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THE MAINTENANCE OF HIGH ATP/ADP LEVEL IN THERMALLY DEGRADED MITOCHONDRIA BY THE 16,16-DIMETHYL-15-KETO-PGB$_1$ TRIMER

Ivar MARTIN, Erik MÄNNIK, Ülo LILLE. KÕRGE ATP/ADP TASEME SÄILIMINE TERMILISELT DEGRADEERITUD MITOKONDRITES 16,16-DIMETÜÜL-15-KETO-PGB$_1$ TOIMEL

Ивар МАРТИН, Эрик МЯННИК, Юло ЛИЛЛЕ. СОХРАНЕНИЕ ВЫСОКОГО УРОВНЯ АТФ/АДФ В ТЕРМИЧЕСКИ ДЕГРАДИРОВАННЫХ МИТОХОНДРИЯХ ТРИМЕРОМ 16, 16-ДИМЕТИЛ-15-КЕТО-ПГВ$_1$

The means expected to protect the heart against the loss of function associated with an ischemic episode include those which maintain the intracellular availability of ATP thereby ensuring that sufficient ATP is available to maintain a relatively low cytosolic Ca^{++}, and which interact directly with the mitochondria thereby preventing them from becoming overloaded with Ca^{++} [1]. The prostaglandin B_1 oligomers synthesized from 15-keto-PGB$_1$ or its 16,16-dimethyl analog have a number of interesting biological activities concerning intracellular Ca^{++} and oxidative phosphorylation in mitochondria (synthesis of ATP). They have been shown to maintain oxidative phosphorylation during the hypotonic degradation in aged mitochondria [2, 3] and stimulate the release of Ca^{++} from mitochondrial pool in isolated hepatocytes (Ca^{++} ionophoric activity) [4, 5]. It has been reported that *in vivo* PG-oligomers exhibit the protection of animals following the cardiac [6] and cerebral ischemia [7], and hypoxia [8]. After taking into account the effects of oligomers *in vitro* and *in vivo* we hope that they will interact with the mitochondrial membrane, thereby preventing mitochondria from losing the oxidative phosphorylation ability during the degradation process.

In this report we describe the protective effect of 16,16-dimethyl-15-keto-PGB$_1$ trimer (further Trimer, Mw = 1086) during the thermal degradation of mitochondria in the absence of phosphate acceptor (ADP) at the 30 °C. We shall demonstrate this protective effect by the measurements of ATP/ADP ratio with the usage of ion-pairing chromatography.

Synthesis, separation and molecular mass determination of the Trimer is presented in [9]. Isolation of rat liver mitochondria and determination of respiratory control index (RCI) of isolated mitochondria, and the effects of the Trimer on the mitochondrial respiration parameters were performed by the methods described in [10].

Degradation of mitochondria. The degradation medium contained 100 mM KCl, 20 mM HEPES buffer pH 7.4, 8 mM K-glutamate, 4mM K-malate, 6 mM $MgCl_2$, 10 mM KH_2PO_4, and 2 mg/ml of mitochondrial protein at 30°C. The Trimer was added before the initiation of the degradation by the addition of mitochondria. Max. degradation time was 60 min.

Oxidative phosphorylation. The 0.5 ml of degradation medium was transferred to the aliquot of 0.3 M sucrose, 20 mM HEPES buffer pH 7.4 and 0.6 mM EGTA at appropriate times. After a 1 min incubation 0.3 μmoles of ADP was added. The phosphorylation was terminated after 4 min by the centrifugation of the reaction medium and a sample of 30 μl was separated from the supernatant layer for the chromatographic analysis of AMP, ADP and ATP.

HPLC analysis of AMP, ADP and ATP was performed by ion-pairing chromatography on the Zorbax ODS column with 5 mM tetrabutylammonium phosphate, 20 mM phosphate buffer pH=6.2 and 15% acetonitrile as an eluent system at 254 nm. The LKB 2220 Recording Integrator was used for the integration of peaks.

Results and discussion

The ATP/ADP ratio of intact mitochondria (degradation time 0 min) after the state of phosphorylation (Steady State 4) was determined to be above 15 (16—30) depending on the quality of separation and handling of mitochondria. The RCI values in the range of 6—7 and respiration of 72 ngatoms O/(mg·min) in the state of phosphorylation were determined in 1 hour after the isolation. Preincubation of mitochondria in the absence of phosphate acceptor at 30°C (thermal degradation) leads to the time-dependent decrease of the ATP/ADP ratio down to 0.75. The ATP/ADP

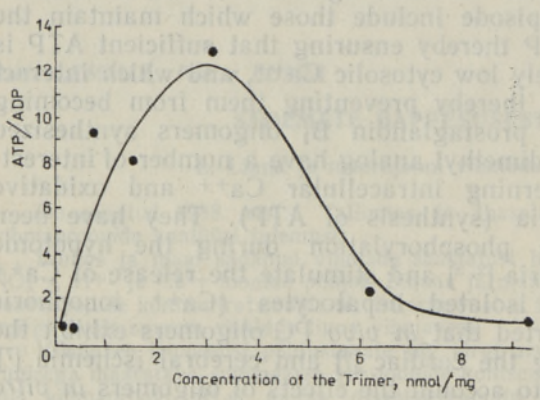

Fig. 1. The ATP/ADP level of 25 min degraded mitochondria by the 16,16-dimethyl-15-keto-PGB_1 trimer.

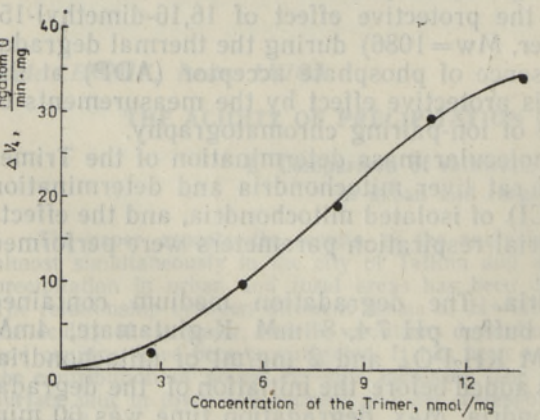

Fig. 2. Stimulation of mitochondrial State 4 respiration (ΔV_4) by the 16,16-dimethyl-15-keto-PGB_1 trimer.

ratio 1.4 was achieved after the 25 min degradation (the Table). The presence of the Trimer in the degradation medium prolongs the mitochondrial degradation time. For example, the 45 min incubation was needed to decrease the ATP/ADP ratio to the same level. However, the bell-shaped relationship between ATP/ADP ratio and the concentration of the Trimer in the degradation medium with the maximum at the 3.3 nmol/mg mitochondrial protein at the 25 min degradation was observed (Fig. 1). This fact is consistent with the results of [10, 11] where the inhibition and uncoupling of oxidative phosphorylation by the oligomers of 15-keto-PGB_1 (PGB_x) at higher concentrations has been shown. Our results on the mitochondrial respiration at Steady State 4 (Fig. 2) suggest that the Trimer caused the increased State 4 respiration and consequently induced the uncoupling of phosphorylation at the concentration of 6—12 nmol/mg as well. Apparently, at higher Trimer concentrations the protective effect of the Trimer during the degradation was suppressed by the uncoupling. Finally, we conclude that the optimum amount of the Trimer (3 nmol/mg) is needed for the demonstration of its beneficial effect to protect the mitochondrial from the degradation.

The effect of the 16,16-dimethyl-15-keto-PGB_1 trimer on the ATP/ADP level during the thermal degradation

Degradation time, min	ATP/ADP	
	Control	Trimer 3.11 nmol/mg protein
0	>15	>15
10	>15	>15
15	11.9	>15
20	3.3	>15
25	1.4	>15
30	1.0	11.3
40	0.75	1.7
50	—	0.9

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