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Review

Diffusion of molecules and macromolecules in thylakoid membranes ☆

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ABSTRACT

The survival and fitness of photosynthetic organisms is critically dependent on the flexible response of the photosynthetic machinery, harbored in thylakoid membranes, to environmental changes. A central element of this flexibility is the lateral diffusion of membrane components along the membrane plane. As demonstrated, almost all functions of photosynthetic energy conversion are dependent on lateral diffusion. The mobility of both small molecules (plastoquinone, xanthophylls) as well as large protein supercomplexes is very sensitive to changes in structural boundary conditions. Knowledge about the design principles that govern the mobility of photosynthetic membrane components is essential to understand the dynamic response of the photosynthetic machinery. This review summarizes our knowledge about the factors that control diffusion in thylakoid membranes and bridges structural membrane alterations to changes in mobility and function. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

1. Introduction

Life on earth requires constant energetic fueling of cell metabolism that is ensured by photosynthetic conversion of solar radiation into metabolic energy forms. Along the trajectory of photosynthetic evolution, the invention of oxygenic photosynthesis by ancient cyanobacteria is considered a big bang of evolution because it made available the almost infinite reservoir of water as an electron donor [1]. Geological records show a rise in the atmospheric oxygen level about 1.8 billion years ago indicating that oxygenic photosynthesis must have occurred way before this time [2,3]. Indeed, it was predicted that oxygenic photosynthesis was already operative more than three billion years ago [4]. During these eons, photosynthetic energy conversion was optimized and tuned to tackle the multiple challenges dictated by the dynamic environment where terrestrial and aquatic photosynthetic organisms lived and still live in. An understanding of photosynthetic energy conversion must appreciate that natural photosynthesis was shaped by evolution to flexibly react to environmental alterations that occur on different time scales. The dynamic response of the photosynthetic machinery depends on diffusion processes. Therefore, knowledge about diffusion of molecules and macromolecules is essential to understanding the plasticity of photosynthetic membranes in a challenging nature.

The evolution of the photosynthetic machinery leads to the highly specialized thylakoid membrane system that separates two aqueous phases, the stroma (plants and green algae) or cytoplasm (cyanobacteria) and the thylakoid lumen. Four highly conserved protein complexes are responsible for energy conversion. They are all membrane integral multi-subunit complexes embedded in the thylakoid membrane bilayer. Three of the four complexes, photosystem II (PSII), cytochrome b_6f complex (cyt *bf*), and photosystem I (PSI) constitute the light-driven electron transport chain from water to ferredoxin. Reduced ferredoxin is a universal reducing equivalent used for many metabolic reactions but mainly for assimilation of CO_2 via NADPH + H^+. The electron transport is strictly coupled to proton translocation from the stroma side of the thylakoid membrane to the lumen that acidifies the thylakoid lumen. The resulting proton motive force is used for ATP formation catalyzed by the fourth protein complex, the ATPase. The photosynthetic machinery is complemented by light harvesting antenna complexes (LHC) that are connected to the photosystems and increase their apparent absorption cross section for harvesting sunlight. In contrast, to the conserved electron transport complexes and the ATPase, LHC complexes show a high degree of variability [5] ranging from hydrophobic membrane embedded LHCs (plants, green and red algae) to extrinsic hydrophilic phycobilisomes (red algae and cyanobacteria). Finally, in addition to this core set of protein complexes, low-abundance small proteins exist in thylakoid membranes. They have come more and more into focus since they are often involved in regulation, repair, turnover, and biogenesis processes.

This review focuses on diffusion-dependent processes in thylakoid membranes of plants and cyanobacteria because of the high knowledge base that exists for these organisms. Care must be taken to extrapolate data from these organisms to other photosynthetic organisms (diatoms, red and green algae) because their thylakoid membrane architecture can deviate. As will be seen below, almost all functional aspects of photosynthetic energy transformation are dependent on the migration of metabolites and protein complexes. This includes diffusion of the small electron carriers, plastoquinone (PQ) and plastocyanin (PC), which wire

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electron transport between the large protein complexes as well as the mobility of protein supercomplexes necessary for adaptation and repair processes. The efficiency of these multiple diffusion processes is highly dependent on the exact structural boundary conditions in thylakoid membranes that in turn are dynamic and controlled by the environment. It will be demonstrated that small structural changes cause switches between efficient and inefficient diffusion. In this context, this section has strong connections to other sections in this special issue dealing with supramolecular protein organization (chapters 12, 13), the dynamic changes of thylakoid membranes (chapters 16, 17), and its modulation by posttranslational protein modifications (chapter 18). Our knowledge about the plasticity of diffusion processes in thylakoid membrane is fragmentary. This is partially caused by technical limitations and partially because the importance to understand the interdependency between membrane structure and mobility and its functional consequences is often underestimated. However, recent reviews on this emergent topic exist [6,7] and the interested reader should refer to these reviews.

2. Diffusion theory

In 1906, Albert Einstein published a theory on diffusion that describes how far a particle will move from a given position if it performs random Brownian type motion [8]. For two-dimensional (2D) systems like biomembranes, the Einstein equation reads:

$$\langle r\rangle^2 = 4 \cdot D \cdot t \tag{1}$$

where $\langle r\rangle^2$ is the mean squared displacement of the particle, t is the time, and D a constant (diffusion coefficient). It follows that in this ideal case of infinite diluted membranes, the plot $\langle r\rangle^2$ versus time gives a straight line where slope is $4D$. From Eq. (1), it follows that for quantitative predictions of lateral diffusion processes, knowledge of the diffusion coefficient D is necessary. The hydrodynamic Saffman–Delbrück theory [9] calculates D for membrane proteins as a function of the membrane thickness (h), membrane viscosity (μm), viscosity of the aqueous fluid around the membrane (μw), and the radius (R) of the diffusing cylinder (approximating that the protein has a cylindrical shape):

$$D = \frac{k_B \cdot T}{4 \cdot \pi \cdot \mu m \cdot h} \cdot \left[ln\left(\frac{\mu m \cdot h}{\mu w \cdot R}\right) - 0.5772 \right]. \tag{2}$$

An important conclusion is that Eq. (2) predicts a weak logarithmic dependence of D on the radius of the protein. Recent experimental evidence indicates a stronger dependency of D on the radius of the protein, i.e. instead of D being proportional to $\ln(1/R)$, it is proportional to $1/R$ [10,11]. However, these theories (i.e. Einstein's diffusion theory) are valid only for very diluted membranes in contrast to most biomembranes. In particular, bioenergetic membranes [12] are the opposite to the diluted Singer Nicolson membrane model suggesting that a few proteins are dispersed in a sea of lipids (see the Macromolecular crowding section below). Since diffusion in crowded membranes can be very different to diffusion in diluted membranes, knowledge of the impact of crowding on mobility is central to understanding diffusion-dependent processes in photosynthetic membranes.

A diffusion theory that includes the protein packing density (obstacle concentration) in membranes is percolation theory [13,14]. Percolation theory predicts that D is no longer a constant but can become distance dependent if obstacles hinder diffusion. The higher the obstacle concentration (c) the stronger the decline in D as a function of diffusion distances. At a critical obstacle concentration (protein density) called percolation threshold (cP), the obstacles form enclosed diffusion domains that prevent any long-range diffusion of a small tracer (e.g. plastoquinone). This can be regarded as a phase transition because of an abrupt change of a fundamental physicochemical membrane property, i.e. a switch from long-range to short range diffusion. In the following, the enclosed diffusion areas will be named microdomains. cP is dependent not only on obstacle density but also on obstacle shape, interaction, self-diffusion, and more [13,14], i.e. on many structural and physicochemical parameters that make it difficult to predict. However, as a rule of thumb, cP occurs at relative obstacle densities (area occupations) around 0.6 to 0.7. As will be seen below, these are interesting values because they are close to protein densities in thylakoid membranes. For an understanding of the multiple diffusion-dependent processes in photosynthetic membranes, percolation theory is a helpful concept because it guides us to ask the right questions about critical parameters that determine mobility of photosynthetic components. In particular, it asks for the exact molecular architecture of membranes because small changes in structural parameters like protein density can have a huge impact on long-range diffusion [14].

3. Structural boundary conditions for diffusion in thylakoid membranes

There is a good knowledge base about structural boundary conditions realized in photosynthetic thylakoid membranes. Since these are addressed in other contributions of this special issue, only aspects related to diffusion processes are summarized in the following and the reader is referred to chapters 12, 13, 16, and 17 of this special issue for more detailed information.

3.1. Overall thylakoid membrane architecture

In oxygenic photosynthetic organisms, the energy converting apparatus is localized in the thylakoid membrane system. In plants and green algae, the thylakoid membrane partially folds to characteristic grana stacks (Fig. 1) that are interconnected by unstacked stroma lamellae [15–17]. The cylindrical grana stacks are missing in free-living aquatic cyanobacteria. It was hypothesized that membrane stacking to grana was invented after photosynthetic organism colonized terrestrial habitats and reflect an adaptation to life on land [18]. In grana-containing organisms, the photosynthetic protein complexes are not homogenously distributed between stacked and unstacked thylakoid regions [17,19–21] (see also chapter 12 of this special issue). PSII with LHCII complexes are concentrated in grana thylakoids whereas PSI with LHCI and the ATPase are found preferentially in unstacked regions. The existence of grana thylakoid membranes sets special structural constraints to molecular traffic between stacked and unstacked regions. For example, for the lateral movement of protein complexes between stacked and unstacked regions, the relation between the width of the stromal gap in grana stacks and the stromal protrusion of proteins is critical. Recent cryo-electron microscopic (EM) image analysis [22,23] shows that the stromal gap in grana is only about 3.5 nm (Fig. 1). This value gives a structural basis for the exclusion of PSI and the ATPase from stacked grana because their stromal protrusions are larger [24,25]. Critical for protein diffusion is also the width of the thylakoid lumen on the opposite membrane side (Fig. 1). This width was determined to be variable between 4.5 and ~10 nm [22,23]. The implication of the luminal width on proteins localized in this tiny compartment will be addressed in Section 4.3.

3.2. Macromolecular crowding

The density of the membrane integral protein complexes in photosynthetic membranes is one of the highest for biomembranes [12,26]. In the light of the percolation theory, this is a critical quantity because if the density is below or above the percolation threshold, it determines whether long-distance diffusion is possible or not. Estimates of the actual number for the relative protein area for grana and whole thylakoids were derived from EM, atomic force microscopy and biochemical data [27–29]. These different approaches give a remarkable constant value of 0.7 to 0.8 for grana thylakoid membranes and about 0.69 (corresponding to a lipid/protein ratio of 0.34 [w/w], [27]) for whole thylakoid

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