., 2009; Yamamoto et al., 2011) (Fig. 1). Other chromatographic peaks having fluorescence and absorbance properties suggestive of bisretinoid, can be observed but the structures of the corresponding compounds are not yet known. The 25 peaks mentioned above include known isomers (e.g. *cis* isomers of A2E), some of the photooxidized forms of these bisretinoids and biosynthetic intermediates such as A2PE (Fig. 1, compound 7) and dihydropyridinium-A2PE (Fig. 1, compound 5) (Section 3.2) (Fig. 1). Not included in this number, however, are bisretinoids differing in terms of the variety of fatty acid moieties that constitute the phospholipid-derived tails of some of these fluorophores (Section 3.2). The bisretinoids in RPE that retain the phospholipid moiety include all-*trans*-retinal dimer-PE and A2-DHP-PE (Fig. 1, compounds 6 and 10). A2E (Fig. 1, compound 8) on the other hand, does not carry fatty acids, since it is generated when its immediate precursor, the phosphatidylethanolamine-bisretinoid A2PE, undergoes phospholipase D-mediated enzymatic hydrolysis (Ben-Shabat et al., 2002b; Liu et al., 2000; Sparrow et al., 2008).