FEBS Open Bio 4 (2014) 722–729

FEBS openbio

journal homepage: www.elsevier.com/locate/febsopenbio

A dodecylamine derivative of cyanocobalamin potently inhibits the activities of cobalamin-dependent methylmalonyl-CoA mutase and methionine synthase of *Caenorhabditis elegans*

Tomohiro Bito [1], Yukinori Yabuta, Tsuyoshi Ichiyanagi, Tsuyoshi Kawano, Fumio Watanabe *

Division of Applied Bioresources Chemistry, The United Graduate School of Agricultural Sciences, Tottori University, Tottori 680-8533, Japan

ARTICLE INFO

Article history:
Received 25 June 2014
Revised 21 July 2014
Accepted 28 July 2014

Keywords:
Caenorhabditis elegans
Cyanocobalamin
Methionine synthase
Methylmalonic acid
Methylmalonyl-CoA mutase
Vitamin B_{12}

ABSTRACT

In this study, we showed that cyanocobalamin dodecylamine, a ribose 5′-carbamate derivative of cyanocobalamin, was absorbed and accumulated to significant levels by *Caenorhabditis elegans* and was not further metabolized. The levels of methylmalonic acid and homocysteine, which serve as indicators of cobalamin deficiency, were significantly increased in *C. elegans* treated with the dodecylamine derivative, indicating severe cobalamin deficiency. Kinetic studies show that the affinity of the cyanocobalamin dodecylamine derivative was greater for two cobalamin-dependent enzymes, methylmalonyl-CoA mutase and methionine synthase, compared with their respective coenzymes, suggesting that the dodecylamine derivative inactivated these enzymes. The dodecylamine derivative did not affect the levels of mRNAs encoding these enzymes or those of other proteins involved in intercellular cobalamin metabolism, including methylmalonyl-CoA mutase (*mmcm-1*), methylmalonic acidemia cobalamin A complementation group (*mmaa-1*), methylmalonic aciduria cblC type (*cblc-1*), and methionine synthase reductase (*mtrr-1*). In contrast, the level of the mRNAs encoding cob(I)alamin adenosyltransferase (*mmab-1*) was increased significantly and identical to that of cobalamin-deficient *C. elegans*. These results indicate that the cyanocobalamin-dodecylamine derivative acts as a potent inhibitor of cobalamin-dependent enzymes and induces severe cobalamin deficiency in *C. elegans*.

1. Introduction

Cobalamin (vitamin B_{12}, Cbl), an essential nutrient for humans, undergoes a complex process of gastrointestinal absorption when provided in the diet [1,2]. After uptake by its target cells, Cbl is converted into 5′-deoxyadenosylcobalamin (AdoCbl) and methylcobalamin (CH_3-Cbl), which function as coenzymes for methylmalonyl-CoA mutase (MCM; EC 5.4.99.2) [3] and methionine synthase (MS; EC 2.1.1.13) [4], respectively. The major symptoms of Cbl deficiency are megaloblastic anemia and neuropathy [5], and the underlying cause(s) of the associated developmental disorders, metabolic abnormalities, and neuropathy are poorly understood [6].

Animal models of Cbl deficiency are required for investigating the molecular mechanisms of these metabolic disorders; however, they are difficult to establish, because animals (e.g., rats) must be fed a Cbl-deficient diet for long periods to achieve Cbl deficiency [7]. Our recent study indicates that *Caenorhabditis elegans* grown under conditions of Cbl deficiency for five generations (approximately 15 days) develops severe Cbl deficiency associated with various phenotypes that include decreased egg-laying capacity (infertility), prolonged life cycle (growth retardation), and reduced lifespan [8]. These phenotypes resemble those of Cbl-deficient mammals.

McEwan et al. [9] synthesized ribose 5′-carbamate derivatives of cyanocobalamin (CN-Cbl) and demonstrated high-affinity binding of intrinsic factor (IF, the gastric Cbl-binding protein) to certain alkylamine derivatives. Our preliminary experiments indicate that

Abbreviations: AdoCbl, 5′-deoxyadenosylcobalamin; Cbl, cobalamin; *C. elegans*, *Caenorhabditis elegans*; CH_3-Cbl, methylcobalamin; CN-Cbl, cyanocobalamin; Hcy, homocysteine; IF, intrinsic factor; MCM, methylmalonyl-CoA mutase; MMA, methylmalonic acid; MMAA, methylmalonic acidemia cobalamin A complementation group; MMAB, cob(I)alamin adenosyltransferase; MMACHC, methylmalonic aciduria cblC type; MS, methionine synthase; MSR, methionine synthase reductase; NGM, nematode growth medium; qPCR, quantitative PCR analysis

* Corresponding author. Address: The United Graduate School of Agricultural Sciences, Tottori University, 4-101 Koyama-minami, Tottori 680-8553, Japan. Tel./fax: +81 857 31 5412.

E-mail address: watanabe@muses.tottori-u.ac.jp (F. Watanabe).

[1] A research fellow of Japan society for the promotion of science.

http://dx.doi.org/10.1016/j.fob.2014.07.008
2211-5463/

these alkylamine derivatives lack detectable biological activity in certain microorganisms that require Cbl for growth, such as *Escherichia coli* 215, *Lactobacillus delbrueckii* subsp. *lactis* ATCC 7830, and *Euglena gracilis* Z. Further, CN-Cbl dodecylamine derivative significantly decreases the levels of Cbl-dependent enzymes in mammalian cells cultured in vitro. Therefore, development of animal models of Cbl deficiency may be facilitated if the dodecylamine derivative acts as a potent inhibitor of Cbl-dependent enzymes. In the present study, we show that CN-Cbl dodecylamine derivative potently inhibited the Cbl-dependent enzymes MCM and MS of *C. elegans*.

2. Results and discussion

2.1. Effects of the CN-Cbl dodecylamine derivative on Cbl-related biomarkers of C. elegans

Although McEwan et al. [9] demonstrated high affinity binding of the gastric Cbl-binding protein IF to the CN-Cbl dodecylamine derivative (Fig. 1), our preliminary experiments indicated that this derivative was inactive in Cbl-dependent microorganisms typically employed in Cbl the bioassay. The dodecylamine derivative significantly inhibited the activities of Cbl-dependent enzymes (MCM and MS) in mammalian cells cultured in vivo (our unpublished data). Therefore, we evaluated effects of the CN-Cbl dodecylamine derivative on Cbl-related phenotypes using *C. elegans* as a model. Table 1 shows the concentrations of Cbl and its dodecylamine derivative in homogenate prepared from adult worms grown in the presence of the CN-Cbl derivative for 3 days. Remarkably, the Cbl concentration of worms grown in the presence of the CN-Cbl derivative was only 35% compared with that of control worms and was similar to that of worms grown for two generations (6 days) in the absence of Cbl [8]. In contrast, the CN-Cbl dodecylamine derivative was absorbed and accumulated by worms grown in the presence of the CN-Cbl derivative (approximately 110 ng/g wet weight). These results suggest that the CN-Cbl dodecylamine derivative did not inhibit the uptake of Cbl in the intestine, but it was readily accumulated in worms and significantly decreased their Cbl concentrations.

Table 1
Contents of CN-Cbl and the CN-Cbl dodecylamine derivative in worms.

	CN-Cbl (ng/g wet weight)	CN-Cbl dodecylamine derivative
Control worms	132.2 ± 26.7	–
Treated worms	46.5 ± 7.8	110.0 ± 17.2

Control and treated worms were grown on plates containing CN-Cbl- and CN-Cbl dodecylamine derivative-supplemented (each at 100 μg/L) M9 media for 3 days, respectively. Corrinoids were extracted from the treated worms by boiling with KCN at acidic pH. CN-Cbl and the CN-Cbl dodecylamine derivative were separated each other using a Sep-Pak Plus C18 cartridge and their levels were determined using the microbiological assay and HPLC, respectively. Data represent the mean ± SD of five independent experiments.

To determine whether the dodecylamine derivative detected in the treated worms was converted into other forms of Cbl, corrinoid compounds were extracted using 80% (v/v) ethanol from worms grown in the presence of the dodecylamine derivative and analyzed using reversed-phase HPLC. The retention times of authentic OH-Cbl, CN-Cbl, AdoCbl, CH_3-Cbl, OH-Cbl dodecylamine, CN-Cbl dodecylamine, and AdoCbl dodecylamine were 3.5, 8.1, 9.5, 12.8, 22.0, 30.6, and 36.2 min, respectively (Fig. 2A–D). The compounds extracted from worms exposed to the derivative eluted with retention times of 3.8–30.3 min (Fig. 2E). A major peak with the retention time of 30.3 min was identical to that of authentic CN-Cbl dodecylamine, and peaks were not detected with retention times of CN-Cbl (8.1 min), OH-Cbl dodecylamine (22.0 min), or AdoCbl dodecylamine (36.2 min). These results indicate that the CN-Cbl dodecylamine derivative accumulated by worms was not converted to any other Cbl-related compound, including its coenzyme forms.

The levels of MMA and Hcy were assayed in *C. elegans* grown for 3 days in the presence of the CN-Cbl dodecylamine derivative (Fig. 3A and B). The levels of both indicators were significantly increased in worms exposed to the dodecylamine derivative compared with those of the control worms. The increased MMA and Hcy levels were identical to those of Cbl-deficient worms. These results show that the worms developed severe Cbl deficiency when they were treated with the CN-Cbl dodecylamine derivative for only 3 days.

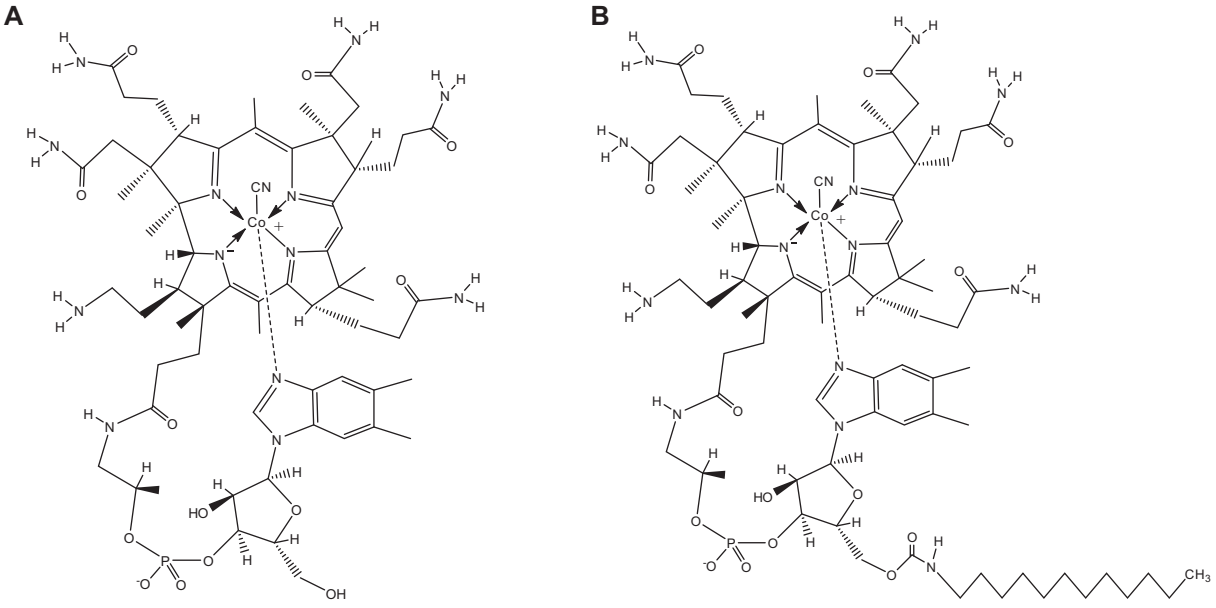

Fig. 1. Structures of cyanocobalamin and its dodecylamine derivative. (A) CN-Cbl; (B) CN-Cbl dodecylamine derivative.