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Review

Xanthophylls as modulators of membrane protein function

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ABSTRACT

This review discusses the structural aspect of the role of photosynthetic antenna xanthophylls. It argues that xanthophyll hydrophobicity/polarity could explain the reason for xanthophyll variety and help to understand their recently emerging function – control of membrane organization and the work of membrane proteins. The structure of a xanthophyll molecule is discussed in relation to other amphiphilic compounds like lipids, detergents, etc. Xanthophyll composition of membrane proteins, the role of their variety in protein function are discussed using as an example for the major light harvesting antenna complex of photosystem II, LHCII, from higher plants. A new empirical parameter, *hydrophobicity parameter* (H-parameter), has been introduced as an effective measure of the hydrophobicity of the xanthophyll complement of LHCII from different xanthophyll biosynthesis mutants of *Arabidopsis*. Photosystem II quantum efficiency was found to correlate well with the H-parameter of LHCII xanthophylls. PSII down-regulation by non-photochemical chlorophyll fluorescence quenching, NPQ, had optimum corresponding to the wild-type xanthophyll composition, where lutein occupies intrinsic sites, L1 and L2. Xanthophyll polarity/hydrophobicity alteration by the activity of the xanthophyll cycle explains the *allosteric* character of NPQ regulation, *memory of illumination* history and the *hysteretic* nature of the relationship between the triggering factor, ΔpH, and the energy dissipation process.

Introduction

Oxygenated carotenoids, xanthophylls, are the most common pigments that occur in nature. Indeed, xanthophylls are found in almost all types of life forms from bacteria, fungi and algae, to plants and animals, including humans. The wide distribution of xanthophylls among different phylogenetic kingdoms and indeed their broad structural variety likely reflect the multiple functions of these organic molecules in living organisms. Apart from color and odor, two crucially important features exploited by life forms in various adaptive and communicative traits, xanthophylls have been proposed to possess strong photoprotective and antioxidant properties. Indeed, the literature dedicated to these two related functions of xanthophylls based on their interaction with light and reactive oxygen species is abundant (for reviews see [1–8]). There is, however, another property of these molecules – hydrophobicity, which remains largely overlooked in studies of the functional aspects of xanthophylls. Molecular structure is not just a determinant of reactivity or interaction with light but is also likely to be an important factor governing partitioning, orientation and interaction of xanthophylls with other biological molecules such as lipids and in particular, proteins. This review will argue that the structural property of xanthophylls, hydrophobicity, explain both their variety and their emergent function as modulators of membrane protein organization and function.

Amphiphilic nature of the xanthophyll structure

Xanthophylls can be considered to be amphiphilic molecules, since they possess hydrophobic as well as hydrophilic structural components (Fig. 1), similar to membrane lipids. Indeed, xanthophylls are often found in biological membranes. Observation of the molecular structures of various membrane-associated hydrocarbon molecules reveals certain structural similarities between them. Membrane lipids possess fatty acid hydrocarbon tails, often saturated, attached to the hydrophilic component via ester bonds (Fig. 1a). The saturation allows molecular structure to be flexible, enabling free rotations around single C–C bonds. Non-polar detergents mimic the lipid structure with the exception of having normally shorter and unbranched fatty acid tails (Fig. 1a). The fatty acid residues in the lipids of *Archaea* possess hydrocarbon tails which are partially *methylated* (Fig. 1a), which enhances the strength of hydrophobic interaction between the fatty acid tails making the membrane of *Archaea* generally more resistant to a number of abiotic environmental stress factors, such as extremes of temperature, pH, etc.

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The major photosynthetic pigments, chlorophylls, can themselves be considered to be lipid-like molecules. Their hydrophobic components, phytol tails, are almost entirely identical to the fatty acids of *Archaea*, with methylation of the hydrocarbon chain (Fig. 1a). In addition, the phytol tail has a non-saturated carbon bond. Many fatty acids also possess a certain number of C=C bonds, which make the molecular structure more rigid since the rotation around those bonds is restricted.

The xanthophyll molecules of certain prokaryotes, such as purple bacteria, possess certain features that resemble the structure of non-polar detergents albeit with the long non-saturated fatty acid tails. One such example is rhodopin glucoside of LH2[1], shown in Fig 1b. The xanthophyll spiriloxanthin of LH1 complex is, however, a more symmetric structure compared to rhodopin glucoside with polar groups present at both ends of the molecule. The length of this molecule is such that it can easily span the membrane. While the polar groups enable transmembrane orientation, the long methylated hydrocarbon chain can build strong interactions with fatty acid tails. Indeed, experimental evidence suggests that xanthophylls affect membrane fluidity and permeability to ions [8,9] – a consequence of strong binding to lipids. Regular unsaturation of C–C bonds makes the xanthophyll molecules very inflexible, enabling them to act as "pillars" supporting and, most likely, shaping the tertiary structure of membrane proteins. Indeed, xanthophylls have been found to be vital for assembly of several photosynthetic membrane proteins (for review see [10]). Presence of polar groups on both ends of generally hydrophobic xanthophyll structure creates the balance of forces, which ensure membrane-spanning orientation similar to that for the integral transmembrane helices of membrane proteins.

The photosynthetic membranes of eukaryotes, such as higher plants, often contain a wider variety of xanthophylls than those of prokaryotes and their end-groups are normally cyclized [11]. Fig. 1c shows structures of four major xanthophylls of higher plants: zeaxanthin (Zea), lutein (Lut), violaxanthin (Vio) and neoxanthin (Neo). All structures are fairly symmetric apart from Neo, which possesses an allene group on one end and is 9-*cis* on the opposite end. The lutein molecule is also asymmetric. It possesses two different types of end-groups, β- and ε-rings, which differ by the position of the double bond within the ring, whilst zeaxanthin contains two β-ring end-groups. Violaxanthin is enzymatically de-epoxidized into zeaxanthin upon exposure of the photosynthetic membrane to excess light, a process that is reversed upon restoration of low light conditions [12,13]. As a result, an intermediate xanthophyll, antheraxanthin, which carries only one epoxy group, is transiently formed. Hence, the variety of xanthophylls in the higher plant photosynthetic membrane is achieved mainly by variation in the number and positioning of polar groups/oxygen atoms and local isomerization of the end-groups relative to the conjugated C=C chain.

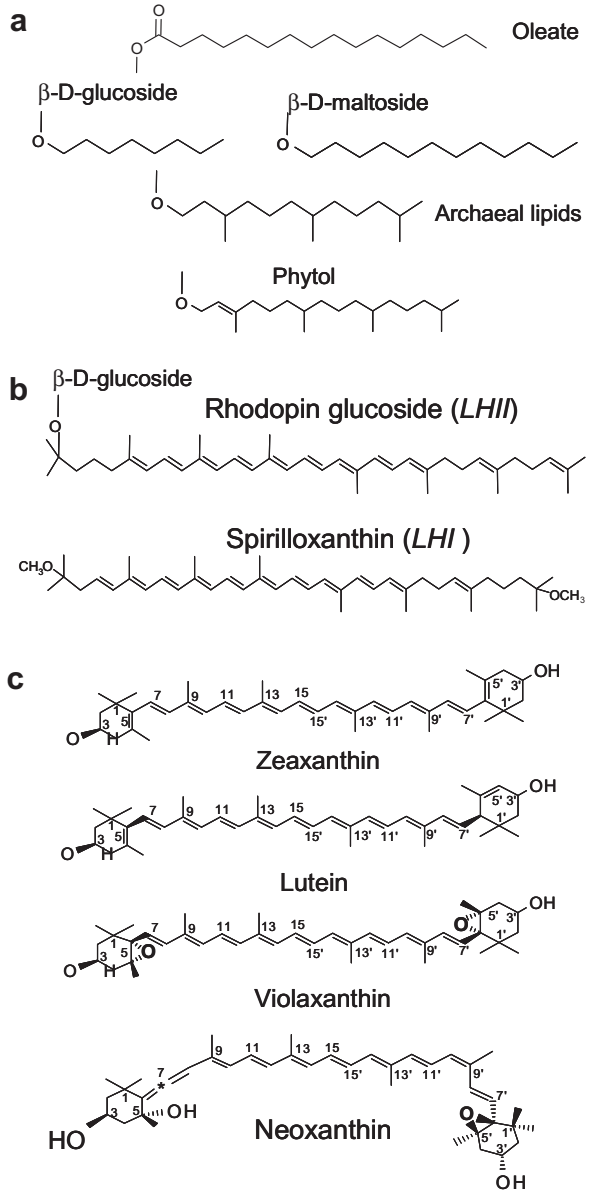

Fig. 1. Structures of various membrane-associated hydrocarbon molecules. Molecular evolution of the xanthophyll structure.

Xanthophyll binding to membrane proteins

Xanthophylls are often isolated as intrinsic components of membrane proteins. Photosynthetic light harvesting antenna complexes are the most investigated xanthophyll-binding proteins. The major trimeric light harvesting complex of photosystem II (LHCII) of higher plants binds two Lut, one Neo and one Vio/Zea molecules per monomer (which can be abbreviated as NLLV or NLLZ compositions) (Fig. 2) [14]. Certain symmetry of xanthophyll domain structure in the trimer can be observed. Two Lut molecules have very symmetric arrangement around two cross-braced transmembrane helixes A and B (Fig. 2). The major interaction forces of the carbon backbone with the protein are based on hydrophobic and van-der-Waals forces while the hydroxyl groups of the head groups form hydrogen bonds with amino acid side chain residues of the helices [14]. Neo is bound into the C-helix domain. Its 9-*cis* end is firmly embedded like a foot into the cleft built by chlorophyll *b* pigments and anchored by a hydrogen bond to Tyrosine 112. Affinity experiments also showed that Neo had the strongest binding of all LHCII xanthophylls. The fourth xanthophyll, Vio, is bound at the monomer–monomer interface within the trimer (Fig. 2). It is partially associated with the C-helix of a neighboring monomer of LHCII and interacts hydrophobically with only a few side chain residues but with several chlorophylls (*b*601, *b*607 and *a*614) and a bound phospholipid [14]. The four oxygen atoms of

[1] *Abbreviations used:* LH1 and LH2, bacterial light harvesting complexes; PSII, photosystem II; RCII, reaction center of photosystem II; LHCII, the major light harvesting complex of PSII; Chl, chlorophyll; Xan, xanthophyll; Neo, Vio, Lut and Zea, neoxanthin, violaxanthin, lutein and zeaxanthin, respectively; N1, V1, L1 and L2, xanthophyll-binding domains of LHCII; XBP, xanthophyll-binding proteins of the human retina; MMX, molecular mechanics force field X; HPLC, high pressure liquid chromatography; PAM, pulse amplitude modulated; NPQ, non-photochemical chlorophyll fluorescence quenching; ΔpH, proton gradient across the photosynthetic membrane.