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Note

Mutagenicity of Coenzyme Q_{10}

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Summary Mutagenicity of organically synthesized coenzyme Q_{10} (CoQ_{10}) was determined by Ames assay in the presence and absence of S9 mix. The tester strains were *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2 *uvr A*. CoQ_{10} displayed no mutagenicity in any tester strain at any dose tested. Therefore, organically synthesized CoQ_{10} was considered to possess no mutagenicity.

Key Words coenzyme Q_{10}, CoQ_{10}, ubiquinone, ubidecarenone, Ames

Coenzyme Q_{10} (CoQ_{10}, ubiquinone-10, or ubidecarenone) is a biofactor playing fundamental roles in the electron transport system and anti-oxidative system.

As Folkers mentioned in the 1985 review (*1*), "It was Dr. Hideaki Fukawa himself who synthesized CoQ_{10} at Nisshin and developed all aspects of the commercial synthesis, which made possible the clinical development and introduction of CoQ_{10} by the Eisai Co., Ltd., in 1974 to treat congestive heart failure, with approval by the governmental agency in Japan." The original manufacturing procedure was the partial synthesis from the solanesol (*all-trans*-nanoprenol) of plant origin (*2*). Since the Nisshin CoQ_{10} was the only available CoQ_{10} for the first decade, most of the basic and clinical studies had been conducted by using the CoQ_{10}, and it was approved as a prescribed drug in Japan. Ever since then the Nisshin-Eisai CoQ_{10} has been dominant in Japanese clinical practices. In 1994, the US government amended the Federal Food, Drug, and Cosmetic Act to approve dietary supplements to hold structure function claim, known as the Dietary Supplement Health and Education Act (DSHEA), which made CoQ_{10} a popular dietary supplement in the USA. CoQ_{10} was also listed in EP and USP, in 2001 and 2002, respectively. And in 2001, the Japanese government declared CoQ_{10} to be categorized as food.

Now CoQ_{10} is widely used in both the clinical field and consumer market around the world. In the USA, sales of CoQ_{10} as a supplement has kept increasing with annual growth ranging from 8 to 27% since 1997, and reaching 258 million dollars in 2003 (3). In Japan, dozens of supplements have appeared since 2001.

Although the preclinical safety data obtained using organically synthesized CoQ_{10} were reviewed by the Japanese Ministry of Health and Welfare (presently the Ministry of Health, Labour and Welfare) in the 1970s and also by the US FDA and EP more recently, they have not been fully published yet. Since the original CoQ_{10} utilized in research and clinical practice was organically synthesized CoQ_{10}, most of the safety reports were conducted with the organically synthesized CoQ_{10}. Recently, however, a few reports appeared to confirm the safety of fermented CoQ_{10}. Williams et al. reported that CoQ_{10} prepared by fermentation was well tolerated by rats at dose levels up to 1,200 mg/kg in a 52-wk oral chronic toxicity study (*4*). Kitano et al. published that microorganism biomass used as a new source in CoQ_{10} production has a no-observable-adverse-effect level of over 2,000 mg/kg/day (*5*). From these results, CoQ_{10} is generally recognized as safe. However, the mutagenicity data for CoQ_{10} have not been reported. This paper presents the mutagenicity of organically synthesized CoQ_{10}, which was re-evaluated in 2001.

Materials and Methods

Chemicals. CoQ_{10} (Nisshin Pharma, Tokyo, Japan; Lot. UEB10HR) used in the present investigation had a purity of 99.2% as analyzed by the Japanese Pharmacopeia method for ubidecarenone.

The following positive controls were used in the bacterial mutation assay: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2; Lot. WTH1046), sodium azide (NaN_3; Lot. DJC6398), 2-methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine·2HCl (ICR-191; PolySciences, IL, USA; Lot. 52300), 2-aminoanthracene (2-AA; Lot. KPQ0892), and benzo(a)pyrene (B[a]P; Lot. TPM5259). Acetone (Lot. ELP5465) was used as the solvent and negative control.

AF-2, NaN_3, 2AA, B[a]P, and acetone were obtained from Wako Pure Chemicals (Osaka, Japan).

Solutions. AF-2, ICR-191, 2AA, and B[a]P were dissolved in dimethyl sulfoxide (DMSO; Wako Pure Chemicals; Lot. SEG4422), water of injection (Hikari Pharmaceutical, Tokyo, Japan; Lot. A0011AA) was used as a solvent for NaN_3. The control solutions were kept at −20°C until use.

CoQ_{10} was dissolved in acetone just before use.

Bacterial Strains. *Salmonella typhimurium* TA98, TA 100, TA1535, and TA1537, and *Escherichia coli* WP2 *uvr A* were used in the study. *Salmonella typhimurium*

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TA98, TA1535, and TA1537 were provided by Prof. B. N. Ames, University of California, Barkley, CA, USA. *Salmonella typhimurium* TA100 and *Escherichia coli* WP2 *uvr A* were obtained from Japanese National Institute of Health and the Institute of Medical Science, the University of Tokyo, respectively.

Bacterial media. Minimum glucose agar M (Oriental Yeast Co., Tokyo; Lot. BM050HQ) and DIFCO BACTO-AGAR (Beckton Dickinson, NJ, USA; Lot. 139900XA) were used for the assay. Nutrient Broth No. 2 (Oxioid Limited, Hampshire, UK; Lot. 218041) was used for pre-incubation.

Bacterial reverse mutation assay. The mutagenicity assay was followed according to the standard procedure (*6*) using the 20-min preincubation modification (*7*). The tester strains were cultured overnight in Oxioid nutrient broth No. 2. The S9 was prepared from male Sprague-Dawley rat liver pretreated with phenobarbital and 5,6-benzofravone, and the cofactors were purchased from Oriental Yeast Co. (Lot. 999101). Two plates were used for each dose. A positive response in the test is defined to be a response where the maximum number of revertants per plate relative to the number produced spontaneously represented an increase of at least 2-fold, and a reproducible dose-response curve was observed.

All the assay procedures were performed at General Laboratory, BML (Saitama, Japan) under the supervision of the New Drug Development Research Center (Hokkaido, Japan).

Results and Discussion

The assay was performed twice, and the results are summarized in Tables 1 and 2. In the initial assay (Table 1), precipitates were observed at a dose not less than 78 μg/plate (−S9 mix) and 1,250 μg/plate (+S9 mix). Therefore the second assay (Table 2) was performed at 5 concentrations not more than 78 μg/plate (−S9 mix) and 1,250 μg/plate (+S9 mix).

As apparently shown in the tables, the numbers of revertant colonies observed in CoQ_{10} were not different from those of negative controls (none), and did not show any dose-dependency, with or without S9 mix, or in either type of mutation, base substitution type or frame-shift type. Therefore, CoQ_{10} was considered to have no mutagenic activities under these conditions.

These results were consistent with the previous study conducted by Eisai in the 1970s using organically synthesized CoQ_{10} (unpublished). Thus our results confirmed the original study ensuring the absence of

Table 1. Observed revertant count in tester strains in the initial assay.

S9 mix	Dose (μg/plate)	Base-substitution type			Frame-shift type	
		TA 100	TA1535	WP2uvrA	TA98	TA1537
Coenzyme Q_{10}						
−S9 mix	none[1]	136, 126	10, 13	22, 29	25, 25	15, 14
	1.2	150, 139	13, 15	24, 27	21, 25	10, 7
	4.9	120, 130	17, 12	25, 32	25, 22	10, 13
	20	128, 141	15, 20	30, 19	17, 19	8, 10
	78[2]	124, 118	12, 14	30, 21	20, 17	8, 13
	313[2]	117, 134	16, 12	24, 26	28, 17	12, 8
	1,250[2]	107, 136	17, 12	29, 21	16, 17	10, 7
	5,000[2]	115, 106	12, 8	23, 20	17, 18	6, 10
+S9 mix	none[1]	144, 134	20, 11	30, 31	36, 30	26, 19
	1.2	130, 150	13, 16	29, 24	29, 40	19, 16
	4.9	153, 138	6, 14	34, 30	44, 38	28, 33
	20	154, 150	10, 11	31, 30	35, 39	21, 22
	78	154, 169	13, 12	30, 33	41, 32	20, 18
	313	159, 136	13, 8	31, 30	26, 33	20, 26
	1,250[2]	140, 124	12, 8	28, 31	29, 33	10, 20
	5,000[2]	124, 119	11, 10	30, 25	26, 32	14, 16
Positive Controls						
−S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	dose[3]	0.01	0.5	0.01	0.1	1.0
	revertants	437	459	153	548	1268
		504	506	166	509	1799
+S9 mix	Chemical	B[a]P	2AA	2AA	B[a]P	B[a]P
	dose[3]	5.0	2.0	10.0	5.0	5.0
	revertants	1054	322	718	282	93
		1102	277	699	277	103

[1] none: acetone alone (negative control).
[2] Precipitates were observed.
[3] unit: μg/plate.

Table 2. Observed revertant count in tester strains in the second assay.

S9 mix	Dose (μg/plate)	Base-substitution type			Frame-shift type	
		TA 100	TA1535	WP2uvrA	TA98	TA1537
Coenzyme Q_{10}						
−S9 mix	none[1]	132, 129	13, 9	18, 19	23, 18	8, 11
	4.9	148, 144	8, 17	22, 12	28, 22	6, 11
	10	131, 132	15, 10	18, 21	16, 16	7, 9
	20	153, 163	11, 12	22, 24	25, 26	10, 7
	39	162, 138	14, 13	31, 23	19, 29	7, 8
	78[2]	129, 137	10, 16	26, 16	20, 20	10, 10
+S9 mix	none[1]	152, 126	8, 11	36, 26	32, 32	18, 18
	78	152, 170	13, 14	40, 37	36, 31	18, 16
	156	162, 144	11, 9	29, 33	29, 26	23, 17
	313	162, 158	8, 13	26, 26	33, 28	12, 21
	625	153, 151	8, 7	30, 32	28, 36	19, 18
	1,250[2]	148, 148	12, 5	24, 28	27, 24	18, 16
Positive Controls						
−S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	dose[3]	0.01	0.5	0.01	0.1	1.0
	revertants	588	504	152	527	1390
		570	442	146	505	1452
+S9 mix	Chemical	B[a]P	2AA	2AA	B[a]P	B[a]P
	dose[3]	5.0	2.0	10.0	5.0	5.0
	revertants	1195	311	739	262	108
		1083	332	818	270	98

[1] none: acetone alone (negative control).
[2] Precipitates were observed.
[3] unit: μg/plate.

mutagenicity of synthetic CoQ_{10}.

REFERENCES

1) Folkers K. 1985. Basic chemical research on coenzyme Q_{10} and integrated clinical research on therapy of disease. *In*: Coenzyme Q (Lenaz G, ed), p 457–478. John Wiley and Sons, New York.
2) Fukawa H. 1981. Coenzyme Q_{10} by partial synthesis. *In*: *Biomedical Clinical Aspects CoQ* (Folkers K, Yamamura Y, eds), Vol 3, p 19–30. Elsevier Science Publishers, Amsterdam.
3) Anonymous. 2004. Top 100 selling U.S. supplements sales and growth 1997–2003, Chart 14. Nutrition Business Journal, San Diego, CA (http://www.nutritionbusiness.com).
4) Williams KD, Maneke JD, AbdelHameed M, Hall RL, Palmer TE, Kitano M, Hidaka T. 1999. 52-Week oral gavage chronic toxicity study with ubiquinone in rats with a 4-week recovery. *J Agric Food Chem* **47**: 3756–3763.
5) Kitano M, Hosoe K, Fukutomi N, Hidaka T, Imai N, Kawabe M. 2004. 28-Day repeated dose toxicity study of dried microorganism in rats. *Food Chem Toxicol.* **42**: 1817–1824.
6) Maron DM, Ames BN. 1983. Revised methods for the *Salmonella* mutagenicity test. *Mutat Res* **113**: 173–215.
7) Yahagi T, Matsushima T, Nagao M, Seino Y, Sugimura T, Bryan GT. 1976. Mutagenicities of nitrofuran derivatives on a bacterial tester strain with an R factor plasmid. *Mutat Res* **40**: 9–14.