Spatial structures in mitochondrial suspension induced by cation efflux

E.L. Holmuhamedov and Yu.V. Evtodienko

Institute of Biological Physics, USSR Academy of Sciences, 142292 Pushchino, Moscow Region, USSR

Received 31 October 1985

The formation of spatial structures in a thin unstirred layer of a mitochondrial suspension has been studied. It is shown that the structure formation depends on the state of the ion-transporting systems of mitochondria and that pattern development coincides with the activation of cation efflux from preloaded mitochondria. Spatial structure formation is an energy-dependent process and is suppressed by respiratory chain inhibitors. Patterning is also inhibited by EGTA, EDTA and ruthenium red, reflecting the requirement for divalent cation translocation in mitochondria for the studied phenomenon.

(Rat liver mitochondria) Spatial structure Inhibitor effect

1. INTRODUCTION

The formation of dissipative structures in non-equilibrium physico-chemical and biochemical systems has been demonstrated [1–4]. We have recently described a new phenomenon representing dissipative structure formation in an oscillating mitochondrial suspension [5]. However, it is quite difficult to elucidate the actual mechanism of patterning in such mitochondria due to the complex interaction of events involved in the oscillatory processes. Here we present new data on spatial structures observed in a suspension of non-oscillating mitochondria. The spontaneous release of preliminary accumulated cations is shown to be followed by structuring of the initially homogeneous mitochondrial suspension. Patterning is observed in mitochondria preloaded with both divalent cations (Ca^{2+} or Sr^{2+}) and/or K^+ in the presence of valinomycin.

2. MATERIALS AND METHODS

The formation of spatial structures was detected with the experimental setup shown in fig.1. The thin unstirred layer of the mitochondrial suspension (depth 1.0 mm) placed on the thermostabilized open cell (∅ 24 mm, 30°C) was illuminated below by a 530 nm light source and photographed directly with a camera. Extramitochondrial concentrations of H^+, K^+ and M^{2+} in the bulk phase were measured by the corresponding ion-selective electrodes [6]. Standard techniques were used for preparation of mitochondria and estimation of

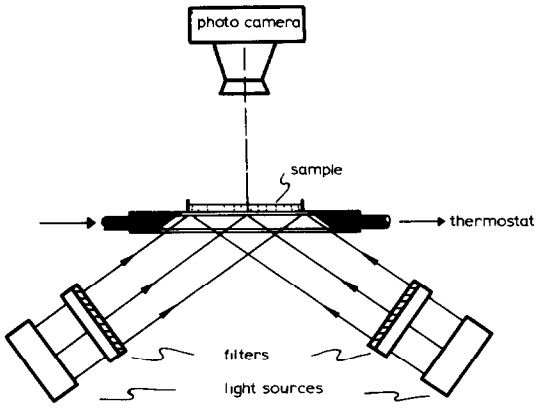

Fig.1. Experimental setup for direct observation of the spatial pattern in a thin unstirred layer of a mitochondrial suspension. For details see text.

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00145793/86/$3.50

protein concentration in a sample. The experimental conditions are given below as well as in the figure legends.

3. RESULTS

3.1. *Spatial structures in suspension of mitochondria treated with valinomycin*

Fig.2 shows the changes of K^+ concentrations in a mitochondrial suspension in the absence (fig.2A) and presence of added valinomycin (fig.2B). As can be seen in fig.2A no changes of K^+ concentration in the mitochondrial suspension incubated without valinomycin were observed. Entirely different kinetics of K^+ concentration changes were observed in the suspension of valinomycin-treated mitochondria. Energy-dependent potassium accumulation in the matrix of mitochondria is followed by its spontaneous and irreversible release. Addition of surface-active agents like local anesthetics or albumin prevents mitochondrial damage and K^+ release. The spatial structures observed in the thin unstirred layer of the mitochondrial suspension are characterized by different behaviour depending on the state of the mitochondria used. When the layer consists of valinomycin-free mitochondria no spatial optical heterogeneity is observed (fig.3A). In the case of valinomycin-pretreated mitochondria no heterogeneity is initially observed as well as during the energy-dependent accumulation of K^+ (cf. figs 2 and 3B). Simultaneous registration of K^+ kinetics in the stirred mitochondrial suspension and patterning in the layer indicates that the efflux of K^+ in the bulk phase results in the appearance of some heterogeneity in the layer which becomes clear cut as the rate of the ion efflux increases (see figs 2B and 3B). It is of interest that the weak lines formed in the spatial pattern expand rapidly till the whole pattern represents a spatial structure though no new lines appear at that time. Upon completion of ion efflux in the bulk phase, pattern formation is also stopped with no further changes in the depth and brightness of the pattern-forming lines.

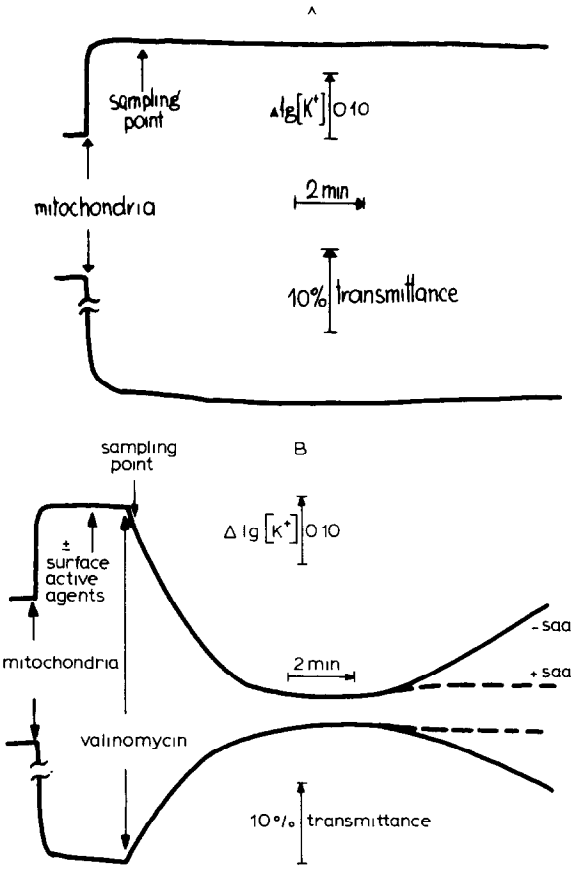

Fig.2. Changes of the extramitochondrial concentration of K^+ and transmittance in the bulk phase of the mitochondrial suspension (2.5 mg protein/ml) incubated in medium containing 20 mM sucrose, 1 mM KCl and 5 mM Tris-succinate, pH 7.5, t = 30°C, in the absence (A) and presence (B) of valinomycin (8 ng/mg mitochondrial protein).

3.2. *Pattern formation in a suspension of mitochondria preloaded with Ca^{2+} or Sr^{2+}*

The spatial structures in the thin layer of the mitochondrial suspension can also be induced in the mitochondria preloaded with divalent cations. Fig.4 presents the changes of the extramitochondrial concentrations of H^+, K^+ and M^{2+} in the bulk phase of the stirred suspension during energy-dependent accumulation of small amounts of Ca^{2+} (or Sr^{2+}). Simultaneous registration of ion translocation kinetics in the bulk phase and patterning in the unstirred thin layer formed from this suspension shows that (as in the case of valinomycin) no changes in the layer are observed during M^{2+} accumulation and H^+ extrusion. The spatial structures in the layer appear at the same time as the spontaneous efflux of accumulated cations in the bulk phase is observed (see figs 4 and 5). Primarily only weak lines appear which serve as a

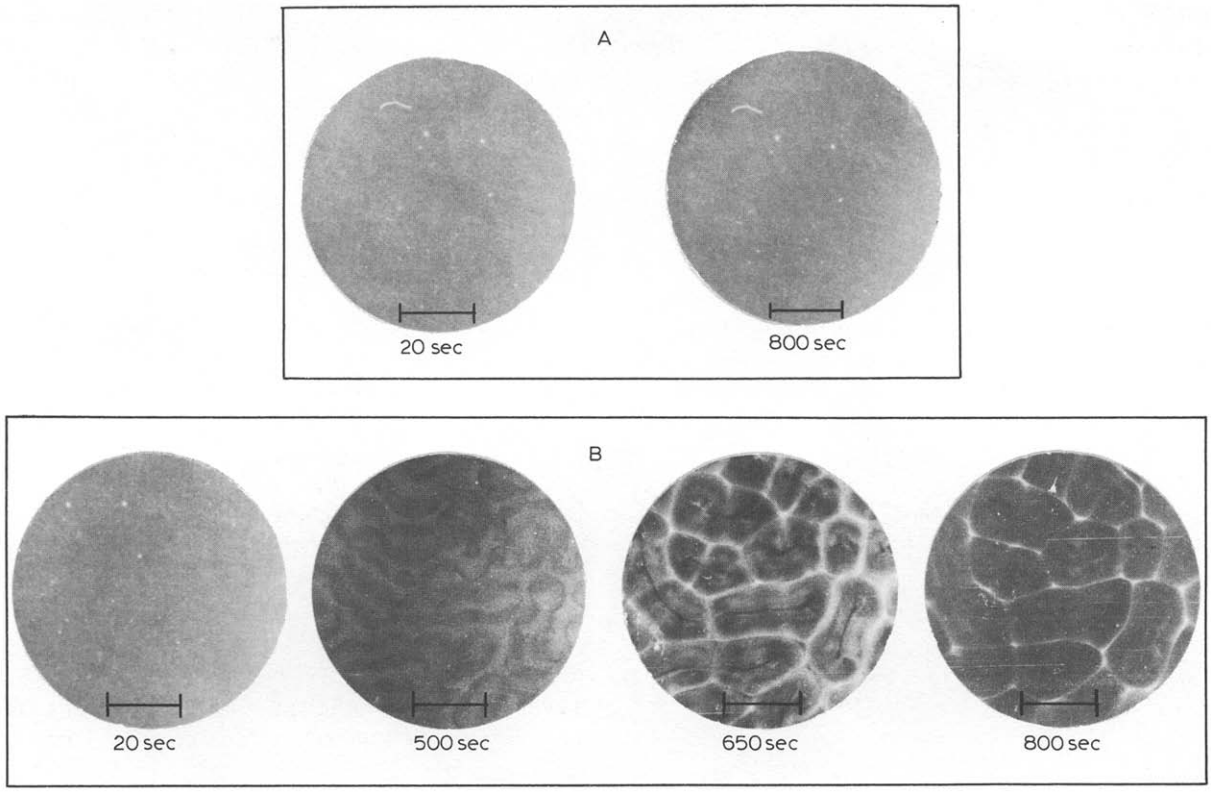

Fig.3. (A) Light scattering picture in a thin layer of valinomycin-free mitochondria sampled from the bulk phase at the indicated time (see fig.2A). The time after sampling is given under the photographs. Length of calibration, l = 4 mm. (B) Sequence of spatial structure appearing in the suspension of valinomycin-treated mitochondria. Sampling was made as indicated (see fig.2B).

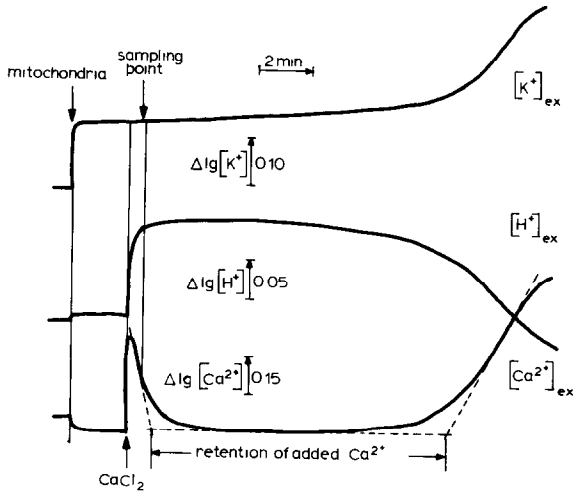

Fig.4. Changes of extramitochondrial concentrations of H^+, K^+ and Ca^{2+} in the bulk phase of the mitochondrial suspension during accumulation of Ca^{2+} (or Sr^{2+}). Incubation as in fig.2 except that 30 nmol Ca^{2+}/mg protein was added.

basis for the future spatial pattern. These specifically shaped lines are subjected to changes developing into the 'bright' and 'dark' zones. These zones grow symmetrically in opposite directions from the initial lines.

3.3. *Effect of respiratory and cation-transfer inhibitors on pattern formation*

The processes of spatial structure formation in the mitochondrial suspension exhibit noticeable sensitivity to specific mitochondrial poisons. The addition to both valinomycin-treated and/or M^{2+}-loaded mitochondria of inhibitors of the respiratory chain just before sampling is followed by suppression of structure formation. Among this the patterning in the suspension of M^{2+}-loaded mitochondria is also suppressed by inhibitors of divalent cation translocation such as ruthenium red, EGTA or EDTA. Structuring in the suspen-

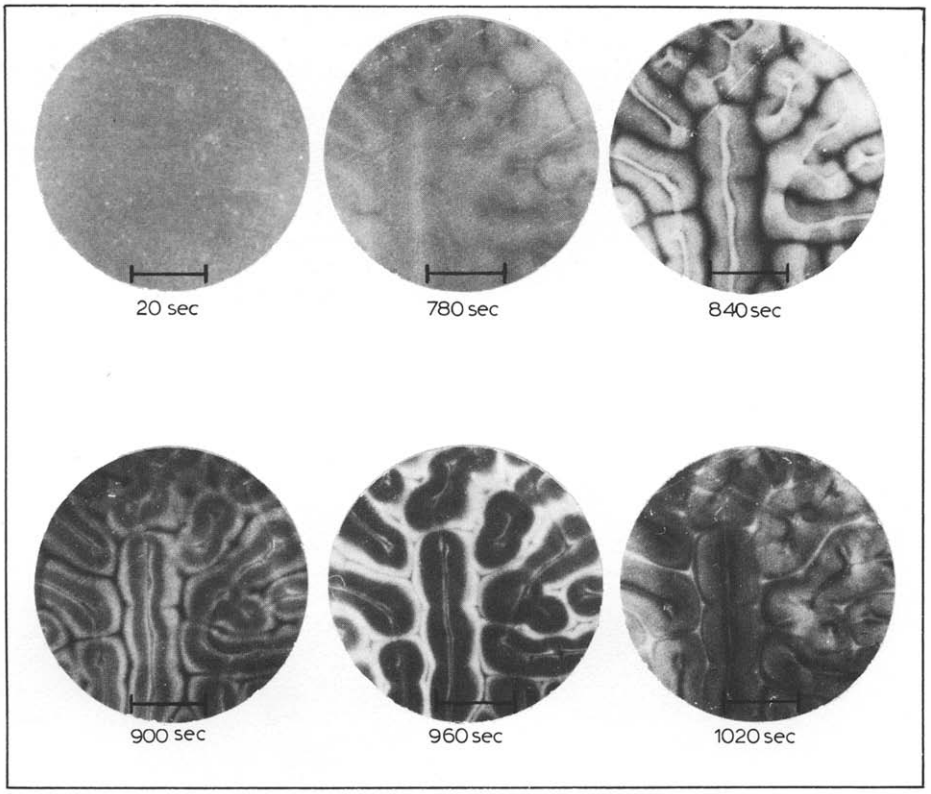

Fig.5. Changes in the spatial structures observed in the thin unstirred layer of the mitochondrial suspension preloaded with Ca^{2+} and sampled from the bulk phase at the indicated time (see fig.4). Length of calibration, l = 4 mm.

sion of valinomycin-treated mitochondria is effectively suppressed by surfactants preventing osmotic damage and subsequent loss of ionic contents from the matrix [7].

4. DISCUSSION

The possible induction of spatial structure in the suspension of non-oscillating mitochondria significantly facilitates the experimental study of its mechanism. The results of this work indicate the dependence of pattern formation in mitochondrial suspensions on the state of the systems of ion transfer via the inner membrane. In our experiments it has been shown that the direction of ion transfer is of great importance for spatial structure formation, i.e. the patterning occurs just in the case of spontaneous efflux of ions from mitochondria preloaded with either K^+ in the presence of valinomycin or Ca^{2+} or Sr^{2+}. Pattern formation induced by K^+, Ca^{2+} or Sr^{2+} is also shown to be suppressed by inhibitors of the respiratory chain or divalent cation accumulation in mitochondria. Therefore divalent cations seem to play an important role in the formation of spatial dissipative structures and the effect is exerted not only by the extramitochondrial concentration of ions but also by its changing during the process of spontaneous efflux because no patterns are observed during the energy-dependent accumulation of K^+, Ca^{2+} or Sr^{2+}. Such a complex interaction between the state of energy-dependent ion-transporting systems of mitochondria and the process of spatial structure formation means that the patterning cannot be accounted for by the effect of hydrodynamic fluxes in the open liquid layer alone. In fact it seems quite difficult to suggest a mechanism that describes the effect of the mitochondrial state on the appearance of hydrodynamic fluxes as it is known to be due to external factors [8,9]. We believe that the formation of spatial structures in mitochondrial suspensions

is due to the latent properties of mitochondria themselves and thus seems to be of particular interest in biology. In fact, if this phenomenon takes place in a cell it means that the cytoplasm possesses a system providing intracellular communication of metabolic processes with Ca^{2+} as one of the strongest metabolic regulators.

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