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UNCOUPLING AND INHIBITION OF OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA BY OLIGOMERS OF 15-KETO-PGB$_1$ (PGB$_x$)

The polymeric prostaglandin PGB$_x$ with a molecular weight range of 2200—2500 (6—7 monomeric units), synthesized from 15-keto-PGB$_1$, was able to maintain oxidative phosphorylation during hypotonic degradation in aged mitochondria isolated from rat liver [1]. It stimulates the release of Ca^{2+} from fragmented sarcoplasmic reticulum and heart mitochondria. Ionophore activity of PGB$_x$ is almost two orders of magnitude greater than that of other prostaglandins [2]. It was found that PGB$_x$ *in vivo* facilitates and significantly increases the survival of monkeys after myocardial infarction with ventricular fibrillation [3], as well as the survival of the heart of the hypoxic mouse [4]. Also, rabbits treated with PGB$_x$ showed improved recoveries from the effects of experimentally induced cerebral ischemia [5].

The association between the increase in cytosolic Ca^{2+} level and tissue damage in ischemic myocardium does exist, because mitochondria are avid accumulators of Ca^{2+} [6] and when overloaded with Ca^{2+}, they rephosphorylate ADP at a relative slow rate [1]. At the same time the energy in the form of ATP is required for the maintenance of intracellular homeostasis with respect to Ca^{2+} and hence for the maintenance of a low cytosolic Ca^{2+} [7]. After taking into account the effects of PGB$_x$ *in vivo* and *in vitro* we hope that PGB$_x$ will interact with mitochondria, thereby preventing the latter from becoming overloaded with Ca^{2+}. The beneficial effect of PGB$_x$ showed *in vitro* with aged mitochondria (3—5 days at 0 °C), but failed with fresh mitochondria [8]. Therefore the examination of the effects of PGB$_x$ on the energetic parameters of oxidative phosphorylation of Ca^{2+}-loaded and nonloaded mitochondria is of great interest.

Methods

PGB$_x$ was synthesized by Michael reaction catalyzed by alkali via 15-keto-PGB$_1$ starting with biosynthetic PGE$_1$. Oligomers were separated, and the molecular weights were determined by gel chromatography [9]. Fractionated oligomers with mean molecular weights of 1336, 2400, 3040 and 3370 were stored at −20 °C in ethanol. We termed those fractions as Fractions I, II, III and IV, respectively.

Mitochondria were isolated from rat liver by differential centrifugation and stored at 0 °C for 2 hours prior to polarographic analysis. The effects of the oligomers of 15-keto-PGB$_1$ on oxidative phosphorylation of Ca^{2+}-loaded and nonloaded rat liver mitochondria were studied polarographically by using a Clark oxygen electrode (produced at Moscow State University), polarographic analyzer PA2 (Laboratorni Pristroje, Praha), and Recorder 2210 (LKB). The specific activities of oligomers (A_3) and (A_4) were defined as changes in the mitochondrial respiration rate in

State 3 and State 4 respectively, caused by the increase of oligomer concentration in the incubation medium by 1 μM, and was dimensioned as (ngatoms O/mg · min)/μM. **Chemicals**: HEPES, EGTA, potassium malate, potassium glutamate, magnesium acetate were products of Sigma. BSA, TRIS and sucrose were purchased at Serva. $CaCl_2$, KH_2PO_4 and EDTA were products of "Реахим", and ADP was from Reanal. **Abbreviations**: PG — prostaglandin, ADP — adenosine-5′-diphosphate, ATP — adenosine-5′-triphosphate, HEPES — N-(2-hydroxyethyl) piperazine-N′-2-ethanesulfonic acid, EGTA — ethylene glycol bis(2-aminoethylether)-N,N-tetra-acetic acid, EDTA — ethylene diaminetetra-acetic acid, TRIS — tris(hydroxymethyl)aminomethane, BSA — bovine serum albumin, RCI — respiratory control index.

Experimental

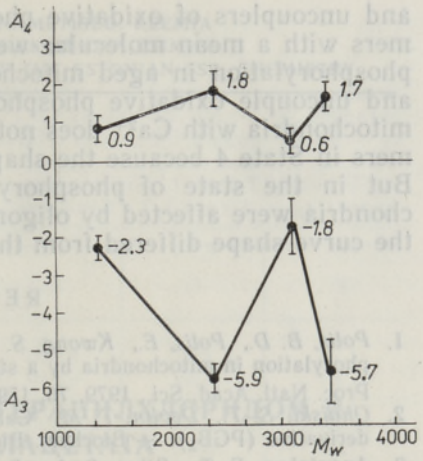
Isolation of rat liver mitochondria was performed in a cold room at +2 °C. The female Wistar rats (200—250 g) were decapitated, their livers excised as rapidly as possible and washed with ice-cold 0.3 M of sucrose solution. The livers were cut with scissors on a Petri dish and washed carefully with the same solution. The chopped livers were homogenized (glass barrel, Teflon pestle) in a medium that contained 0.3 M of sucrose, 0.2 mM of EDTA and 10 mM of TRIS · HCl pH 7.4. The nuclei were sedimented at 700 × g during 10 min, and mitochondria at 8000 × g during 20 min. The mitochondrial pellet was washed twice with a homogenization medium that consisted of 1 mg/ml of BSA and was homogenized with a pipette in the same solution. The centrifugation, washing and homogenization procedures were repeated at 5300 × g and 4400 × g for 10 min. After rehomogenization of the final mitochondrial pellet, the content of protein was measured by the Biuret method [10] with BSA used as a standard.

Polarographic analysis of oxidative phosphorylation. The standard incubation medium contained 250 mM sucrose, 20 mM HEPES buffer pH 7.4, 4 mM potassium glutamate, 2 mM potassium malate, 3 mM magnesium acetate, 4 mM KH_2PO_4, 0.3 mM EGTA, and 1 mg of mitochondrial protein in a final volume of 1.0 ml at 28 °C. Additions were made with microsyringes through a small opening in the cell: 150 μM of $CaCl_2$ for the loading of mitochondria, 0.6—15 μM of oligomers and 120 μM of ADP for the initiating oxidative phosphorylation. We also determined the oxygen uptake and respiratory control index (RCI) which was defined as the ratio between the respiratory rate during the active state of respiration and the respiratory rate after the phosphorylation of ADP. The mean values of A_3 and A_4 for metabolic states 3 and 4 were obtained with the help of five measurements in the oligomer concentration range of 0.6—15 μM.

Results

The RCI values in the range of 5—6 and respiration of 72 ngatoms O/mg · min in the steady State 3 were determined 1 hour after the isolation of mitochondria. Addition of oligomers to the incubation medium led to the uncoupling and inhibition of oxidative phosphorylation. For example, a decrease of respiration to the level of 32.4 ngatoms O/mg · min and respiratory control index 1.4 was caused by an addition of 5.85 nmoles of Fraction II per 1 mg of protein. The increases and decreases of mitochondrial respiration are illustrated in Fig. 1. It was unexpected that affecting ability of 15-keto-PGB_1 oligomers appears periodically, because the bell-shaped activity curve of PGB_x with a maximum at 2500 Dalton is known [1]. Mitochondria loaded with 150 μM Ca^{2+} showed respiration of 36 ngatoms O/mg · min in the active state and RCI 2.5 1 hour after

Fig. 1. Influence of 15-keto-PGB_1 oligomers on mitochondrial respiration in the state of phosphorylation (State 3) and at rest (State 4). Corresponding activities of oligomers in both states are A_3 and A_4. For definition and dimensions see Methods.



isolation. Activities A_3 and A_4 for the Ca^{2+}-loaded mitochondria are shown in Figs 2 and 3. The A_4 values for Ca^{2+}-loaded mitochondria are approx. two times greater than for nonloaded mitochondria, and Fraction III (M_w 3040) differs from the others by its lowest A_4 value.

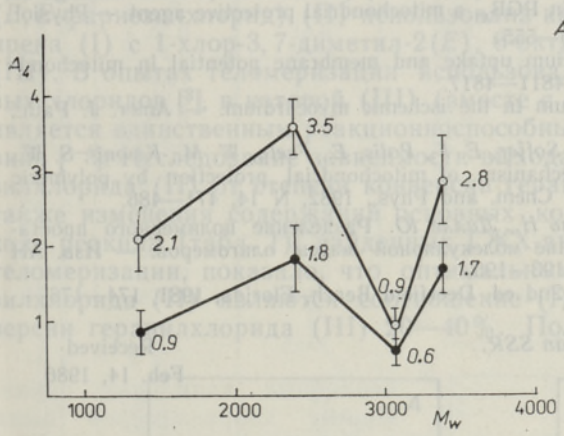


Fig. 2. Activities of oligomers on mitochondrial respiration for Ca^{2+}-loaded (—○—○—) and intact (—●—●—) mitochondria in State 4 (A_4).

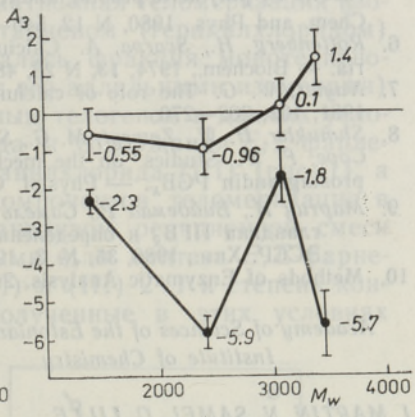


Fig. 3. Activities of oligomers on mitochondrial respiration for Ca^{2+}-loaded (—○—○—) and intact (—●—●—) mitochondria in State 3 (A_3).

In the active state, the intramitochondrial Ca^{2+} prevents the inhibition of phosphorylation by oligomers. Furthermore, an increase of the concentration of Fraction IV (M_w 3370) by 1 μm stimulates respiration by 1.36 ngatoms O/mg · min (Fig. 3).

Discussion

The beneficial effect of PGB_x serving to protect oxidative phosphorylation during hypotonic degradation was revealed in aged (4 days at 0 °C) mitochondria. An addition of PGB_x also prevents the Ca^{2+}-inhibition of phosphorylation of two-day-old mitochondria [1]. On the contrary, the inhibition of respiration in State 3 and stimulation in State 4 of two-hour-old mitochondria by PGB_x were observed during the present study. By our results, the oligomeric derivatives of 15-keto-PGB_1 act like inhibitors

and uncouplers of oxidative phosphorylation. It is surprising that oligomers with a mean molecular weight of 2400 are able to protect oxidative phosphorylation in aged mitochondria, but at the same time they inhibit and uncouple oxidative phosphorylation in intact mitochondria. Loading mitochondria with Ca^{2+} does not change the mechanism of action of oligomers in State 4 because the shapes of activity curves are similar (Fig. 2). But in the state of phosphorylation (State 3), the Ca^{2+}-loaded mitochondria were affected by oligomers only to an inconsiderable extent and the curve shape differed from that obtained with nonloaded mitochondria.

REFERENCES

1. *Polis, B. D., Polis, E., Kwong, S. W.* Protection and reactivation of oxidative phosphorylation in mitochondria by a stable free-radical prostaglandin polymer (PGB_x). — Proc. Natl. Acad. Sci., 1979, **76**, 1598—1602.
2. *Ohnishi, S. T., Devlin, T. M.* Calcium ionophore activity of a prostaglandin B_1 derivative (PGB_x). — Biochem. Biophys. Res. Communs, 1979, **89**, N 1, 240—245.
3. *Angelakos, E. T., Riley, R. L., Polis, B. D.* Recovery of monkeys from cardiogenic shock after myocardial infarction with ventricular fibrillation; effects of PGB_x. — Physiol. Chem. and Phys., 1980, N 12, 81—96.
4. *Polis, E., Cope, F. W.* Polymeric prostaglandin PGB_x and other prostaglandin polymers prolong survival of the heart of the hypoxic mouse. — Aviation, Space and Environ. Med., 1983, **54**, N 5, 420—424.
5. *Kolata, R. J., Polis, B. D.* Facilitation of recovery from ischemic brain damage in rabbits by polymeric prostaglandin PGB_x, a mitochondrial protective agent. — Physiol. Chem. and Phys., 1980, N 12, 545—555.
6. *Rottenberg, H., Scarpa, A.* Calcium uptake and membrane potential in mitochondria. — Biochem., 1974, **13**, N 23, 4811—4817.
7. *Nayler, W. G.* The role of calcium in the ischemic myocardium. — Amer. J. Path., 1981, **102**, 262—270.
8. *Shmukler, H. W., Zawryt, M. G., Soffer, E. F., Polis, E., Feely, W. M., Kwong, S. W., Cope, F. W.* Studies on the mechanism of mitochondrial protection by polymeric prostaglandin PGB_x. — Physiol. Chem. and Phys., 1982, N 14, 471—486.
9. *Мартин И., Вийдемаа Р., Самель Н., Лилле Ю.* Разделение полимерного простагландина $ПГВ_x$ и определение молекулярной массы олигомеров. — Изв. АН ЭССР. Хим., 1986, **35**, № 3, 193—196.
10. Methods of Enzymatic Analysis. 2nd ed. Deerfield Beach, Florida, 1981, 174—176.

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OKSÜDATIIVSE FOSFORÜÜLIMISE ANKAPLING JA INHIBEERIMINE MITOKONDRITES 15-KETO-PGB_1 OLIGOMEERIDE (PGB_x) POOLT

On näidatud, et oligomeerne prostaglandiin PGB_x molekulmassiga 2200—2500, mis on võimeline kaitsma oksüdatiivset fosforüülimist mitokondrites hüpotoonilise degradatsiooni käigus, käitub intaktses mitokondris kui oksüdatiivse fosforüülimise ankapler ja inhibiitor. 5,85 nmooli PGB_x (M_w 2400) lisamine 1 mg mitokondritele põhjustab 50%-list fosforüülimise inhibeerimist. Pärast mitokondrite inkubeerimist 150 μm Ca^{2+} kahekordistus PGB_x toime neljandas staadiumis, samal ajal aga kolmandas staadiumis inhibeerivat toimet ei täheldatud.

И. МАРТИН, Н. САМЕЛЬ, Ю. ЛИЛЛЕ

РАЗОБЩЕНИЕ И ИНГИБИРОВАНИЕ ОКИСЛИТЕЛЬНОГО ФОСФОРИЛИРОВАНИЯ В МИТОХОНДРИЯХ ОЛИГОМЕРАМИ 15-КЕТО-$ПГВ_1$ ($ПГВ_x$)

Показано, что олигомерное соединение $ПГВ_x$ с молекулярным весом 2200—2500, оказывающее полезное действие на окислительное фосфорилирование в поврежденных митохондриях, ингибируют его в интактных митохондриях. Добавление 5,85 нмоля $ПГВ_x$ (М 2400) к 1 мг митохондрий вызывает ингибирование фосфорилирования на 50%. В митохондриях, поврежденных 150 мкМ Ca^{2+}, действие $ПГВ_x$ на дыхание в 4-м состоянии увеличивается вдвое, а в 3-м состоянии ингибирующего действия не обнаруживается.