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Mitochondrial creatine kinase adsorption to biomimetic membranes: A Langmuir monolayer study

Nathalie Vernoux [a,1], Ofelia Maniti [a], Françoise Besson [b], Thierry Granjon [a,*], Olivier Marcillat [a], Christian Vial [a]

[a] *CNRS UMR 5246/IMBL, Biomembranes et enzymes associés, Université Lyon 1, 43 boulevard du 11 Novembre 1918, F-69622 Villeurbanne cedex, France*
[b] *CNRS UMR 5246/IMBL, Organisation et dynamique des membranes biologiques, Université Lyon 1, 43 boulevard du 11 Novembre 1918, F-69622 Villeurbanne cedex, France*

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Abstract

Interaction of mitochondrial creatine kinase (mtCK) with either synthetic or natural zwitterionic or acidic phospholipids was monitored by surface pressure measurements. Injection of mtCK beneath a monolayer at very low surface pressure results in a large increase in the apparent area per lipid molecule reflecting the intrinsic surface activity of the protein. This effect is particularly pronounced with anionic phospholipid-containing films. Upon compression to high lateral pressure, the protein is squeezed out of the lipid monolayer. On the contrary, mtCK injected beneath a monolayer compressed at 30 mN/m, does not insert into the monolayer but is concentrated below the surface by anionic phospholipids as evidenced by the immediate and strong increase in the apparent molecular area occurring upon decompression. Below 8 mN/m the protein adsorbs to the interface and remains intercalated until the lateral pressure increases again. The critical pressure of insertion is higher for anionic lipid-containing monolayers than for films containing only zwitterionic phospholipids. In the former case it is markedly diminished by NaCl. The adsorption of mtCK depends on the percentage of negative charges carried by the monolayer and is reduced by increasing NaCl concentrations. However, the residual interaction existing in the absence of a global negative charge on the membrane may indicate that this interaction also involves a hydrophobic component.

Keywords: Mitochondrial creatine kinase; Langmuir monolayer; Dimyristoylphosphatidylcholine; Dimyristoylphosphatidylglycerol; Cardiolipin; Phosphatidylethanolamine; Elasticity coefficient; Protein lipid interaction

1. Introduction

Vertebrate excitable tissues express a dimeric cytosolic form of creatine kinase (CK) and a mitochondrial one (mtCK). The latter exists as two interconvertible oligomeric forms, a dimer and an octamer [1,2]. Both isoenzymes are soluble proteins which can be associated to a variable extent with subcellular structures. Whereas muscle cytosolic CK (MMCK) can be associated with myofibrills [3] or membranes [4], octameric mtCK binds to the outer face of the inner mitochondrial membrane, as well as to anionic phospholipid-containing vesicles [1,5–9], while dimeric mtCK and cytosolic isoforms have no affinity for the mitochondrial membrane. The mtCK level is specially high at the contact sites between the inner and the outer mitochondrial membranes [10–12]. Since this protein has a propensity to bridge vesicles made up of internal and external mitochondrial membrane phospholipids [13], it has been proposed that it could play a role in contact points formation [5,13]. Scanning transmission electron microscopy and X-ray diffraction have given a picture of the mtCK octamer [10,14–17]. It is a highly ordered cube of about $9.3 \times 9.3 \times 8.6$ nm which is made up of four elongated banana-shaped dimers arranged along a four-fold symmetry axis. The top and bottom faces perpendicu-

* Corresponding author. Fax: +33 472 431 557.
E-mail address: thierry.granjon@univ-lyon1.fr (T. Granjon).
[1] Present address: Departement de Biochimie et Microbiologie, CREFSIP, Université Laval, Québec, Canada.

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lar to this axis contain the C-terminal ends rich in basic residues and are responsible for membrane binding via interactions with acidic phospholipids [18].

Release of mtCK from the mitochondrial membrane or from liposomes can be achieved by incubation at high ionic strength, by ADP or ATP [19–21] or thiol-reactive organomercurials [22]. The p*I* of the octamer (around 8.8) is significantly more basic than that of the other isoforms: 8.2 and 7.1 for dimeric mtCK and MMCK, respectively [23,24] and this may be responsible for the binding of the protein to membranes by ionic interactions [6]. A mathematical model describing mtCK binding to mitochondrial membranes by ionic interactions and the effects of solubilizing effectors has been proposed [25]. However, according to hydrophobic interaction chromatography and differential scanning calorimetry data, mtCK seems to be more hydrophobic than cytosolic isoenzymes [6,26].

We have previously shown that the binding of mtCK to liposomes decreased the fluidity of the membrane [27]. Octameric, but not dimeric mtCK, has a marked amphipathic character which allows it to adsorb at the air–water interface [28]. Indeed the saturating surface pressure induced by the protein at a lipid–free air–water interface is 22 mN/m and the saturation is obtained at a concentration of about 40 nM mtCK.

Within the cell CK isoenzymes contribute to a shuttle which efficiently exports chemical energy as phosphocreatine to the cytosol and creatine to mitochondria, so as to maintain a high ADP phosphorylation potential in the vicinity of the active site of energy consuming enzymes [4,29–31].

Since the interaction of mtCK with the cytoplasmic side of the inner mitochondrial membrane is a key factor in the distribution of energy in the cells of excitable tissues [4] and may also play a role in permeability transition pore regulation [32], our aim was to explore the molecular basis of this interaction using a biomimetic model membrane.

The Langmuir phospholipid monolayer at the air–water interface is a good model for half of a cell membrane. Since lipid composition and surface pressure can be varied and the subphase can be adjusted to various conditions of pH and ionic strength, monolayers are a powerful tool in understanding protein–lipid interactions. Langmuir monolayers enable us to study these interactions with phospholipid molecules autoorganized in a well defined arrangement according to the lipid composition, the ionic characteristics of the monolayer and the surface pressure.

The film structural characteristics were studied from $\pi(t)$ adsorption kinetics and π–A isotherms. We first used monolayers made up of DMPC and DMPG, two synthetic phospholipids having the same acyl chains but different headgroups to highlight the impact of electrostatic contribution to the interaction of octameric mtCK with membranes. Then we studied the interaction with a mixture of natural phospholipids PC/PE/CL, mimicking the composition of the inner mitochondrial membrane and looked for an additional hydrophobic contribution to this interaction. Penetration experiments were carried out using phospholipids having different headgroups (zwitterionic or negatively charged) and acyl chains.

2. Materials and methods

2.1. Materials

Tris, DTT, NaCl, phosphatidylcholine (PC) from egg yolk, phosphatidylethanolamine (PE) from egg yolk, cardiolipin (CL) from bovine heart, dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) were obtained from Sigma (Saint Quentin Fallavier, France). They were of the highest purity available. EDTA was purchased from Roche (Meylan, France). Water was purified with a Milli-Q system, its resistivity was greater than 18 MΩ cm and its pH around 5.6.

2.2. Protein expression and purification

Recombinant octameric rabbit heart mtCK (340 kDa) was prepared and purified as previously described [33]. The purified enzyme was obtained in 20 mM Tris, 0.1 mM EDTA pH 7.4 at a protein concentration of about 1 μM. Protein concentration was determined by the Lowry method using bovine serum albumin as standard.

2.3. Film formation and surface pressure measurements

All experiments were performed at constant temperature (21 ± 0.1 °C). The film balance was built by R&K (Riegler & Kiersten, Wiesbaden, Germany) and equipped with a Wilhelmy-type surface-pressure measuring system. Troughs were surrounded by a closed box to avoid dust deposition and water evaporation. For measurements of π–A isotherms, a rectangular trough with a total area of 162 cm^2 was used. The subphase buffer was 20 mM Tris, 0.1 mM EDTA, pH 7.4, in the presence or not of 0.3 M NaCl, this NaCl concentration was chosen to prevent mtCK binding to anionic phospholipids [6]. In all experiments, the subphase was continuously stirred using a magnetic bar spinning at 100 rev/min.

Lipid monolayers were formed on a clean air/buffer interface by spreading known amounts of phospholipid dissolved in chloroform/methanol (4:1). After solvent evaporation (i.e., 20 min after spreading), the monolayer was symmetrically compressed at a velocity of 6 cm^2/min. In our experimental conditions the lipid monolayers were stable and no relaxation occurred even at 30 mN/m. In order to measure π–A isotherms in the presence of protein, a final concentration of 0.9 nM protein was injected in the subphase using a Hamilton syringe at a surface pressure of either 0 or 30 mN/m. The injected volume (<100 μl) was negligible as compared to the trough volume (120 ml). In the case of injection of the protein at 0 mN/m, a new isotherm was recorded 3 h later, i.e., the time necessary for the surface pressure to attain its equilibrium value at 21 °C. When the protein was injected at 30 mN/m, the film was expanded after 3 h and immediately compressed.

Surface compressional moduli were calculated from the pressure–area data obtained from the monolayer compressions, using the following equation: $Ks = -A\, d\pi/dA$, where A is the molecular area and π the corresponding surface pressure. This interfacial parameter is expressed in mN/m.