# Does an Inhibitor of Mitochondrial Adenylate Kinase also Affect Oxidative Phosphorylation?

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*Summary.* Adenylate kinase activity of intact mitochondria is strongly inhibited by $Ap_5A$, i.e. $p^1,p^5$-Di (adenosine-5'-) pentaphosphate, whereas oxidative phosphorylation is not affected. Therefore, $Ap_5A$ is a useful tool to distinguish between oxidative and non oxidative ATP generating reactions.

In 1973 LIENHARD and SECEMSKI[2] showed that $Ap_5A$, i.e. $p^1,p^5$-Di(adenosine-5'-)pentaphosphate, inhibits rabbit muscle adenylate kinase (E.C. 2.7.4.2) with a $K_i$ of $3\times10^{-8}$ *M*. This property makes $Ap_5A$ seem a valuable tool for the investigation of mitochondrial reactions, because its application should allow one to distinguish between oxidative and non-oxidative ATP generating reactions. However, 2 questions arise: 1. Is the inhibitory effect of $Ap_5A$ weakened by the more difficult accessibility of the adenylate kinase, when it is an integral part of the mitochondrial architecture? Till now only the pure enzyme has been shown to be inhibited[2]. 2. If $Ap_5A$ does inhibit mitochondrial adenylate kinase, does it also affect oxidative phosphorylation? According to the hypothesis of OZAWA and MACLENNAN[3,4], postulating a functional relationship between oxidative phosphorylation and adenylate kinase (for details see 'Conclusions'), inhibition of adenylate kinase necessarily causes an inhibition of ATP synthesis. To answer these questions, we carried out the following experiments using intact mitochondria.

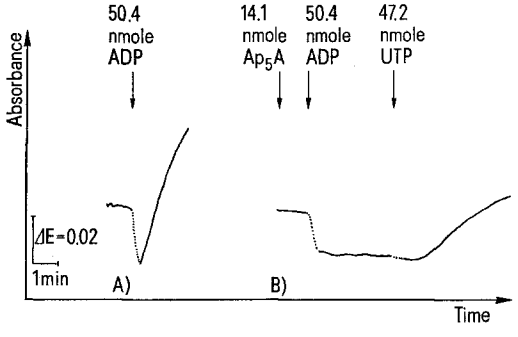


Fig. 1. Mitochondrial ATP formation was measured in a total volume of 0.5 ml. Additions made to the incubation mixture were: glucose: 1 μmole; NADP: 0.12 μmole, atractyloside 4 μg per mg of mitochondrial protein; enzyme mixture (hexokinase/glucose-6-phosphate dehydrogenase): 2 μl. Increase of extinction at 340 nm was recorded by a Leitz photometer. $\varepsilon_{340}^{NADPH_2}$ is 6,220 $M^{-1}$ cm$^{-1}$; path length was 0.2 cm. As atractyloside was added, the ATP formation shown in experiment A must be due to the adenylate kinase reaction. Experiment B shows that $Ap_5A$ inhibits adenylate kinase, but does not inhibit nucleosidediphosphate kinase.

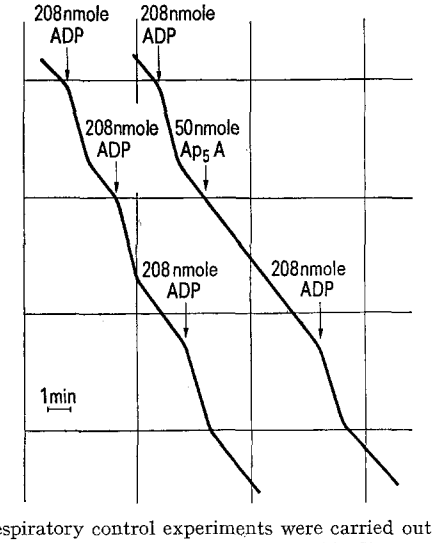


Fig. 2. Respiratory control experiments were carried out in a final volume of 2.0 ml with a commercially available Clark type oxygen electrode (L. Eschweiler & Co., Kiel, GFR). The incubation mixture was preincubated with 10 μmoles of succinate as an electron donator for 2 min. After the addition of $Ap_5A$, no alteration concerning state 4 or state 3 can be recognized.

*Materials.* Rat liver mitochondria were prepared from male 'Wistar' rats (100–150 g) according to well-known procedures[5]. Protein content was determined by the Biuret method. Nucleotides and NADP were obtained from Boehringer Biochemica (Tutzing, GFR). Atractyloside and $Ap_5A$ (the latter now being commercially available) were generous gifts from Dr. G. WEIMANN of Boehringer Biochemica. The concentrations of the $Ap_5A$ solutions used were determined by measuring the extinctions at 259 nm with $\varepsilon_{259}^{Ap_5A}$: 30,000 $M^{-1}$ cm$^{-1}$. A possible hypochromicity due to the structure of $Ap_5A$ was neglected. Enzymes: A mixture of hexokinase (E.C.2.7.1.1; 280 U/ml) and glucose-6-phosphate dehydrogenase (E.C.1.1.1.49; 140 U/ml) was obtained from Boehringer Biochemica (Tutzing, GFR).

*Methods.* All experiments were carried out in an incubation mixture containing 0.25 *M* sucrose, 10 m*M* triethanolamine, 0.2 m*M* EDTA, 10 m*M* KCl, 10 m*M* $MgCl_2$ and 5 m*M* inorganic phosphate at pH 7.4 and 21 °C. Total mitochondrial protein was always about 1 mg/ml. For further additions see legends of the figures; note that the final concentrations of all nucleotides were equal although different absolute amounts had to be added due to the different assay volumes.

Adenylate kinase activity was determined by measuring the ATP formation with the hexokinase/glucose-6-phosphate dehydrogenase system. To exclude ATP formation by oxidative phosphorylation, atractyloside was added to all assays. For further details see legend of Figure 1. Respiratory control experiments and assays of the redox cycles of the endogeneous pyridine nucleotides were carried out as described in the legends of Figures 2 and 3.

*Results.* Figure 1 shows two typical experiments to examine the effect of $Ap_5A$ on the adenylate kinase activity. In the absence of $Ap_5A$ the addition of 50.4 nmole of

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ADP causes an ATP formation of 0.029 ± 0.005 µmole/min per mg of mitochondrial protein (experiment A). After the addition of 14.1 nmole of $Ap_5A$ (i.e. 28.2 nmole per mg of mitochondrial protein) no ATP formation occurs (experiment B). It should be mentioned here that, in another set of experiments, we made sure that $Ap_5A$ does not affect the hexokinase/glucose-6-phosphate dehydrogenase system itself. The amount of $Ap_5A$ added in experiment A is more than sufficient to inhibit the adenylate kinase reaction completely. We have found that the addition of only 0.5 nmole of $Ap_5A$ leads to a 60% inhibition of the ATP formation corresponding to a $K_i$ value between $10^{-6}$ $M$ and $10^{-7}$ $M$.

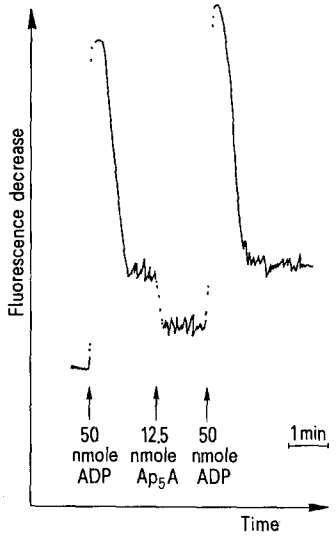


Fig. 3. Redox cycles of the endogenous pyridine nucleotides were carried out in a final volume of 0.5 ml. Excitation filter: 313 + 366 nm, emission filter: 500–3000 nm. The incubation mixture was preincubated with 1 µmole of succinate as an electron donator for 2 min. After the addition of $Ap_5A$, no alterations of the redox cycles occur.

Looking once more at Figure 1 B, one can see that even high concentrations of $Ap_5A$ do not inhibit the mitochondrial nucleosidediphosphate kinase activity. The addition of 47.2 nmole of UTP leads to a clearly recognizable ATP formation, although $Ap_5A$ is present. Figures 2 and 3 show that even high concentrations of $Ap_5A$ do not at all affect oxidative phosphorylation. In respiratory control experiments (Figure 2), neither state 4, state 3, nor the P/O ratio is altered. These findings are confirmed by assaying the redox cycles of the endogenous pyridine nucleotides (Figure 3). Neither their shape, height, nor basic width is affected by $Ap_5A$.

*Conclusions.* 1. Adenylate kinase activity is inhibited by $Ap_5A$, even when the enzyme is an integral component of the mitochondrial architecture, but its more difficult accessibility leads to a higher $K_i$ value than that established by LIENHARD and SECEMSKI[2] for the pure enzyme. Fortunately the decrease of the inhibitory effect is not very striking, probably due to phenomena similar to the 'intramitochondrial intermembranal large amplitude protein movements' described by WAKSMAN and RENDON[6] for mitochondrial aspartate aminotransferase. 2. The generally accepted view that in intact mitochondria ADP is the primary $P_i$-acceptor during oxidative phosphorylation has been challenged by OZAWA and MACLENNAN[3,4]. According to their hypothesis, ADP is generated by oxidative phosphorylation of AMP and then converted to ATP and AMP by adenylate kinase. AMP again acts as $P_i$-acceptor. Our results contradict their conclusions, because $Ap_5A$ inhibits mitochondrial adenylate kinase completely, but does not affect oxidative phosphorylation at all.

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## Karyological Patterm of two Chilean Lizards Species of the Genus *Liolaemus* (Sauria; Iguanidae)

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*Summary.* The karyotypes of Chilean lizards *Liolaemus pictus* and *Liolaemus cyanogaster* is described for the first time. Both species possess 34 chromosomes; 6 pairs of macrochromosomes and 11 pairs of microchromosomes. Karyologically it is possible to differenciate this species because the pair No. 2 is metacentric (*m*) in *L. pictus* and submetacentric (*sm*) in *L. cyanogaster*. It is shortly discussed the signification of formule $2n = 34$ for the species of *Liolaemus* analized karyologically and its possible mechanism of acquisition.

There is little chromosome information concerning the South American lizards of the genus *Liolaemus*. The only known species is *Liolaemus lutzae* from Brazil ($2n = 34$)[1], of which only the chromosomes of one male individual have been reported. In the present paper, new karyological data about lizards of the genus are given. The chromosomes of *Liolaemus pictus* and *Liolaemus cyanogaster*, two species of South Chile, are presented for the first time. Both species overlap geographically (Concepción to Chiloé) and exhibit a spectrum of morphological, ecological and physiological adaptations to certain habitats (forests and meadows). The two species are different in details of scutellation and colour patterm. The classification of *L. pictus* and *L. cyanogaster* follows that of DONOSO-BARROS[2]. Chromosomes were obtained from bone marrow, testes and leukocytes of young animals previously injected with colchicine 0.1%. The chromosome preparations were made according to methods developed by FORD and HAMERTON[3] (bone marrow and testes) and BAKER et al.[4] (culture of whole blood) and stained afterwards with Giemsa solution. Chromosome classification (only macrochromosomes) according

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