アジドユビキノン-2の合成および電子伝達活性の評価

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Note

Synthesis and Electron-Transfer Activity of Azido Ubiquinone-2

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INTRODUCTION

Mitochondrial respiratory chain is one of the possible target sites of synthetic pesticides. Especially, novel types of NADH-ubiquinone oxidoreductase (complex-I) inhibitors such as fenpyroximate (NNI-850, Nihon Nohyaku), tebufenpyrad (MK-239, Mitsubishi Chemical), and fenazaquin (EL-436, Dow AgroSciences) are thought to hold important positions among most modern insecticides and acaricides.[1–3] Many complex-I inhibitors act at or close to the ubiquinone (Q) reduction site,[4,5] whereas our knowledge concerning the binding and redox properties of Q in complex-I is still not sufficient due to the enormous size and complexity of the enzyme. Considering that Q redox reaction is closely coupled to proton pumping of complex-I,[6] identification of the Q binding site(s) is highly important not only to elucidate the mode of action of the inhibitors, but also to get insight into structural and functional features of the enzyme.

Photoaffinity labeling technique using azido-Qs is powerful for this purpose,[7,8] whereas azido-Qs so far synthesized, such as 2-azido-3-methyl-5-methoxy-6-*n*-decyl-1,4-benzoquinone and 2,3-dimethoxy-5-azido-6-*n*-decyl-1,4-benzoquinone, did not necessarily serve as sufficient redox substrate of respiratory enzymes. In this study, we report efficient synthetic proce dures for 2-azido-3-methoxy-5-methyl-6-geranyl-1,4-benzoquinone (2-azido-Q_2, Fig. 1) and 2-methoxy-3-azido-5-methyl-6-geranyl-1,4-benzoquinone (3-azido-Q_2, Fig. 1) on the basis of our previous procedures that enable chemical modifications of the substituents at all positions in the quinone ring to other alkyl or alkoxy groups.[9] We examined electron transfer activities of the two azido-Q_2 with bovine heart mitochondrial complex-I.

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RESULTS AND DISCUSSION

Synthetic procedures of azido-Q_2 are summarized in Scheme 1. Two regioisomers of Q derivatives, wherein the substitution patterns in the 2- and 3-positions on the quinone ring are opposite to each other, are inseparable because of the very similar chromatographic properties.[10,11] Therefore, 2- and 3-azido-Q_2 must be synthesized via independent synthetic procedures. The key intermediates **8a** and **8b** were prepared by the same reaction conditions, but starting from different substituted phenols **2** and **5**, respectively, based on the previous method.[9] In general, when azide anion is used as a nucleophile on the *para*-quinone ring which possesses a methoxy group and some leaving group in vicinal positions, azide anion replaces the leaving group, but not the methoxy group.[12] To introduce azido group into the vicinal position of the methoxy group of **8**, we prepared hydroxy Q_2 (**11**) as a precursor of methanesulfonyl (mesyl) ester **12**. Benzyl group of **10a** was cleaved by $SnCl_4$ (0.5 eq) in CH_2Cl_2 at 0°C (yield, 76%). A half eq of $SnCl_4$ was sufficient to consume benzyloxy Q_2 (**10**). The hydroxy group of **11a** was converted to mesyl ester by mesyl chloride (2 eq) and triethylamine (3 eq) in THF at −20°C (yield, 84%). Mesyl ester **12a** was treated with NaN_3 (2 eq) in MeOH to give 2-azido-Q_2 (**1a**) in 37%. The yields of series **b** are almost the same as those of series **a** described here.

The electron accepting efficiencies of these azido-Q_2 were examined with bovine heart mitochondrial complex-I (Table 1). Their activities in terms of *Vmax*/*Km* value are efficient compared to Q_2 which is one of the best short-chain Qs with bovine complex-I.[9,13] This result is consistent with the previous observation that bovine complex-I loosely recognizes the 2- and 3-positions of quinone ring.[9] Thus, two azido-Q_2 synthesized in this study are expected to be useful probes for photoaffinity labeling study of bovine complex-I. It may be notable that 3-azido-Q_2 is further transformed by $NaBH_4$ reduction into rhodoquinone-2 (2-methoxy-3-amino-5-methyl-6-geranyl-1, 4-benzoquinone) which servers as an electron carrier in mitochondrial respiratory chain of anaerobic organisms like parasites.

EXPERIMENTAL

1. General

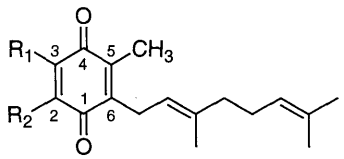
^{1}H NMR spectra; Brucker AC-300. Mass spectra; Jeol JMS HX-110. Column chromatography; YMC silica gel (SIL-60-S75).

2. Synthesis

2.1 2-Azido-3-methoxy-5-methyl-6-geranyl-1,4-benzoquinone; 2-azido Q_2 (1a)

Mesyl ester **12a** (3.19 g, 8.34 mmol) was dissolved in MeOH (70 ml). The subsequent operations were performed in the

dark. To the above solution, NaN_3 (1.08 g, 16.6 mmol) was added and the mixture was stirred for 1.5 hr at room temperature. The reaction was quenched with water and extracted with ether. The extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (9: 1) gave **1a** (1.02 g, 3.10 mmol, 37%) as a dark red oil. Not reacted mesyl ester **12a** (1.00 g, 2.61 mmol) was recovered. ^{1}H NMR ($CDCl_3$) δ ppm: 1.58 (3H, s, CH_3), 1.66 (3H, s, CH_3), 1.73 (3H, s, CH_3), 1.98-2.04 (4H, m, CH_2), 2.03 (3H, s, Ar-CH_3), 3.20 (2H, d, J=7.0 Hz, Ar-CH_2), 4.07 (3H, s, CH_3-O), 4.93 (1H, t, J=6.9 Hz, -CH =), 5.03 (1H, t, J=6.6 Hz, -CH =). HRFABMS (NBA-PEG) m/z [(M+H)$^+$]: Found, 330.1828; Calcd. for $C_{18}H_{24}N_3O_3$, 330.1818.



Q_2 : R_1 = OCH_3, R_2 = OCH_3
2-azide Q_2 (**1a**): R_1 =OCH_3, R_2 = N_3
3-azide Q_2 (**1b**): R_1 = N_3, R_2 = OCH_3

Fig. 1 Structures of azido-Qs synthesized in this study. To clarify the numbering of the positions on 1,4-benzoquinone ring, the numbers were standardized as shown in this figure throughout the text.

2.2 *2-Methoxy-3-azido-5-methyl-6-geranyl-1,4-benzoquinone; 3-azido Q_2 (1b)*

1b was synthesized same as **1a** from **12b** (1.52 g, 3.97 mmol) in the yield of 37%. ^{1}H NMR ($CDCl_3$) δ ppm: 1.58 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.73 (3H, s, CH_3), 1.92-2.04 (4H, m, CH_2), 2.04 (3H, s, Ar-CH_3), 3.19 (2H, d, J=7.0 Hz, Ar-CH_2), 4.08 (3H, s, CH_3-O), 4.91 (1H, t, J=7.0 Hz, -CH =), 5.03 (1H, t, J=6.7 Hz, -CH =). HRFABMS (NBA-PEG) m/z [(M+H)$^+$]: Found, 330.1815; Calcd. for $C_{18}H_{24}N_3O_3$, 330.1818.

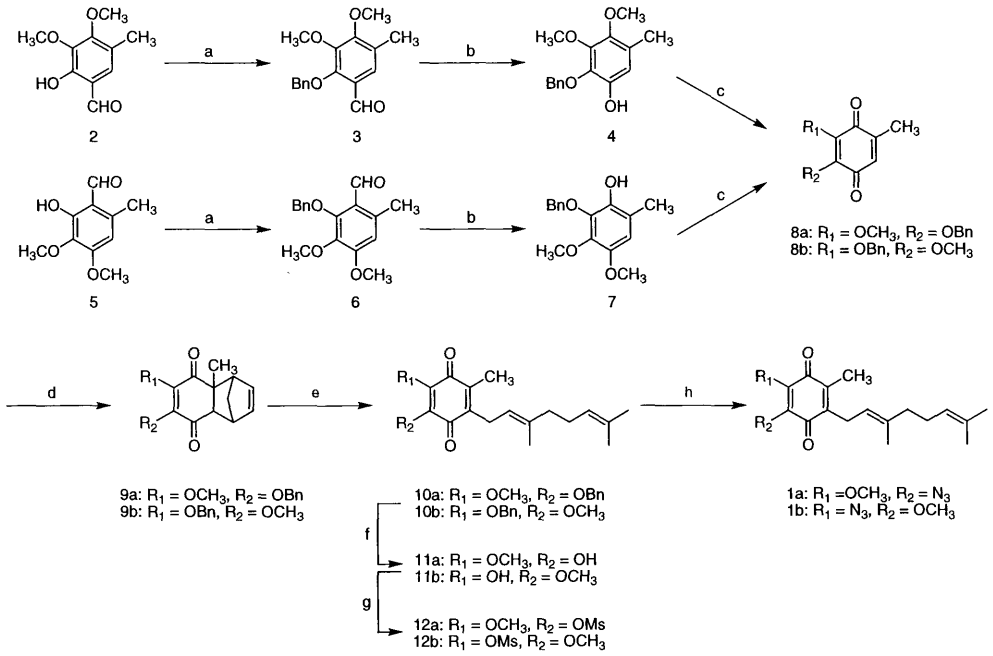
3. *Assay*

Bovine heart submitchondrial particles (SMP) were prepared by the method of Matsuno-Yagi and Hatefi[14] and stored in a buffer containing 0.25 M sucrose and 10 mM Tris-HCl (pH 7.4) at −78°C. NADH-ubiquinone reductase activity was measured as the rate of NADH oxidation at 30°C with a Shimadzu UV-3000 spectrophotometer at 340 nm (ε=6200

Table 1 Kinetic parameters of Q_2 analogues in bovine complex-I[a].

Qs	*Km* (μM)	*Vmax* (μmol/min/mg)	*Vmax/Km*
Q_2	2.0	0.21	0.11
2-Azido-Q_2 (**1a**)	4.3	0.26	0.06
3-Azido-Q_2 (**1b**)	4.1	0.36	0.09

[a]The *Km* and *Vmax* values were obtained by Linewever-Burk plot from two independent experiments.



Scheme 1 Synthesis of azido Q_2

a) BnBr (1.5 eq), K_2CO_3 (3 eq) in DMSO, 60°C, 1.5 hr (97%). b) (i) *m*-CPBA (1.2 eq) in EtOAc, rt, 24 hr. (ii) KOH (2.5 eq) in dioxane/water, rt, 40 min (82%). c) $Ce(NH_4)_2(NO_3)_6$ (2.5 eq), 2,6-pyridinedicarboxylic acid (2.5 eq) in MeCN/water, 0°C, 2 hr (77%). d) cyclopentadiene (10 eq) in CH_2Cl_2, rt, 24 hr (96%). e) (i) *t*-BuOK (1.1 eq), geranylbromide (1.2 eq) in THF/DMF, −20°C, 30 min. (ii) toluene, reflux, 2.5 hr (64%). f) $SnCl_4$ (0.5 eq) in CH_2Cl_2, 0°C, 30 min (76%). g) MsCl (2 eq), Et_3N (3 eq) in THF, −20°C, 1 hr (84%). h) NaN_3 (2 eq), MeOH, rt, 1.5 hr (37%).

$M^{-1}\,cm^{-1}$). The reaction medium, contained 50 mM potassium phosphate (pH 7.4), 0.25 M sucrose, 1 mM $MgCl_2$, 2 mM KCN, 0.2 μM antimycin A, 0.2 μM MOA-stilbene, and 30 μg/ml of mitochondrial proteins. After equilibration of SMP with the ubiquionone analogues, the reaction was started by the addition of 50 μM NADH.

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要　　約

アジドユビキノン-2の合成および電子伝達活性の評価

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ミトコンドリアNADH-ユビキノン酸化還元酵素（複合体-I）の阻害剤の中には現在，実用的な殺虫・殺ダニ剤として重要な位置を占めるものがいくつかある．複合体-Iにおけるユビキノン（Q）結合部位の同定は阻害剤の作用機構研究のみならず，本酵素の構造および機能特性を解明するうえで重要であり，そのための手法としてアジドキノンを用いた光親和性標識実験が有効であると考えられる．そこで，我々の開発したユビキノン類縁体の合成法を応用し，2-benzyloxy-Q_2 および 3-benzyloxy-Q_2 から3段階でそれぞれ 2-azido-Q_2 および 3-azido-Q_2 を効率よく合成した．牛心筋ミトコンドリア複合体-Iにおける電子伝達活性を評価したところ，両化合物とも良好な電子受容体として機能することがわかった．