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Single-molecule studies of the dynamics and interactions of bacterial OXPHOS complexes☆

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ABSTRACT

Although significant insight has been gained into biochemical, genetic and structural features of oxidative phosphorylation (OXPHOS) at the single-enzyme level, relatively little was known of how the component complexes function together in time and space until recently. Several pioneering single-molecule studies have emerged over the last decade in particular, which have illuminated our knowledge of OXPHOS, most especially on model bacterial systems. Here, we discuss these recent findings of bacterial OXPHOS, many of which generate time-resolved information of the OXPHOS machinery with the native physiological context intact. These new investigations are transforming our knowledge not only of the molecular arrangement of OXPHOS components in live bacteria, but also of the way components dynamically interact with each other in a functional state. These new discoveries have important implications towards putative supercomplex formation in bacterial OXPHOS in particular. This article is part of a Special Issue entitled Organization and dynamics of bioenergetic systems in bacteria, edited by Conrad Mullineaux.

1. Introduction

The enzymes and substrates used by bacteria for chemiosmotic ATP synthesis are as varied as the ecological niches they occupy. As autotrophs and/or heterotrophs living phototrophically, chemolithotrophically, and/or organotrophically, bacteria can use light, minerals and organic substrates as sources of reducing electrons for electron transport chains that establish a proton motive force (pmf) by redox reactions coupled to proton translocation, which can be vectoral (where protons are literally pumped through channels in the enzyme) or scalar (where protons are chemically consumed on one side of the membrane and liberated on the other). The pmf in turn is used to power the rotary mechanism of the F_1F_o-ATP synthase which produces ATP [1]. While there is great diversity in the enzymes and electron transport chains that allow bacteria to 'eat' everything from photons and water, to elemental sulfur and sugars, the basic mechanism of chemiosmotic ATP synthesis [2] is same and requires a pmf across a membrane, established by correctly oriented electron transport chain enzymes which effectively move protons across that membrane in the opposite direction to that which protons must flow through the ATP synthase for ATP synthesis.

Historically, the chemiosmotic ATP synthesis systems of the plant chloroplast and mammalian mitochondrion are the bases of understanding the two classical modes of chemiosmosis: photophosphorylation in the former and oxidative phosphorylation (OXPHOS) in the latter [3]. Fig. 1 schematizes chemiosmotic ATP synthesis by oxidative phosphorylation (OXPHOS) in the model heterotrophic bacterium *Escherichia coli*, illustrating the metabolic flexibility of this organism. (For more details on *E. coli* OXPHOS genes, we refer the reader to the review by Magalon and Alberge, BBA 2015, in this special issue and Figure 1 of that review in particular.)

This central metabolic pathway is governed by a multi-enzyme system that is localized to bioenergetic membranes, but the organization and dynamics of bioenergetic complexes in two dimensions in the plane of the membrane is not well understood and has implications for the operation of the system as a whole. Two extreme models of organization and dynamics can be envisaged: solid state or random diffusion [4]. A solid state model implies that protein complexes are locked together and substrates are channeled from one to the other, such that the efficiency of the system would be limited by the turnover of the enzymes, whereas a random diffusion model allows for the possibility that the concentration of components in the membrane limits flux.

The presence of oxidative OXPHOS supercomplexes in mitochondria and bacteria [5–10] and role of supercomplexing for channeling electrons [11] support of a solid state model in mitochondria, but the relevance of supercomplexing to the catalytic kinetics and efficiency of the system is disputed by alternative interpretations [12] of the data of Lapuente-Brun et al. [11]. Blaza et al. [12] in fact suggest that while supercomplexes exist in mitochondria, perhaps they have no physiological function other than to allow optimal enzyme packing and thus

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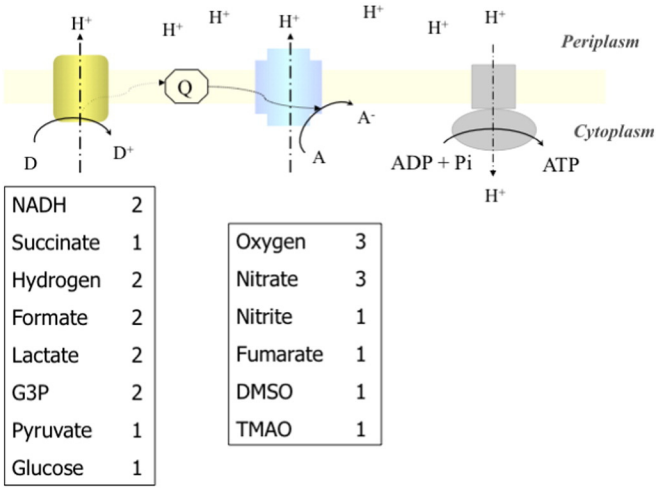

Fig. 1. Schematic representation of the *Escherichia coli* OXPHOS chain. Electrons are transferred from donor substrates (D) to quinones (Q) by quinone reductases (in yellow) and then to acceptor substrates (A) by terminal oxidases (in blue). Electron transport is concomitant with proton translocation, resulting in a pmf. For chemiosmotic ATP synthesis by the F_1F_O ATP synthase (in gray). Below each depicted enzyme is the range of electron donors and acceptors that can be used by the *E. coli* respiratory chain and the numbers indicate the number of known isozymes for each substrate (data adapted from [68]).

improve overall efficiency of OXPHOS at the level of the whole mitochondrion.

What is the situation in bacteria? The operation of bacterial electron transport chains should be of great importance for those interested in killing or manipulating bacteria for disease management, bioproduction and bioremediation. For instance, altering levels of mobile electron carriers might have large effects for bacteria which operate a random collision system, but might have little effect on bacteria that operate at solid state system.

Fluorescence microscopy studies have allowed researchers to address the questions of the organization and dynamics of bacterial OXPHOS components in live cells. Traditional fluorescence imaging techniques involve exciting and collecting the emitted fluorescence from all the fluorophores in the focal plane simultaneously. Images are generated directly by photons landing on a 2-dimensional detector, which translates the signal into a photon intensity map — or takes a photograph (confocal images can be considered to be a photo-collage). Such images are diffraction-limited in terms of spatial resolution to the typical optical resolution limit of ca. 200–300 nm which is determined from the Abbe theory of optical diffraction to be roughly half the wavelength of emitted light. These diffraction-limited fluorescence imaging techniques suggested a heterogeneous distribution of OXPHOS complexes in the plasma membranes of *Bacillus subtilis* [13] and *E. coli* [14] and that OXPHOS complexes are mobile in the bacterial membrane.

Single-molecule imaging approaches aim to build up a picture of the cell by observing many molecules but individually, one at a time. Such approaches not only reveal the overall trend for a population of molecules but also the population structure, showing up heterogeneities that may be averaged out in an ensemble measurement. They also have the advantage of being able to determine the location of molecules 10–50-fold more accurately than in diffraction-limited imaging [15]. This is because the detected emission from a point source of light on a two-dimensional detector is manifested as a point spread function image of 200–300 nm width which can be fitted by an analytical function (typically approximated by a 2D Gaussian profile) to determine the location of the intensity centroid as the best estimate for the location of that source [16]. Such studies on OXPHOS in bacteria have painted a detailed picture of bacterial OXPHOS systems in live cells, which is suggestive of how the many enzymes might work together to achieve ATP synthesis.

As far as we are aware, single-molecule fluorescence studies on bacterial OXPHOS have only been carried out on *E. coli* [17–20]. These studies have characterized the patches of OXPHOS complexes that have been observed by ensemble average imaging to unprecedented levels of detail. They have also cataloged the mobility of complexes in the membrane, taking advantage of the improved spatial resolution of single molecule approaches, revealing that the movement of complexes is not uniform. They have thus begun to address the question of how OXPHOS complexes relate to each other spatially.

2. Heterogeneous patching

To date, the quinone reducing enzymes: type 1 NADH dehydrogenase (NDH-1) [21,22] and succinate dehydrogenase (SDH) [23], quinol oxidizing enzymes: the Cytochrome bo [24] and Cytochrome bd-1 complexes [25–27], and the F_1F_o-ATP synthase [28,29] have been functionally fluorescent-protein labeled and expressed from native loci in *E. coli* cells (Table 1). By ensemble average imaging, all of these complexes were observed to be heterogeneously distributed in the *E. coli* membrane and apparently localized in mobile clusters [17,30]. A more precise single-molecule approach was taken to study these apparent clusters in order to tease out details such as the variation in the number of complexes in each cluster, the distribution of physical sizes of the clusters and the diffusional behavior of individual clusters or complexes [17,19].

Single-molecule counting methods (see Box 1), developed originally from stoichiometry studies of torque-generating components of the bacterial flageller motor [31], revealed that these clusters are heterogeneous in terms of the number of complexes located within them (Table 2). The clusters are also expected to be variable in size as estimated by the comparison of the apparent full width at half maximum (FWHM) of the individual clusters to that of a single fluorophore in the same microscope [17].

PALM (***P***hoto-***A***ctivation ***L***ocalisation ***M***icroscopy) imaging of Cytochrome bd-1 and NDH-1 in fixed cells gives a more detailed picture of the arrangement of these complexes in the membrane [19] (see Box 2). Consistent with the broad distributions that were suggested by diffraction-limited imaging, the clusters of complexes were

Table 1
Functionally labeled *E. coli* OXPHOS complexes.

OXPHOS complex	Subunit	Terminus	Linker	Fluorophore	Reference source
NDH-1	NuoF	N	Thr-Asp-Pro-Ala-Leu-Arg-Ala	GFP, mCherry, mMaple	[30,17,19]
NDH-1	NuoF	C	Gly-Leu-Cys-Gly-Arg	Cerulean	[30]
SDH	SdhC	N	Thr-Asp-Pro-Ala-Leu-Arg-Ser[a]	mCherry	[30]
Cytochrome bd-1	CydB	C	Gly-Leu-Cys-Gly-Arg	GFP, mCherry, mMaple	[17,19]
Cytochrome bo	CyoA	C	No linker	mCherry	[30]
ATP-synthase	AtpB	C	Gly-Ser-Met-Val	GFP	[30]
ATP-synthase	AtpB	C	Gly-Ser	GFP, PAGFP, mEos3.2	[20]

[a] Replaces Met-Ile-Arg-Asn.

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