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Effects of 10-*n*-Butyl-3-methyl-5-deazaflavo-6,9-quinone (5-dFlQ) on Mitochondrial Respiration

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Abstract. 10-*n*-Butyl-3-methyl-5-deazaflavo-6,9-quinone (5-dFlQ) significantly stimulated the state 4 respiration in mitochondria in a concentration-dependent manner. The respiration inhibited by rotenone and antimycin A was recovered by the addition of 5-dFlQ. These facts suggest that 5-dFlQ acts as electron carriers such as flavins or ubiquinone in the mitochondrial respiratory chain and forms a new pathway for electron transfer.

Introduction

Riboflavin in which ribitol is bound to tricyclic isoalloxazine has well been known to play a role as a biological redox agent in transfer of one or two electrons. In 1967 and 1970, 5-deazaflavin, in which N-5 of the flavin is replaced by CH, was synthesized as a potential riboflavin antagonist by Cheng et al.[3,4](Chart 1). Since then, there have been several reports of syntheses of 5-deazaflavin derivatives[5,6,7], and the example of a catalytic autorecycling amine oxidation with synthetic 5-deazaflavins has been reported by Yoneda and co-workers[8]. In 1978, coenzyme F_{420} possessing an 8-hydroxy-5-deazaflavin moiety was isolated from methanogenic bacteria[9] (Chart 1).

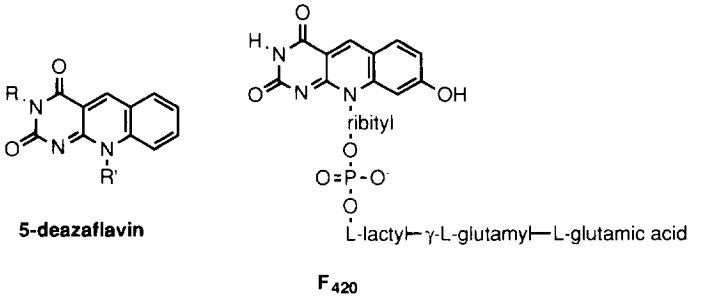
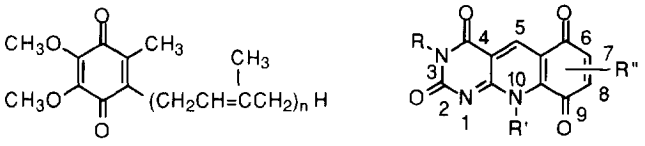


Chart 1. Structures of 5-deazaflavin and coemzyme F_{420}

Kimachi et al. have recently reported the synthesis of 5-deazaflavo-6,9-quinones which are a hybrid model compound of 5-deazaflavin and ubiquinone (coenzyme Q) by introduction of benzoquinone structure to the benzene ring of 5-deazaflavin, and have demonstrated a catalytic amine oxidation with them under mild condition[10] (Chart 2). Most recently some of 5-deazaflavo-6,9-quinone derivatives have been found to have antitumor activities[11], while their mechanistic details were unknown.



Coenzyme Q **5-deazaflavo-6,9-quinone**

Chart 2. Coenzyme Q and 5-deazaflavo-6,9-quinone

In the meantime, it is known that the proton is transferred through the electron transport to the outside of inner mitochondrial membrane, turning out to make the proton gradient and the membrane potential. As the mitochondrial respiration is controlled by the membrane potential, respiratory rate is comparatively slow in the absence of ADP ("state 4" according to Chance and Williams[12]). But when the agent diminishing the proton gradient is added to the mitochondria, the state 4 respiratory rate will be stimulated in order to keep the proton gradient to the appropriate level. In the presence of ADP, ATP formation and proton transportation to the inside of inner membrane by ATP synthase induce comparatively rapid respiratory rate ("state 3"). 5-Deazaflavo-6,9-quinone derivatives seem to become components of the mitochondrial respiratory chain, and considered to be involved in electron transfer like flavins and ubiquinone, which play a role in acceptance of one or two electrons. In the present paper, we report the stimulatory effect of 10-*n*-butyl-3-methyl-5-deazaflavo-6,9-quinone (5-dFlQ) on the respiration of mitochondria.

Materials and Methods

Respiratory rate measurement was carried out according to the procedure described as follows. After the addition of substrates to mitochondrial suspension[13] (2 mg protein), oxygen consumption was traced for 2 minutes to achieve the state 4 respiration. 10-*n*-Butyl-3-methyl-5-deazaflavo-6,9-quinone (5-dFlQ) was chosen as a representative compound of 5-deazaflavo-6,9-quinones because it could be easily prepared in sufficient quantities and had good solubility in the buffer used. 5-dFlQ (10-50 nmol) were added when the state 3 respiration by the addition of 400 nmol of ADP was returned to the state 4, and the respiratory rate of state 4 was traced for at least 2 minutes[14].

Results and Discussions.

5-dFlQ (50 nmol) were added to the state 4 respiration with succinate plus rotenone as substrate. The result is shown as an oxygram (respiratory rate of mitochondria) in Fig 1. The respiratory rate reached maximum immediately after the initial addition with 5-dFlQ, while it had no effect at the subsequent addition of oligomycin (ATP synthase inhibitor). This indicates that 5-dFlQ enhances the state 4 respiration at a site not

involving ATP synthase. As illustrated in Fig. 2, addition of 5-dFlQ (10, 30, and 50 nmol) showed a concentration-dependent stimulation in state 4 respiration rate (Fig. 2).

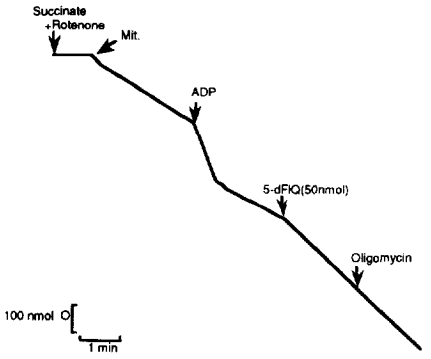


Fig.1. Effect of 5-dFlQ on mitochondrial state 4 respiration.

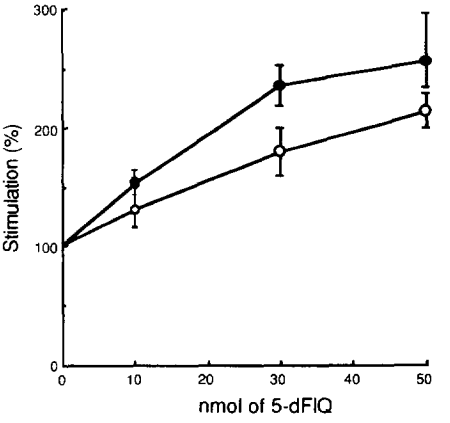


Fig.2. Effects of various concentration of 5-dFlQ on the state 4 respiration rate supported by either glutamate + malate (closed circles) or succinate (open circles).

The redox potential of 5-dFlQ is -790 mV (vs Ag/AgCl, 0.5 mM in 20 ml of DMF containing 0.1 M n-$Bu_4N^+Cl^-$), and that of CoQ is -840 mv (same condition). The similar electrochemical property between 5-dFlQ and coenzyme Q suggests that 5-dFlQ can easily capture electron(s) from electron donors in the respiratory chain. Furthermore the state 4 respiration (supported by glutamate plus malate) stopped by the initial addition of Rotenone restarted by the second addition of 5-dFlQ, and in the case of the state 4 respiration (supported by succinate) stopped by initial addition of Antimycin A, the similar result was obtained (Fig. 3). These suggest that 5-dFlQ forms a new pathway to accept electron(s) at the position upper than their inhibition sites.

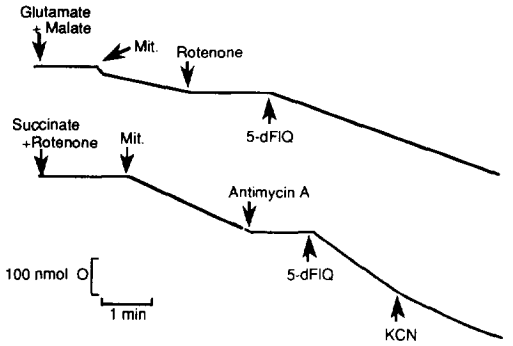


Fig.3. Effects of 5-dFlQ (50 nmol) on inhibited respiration by rotenone (1.6 μM) or antimycin A (0.5 μg/ml) and KCN (5 μM).

In conclusion, 5-dFlQ significantly stimulated the state 4 respiration in mitochondria. It is proposed that 5-dFlQ form the new pathway for electron transfer by bypassing the conjugation site and diminish the formation of the proton gradient, which led to the accerelation of the electron transfer in mitochondria.

References and Notes

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13. Mitochondria were isolated from liver of Sprague Daweley rat aged 7-8 weeks according to the methods of Hoogeboom[15] and Slater[16].
14. Respiration rate measurement was carried out polarographically using a Clark oxygen electrode (Yellow Springs Instruments, U.S.A.) at 30°C in the medium (4.0 ml) containing 200 mM sucrose/2 mM $MgCl_2$/1 mM EDTA-2Na/10 mM Tris-Cl/10mM K-Pi, (pH 7.4). The functional integrity of the mitochondria was tested by the RCR (respiratory control ratio). The RCR was calculated by dividing the respiratory rate measured in the presence of ADP (state 3) by those subsequently obtained when ADP has been used up (state 4). Glutamate together with malate (5 mM eath) or succinate (10 mM) was used as substrate and rotenone (1.6 μM) was added in the case that succinate was used as substrate to prevent hydrogen channeling into the respiratory chain . Oligomycin (5 μg/ml), an inhibitor of oxidative phosphorylation, was introduced 1.5 min after the initial addition of 5-dFlQ (50 nmol), while respiratory inhibitor such as rotenone (1.6 μM), antimycin A (0.5 μg/ml), or KCN (5 μM) was added with 5-dFlQ .
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