Chemical natures and application of 6-formylpterin derivatives

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ABSTRACT

Pterin, an analog of guanine, is an electron transfer compound in biological systems. Among the analogs, 6-formylpterin (6FP) has been demonstrated to have many marked physiological and pharmacological activities. In vitro, 6FP converts molecular oxygen to reactive oxygen species (ROS) in the presence of NADH or NADPH under light illumination, with the oxidation of NADH to NAD⁺. In the present study, it has been elucidated that some of 6FP derivatives in which the 3-position of 6FP is modified have such unique activities even in the dark where the most of in vivo biological events occur.

INTRODUCTION

Pterin whose structure is analogous to guanine is an electron transfer compound in biological systems. Some endogenous pterins are known to play important roles in immune or cytokine systems and also works as a cofactor for the enzymatic hydroxylation of the aromatic amino acid. 6-Formylpterin (6FP) which is one of the pterin derivatives is generated from folic acid, which abundantly exists in nature, by photooxidation in the presence of oxygen. 6FP is produced in vivo from folic acid in some pathological conditions, such as carcinoma. In the previous studies we have demonstrated that 6FP has potent neuroprotective effects against transient ischemia-reperfusion injury (IRI) in gerbils and that the similar effects of 6FP in rat retinal IRI. IRI occurs in cardiac infarction, brain infarction, organ transplantation, and so on that result in apoptotic cell death. The protective agents against the IRI have been important research targets in pharmaceutical studies over the past two decades, so 6FP is attracts researchers. We have also demonstrated that 6FP generated H₂O₂, one of the reactive oxygen species (ROS), in the cells. Since ROS is not only involved in cell death but also modulates a variety of cell functions, this unique property of 6FP remains to be revealed. ROS production by pterin derivatives has been investigated in the field of photochemistry intensively in the last two decades, and under UV irradiation, some pterin derivatives were found to activate O₂ and generate ROS. In vitro, 6FP was also found to generates superoxide anion radicals (O₂⁻) from O₂ in the presence of NAD(P)H under light illumination. We have also demonstrated that 6FP derivatives whose 2- and 3-positions are modified have enhanced water solubility and convert O₂ to O₂⁻ in the presence of NADH under light illumination as 6FP does. In vivo, however, the most of biological reactions proceed in the dark. To elucidate the physiological activities of 6FP in vivo, it is essential to investigate reactivity of 6FP in the dark. In living organisms, the ubiquitously occurring electron source is NADH, which is a central intermediate in oxidative catabolism and acts as a convenient source of readily transferable electrons in cell. If 6FP and 6FP derivatives serve as an electron acceptor in vivo, it will accept electrons from NADH and induce some biological phenomenon in living cells. So, in the present study, we used NADH as an electron source and examined NADH oxidation activities of 6FP and 6FP derivatives in the dark.

RESULTS AND DISCUSSION

We prepared 6FP derivatives described in Fig. 1. DFP and DFM in which the 2-position amino group is modified by N,N-dimethylaminomethylene group and the nitrogen atom at the 3-position by pivaloyl and methyl groups, respectively, DF in which the 2-position is analogously modified and the 3-N free, and FM in which only the 3-N is modified by a methyl group and 2-amino group free were

![Image of chemical structures](https://sample-image-url.com/structure.png)

Fig. 1 6FP and its derivatives studied in this work.
synthesized. PBS solutions (pH 7.4) containing 2 mM of 6FP or 6FP derivatives and 2 mM NADH was allowed to stir in the complete darkness: It should be noted that to increase the concentration to 2 mM, pH of the 6FP sample was 9.7, and that because of the low water solubility, the concentration of DF was 1 mM and therefore the concentration of NADH was also 1 mM (pH 7.6). The time-dependent concentration change of each component in the sample solution was analyzed by HPLC. The results are shown in Fig. 2. 6FP, which catalyze NADH oxidation reaction rapidly under UV or light illumination, and DF did not show NADH oxidation activity in the dark. DFP, DFM and FM which have modified groups on the 3-position oxidized NADH in the dark. From these results, the modification on the nitrogen atom at the 3-position of 6FP is essential for introducing the NADH oxidation activity in the dark. Comparing DFP which has electron-withdrawing group on the 3-position with DFM which has electron-donating group on the same position, it is concluded that the nature of the modified groups is not significant for the NADH to NAD' oxidation activity in the dark.

**CONCLUSION**

We have found that some 6FP derivatives have the NADH oxidation activity in the dark. This activity was observed for DFP, DFM, and FM but not for 6FP or DF. This indicates that to have this activity, modification of the 3-position of 6FP is essential. The present findings would be the key for elucidating the mechanism of unique activity of 6FP that 6FP generate ROS in the cell, and designing the pharmacological compounds that generates appropriate and controllable amount of ROS in vivo. There is possibility that this kind of modification process of 6FP may be involved in biological systems.

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**REFERENCES**