Biochemistry, function, and deficiency of vitamin B_{12} in *Caenorhabditis elegans*

Tomohiro Bito and Fumio Watanabe

Faculty of Agriculture, School of Agricultural, Biological, and Environmental Sciences, Tottori University, Tottori 680-8553, Japan
Corresponding author: Fumio Watanabe. Email: watanabe@muses.tottori-u.ac.jp

Abstract

Caenorhabditis elegans is a nematode that has been widely used as an animal for investigation of diverse biological phenomena. Vitamin B_{12} is essential for the growth of this worm, which contains two cobalamin-dependent enzymes, methylmalonyl-CoA mutase and methionine synthase. A full complement of gene homologs encoding the enzymes associated with the mammalian intercellular metabolic processes of vitamin B_{12} is identified in the genome of *C. elegans*. However, this worm has no orthologs of the vitamin B_{12}-binders that participate in human intestinal absorption and blood circulation. When the worm is treated with a vitamin B_{12}-deficient diet for five generations (15 days), it readily develops vitamin B_{12} deficiency, which induces worm phenotypes (infertility, delayed growth, and shorter lifespan) that resemble the symptoms of mammalian vitamin B_{12} deficiency. Such phenotypes associated with vitamin B_{12} deficiency were readily induced in the worm.

Keywords: *Caenorhabditis elegans*, cobalamin, growth retardation, infertility, vitamin B_{12}

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Introduction

Vitamin B_{12} is well known as a corrinoid compound containing a corrin macrocycle in their structure. Vitamin B_{12} is usually presented as cyanocobalamin (CN-B_{12}). This unnatural form of cobalamin is more chemically stable than naturally occurring cobalamins.[1] However, in this review, the term vitamin B_{12} (B_{12}) refers to cobalamin compounds having B_{12} activity. CN-B_{12} is usually used for human dietary supplements and the CN-B_{12} taken up by living cells is synthesized into the coenzyme forms of cobalamin,[2] methylcobalamin (CH_3-B_{12}), and 5′-deoxyadenosylcobalamin (AdoB_{12}). Methionine synthase (MTR) (EC 2.1.1.13) contains CH_3-B_{12} as a prosthetic group and functions in methionine biosynthesis.[2] AdoB_{12} functions as a coenzyme for methylmalonyl-CoA mutase (MCM) (EC 5.4.99.2) catalyzing the isomerization of *R*-methylmalonyl-CoA to succinyl-CoA in the catabolism of amino acid and odd-chain fatty acid in mammalian cells[3] (Figure 1).

Only certain bacteria have the *de novo* biosynthetic pathway of B_{12}, which is accumulated in the bodies of predatory animals located higher in the food chain.[4] Thus, B_{12} is the only vitamin that does not exist in plant-derived foods. In particular, the best dietary sources of B_{12} include meat, milk, fish, and shellfish.[5] The recommended dietary allowance of B_{12} is 2.4 μg per day for adults.[6,7] People with atrophic gastritis developing low stomach acid output easily present the food protein-bound B_{12} malabsorption, which prevails in elderly people.[8] Thus, strict vegetarians and elderly people have an increased risk of developing B_{12} deficiency.[6,9] Megaloblastic anemia and neuropathy are well-known symptoms of B_{12} deficiency,[6] but the molecular mechanism underlying such diseases is still poorly understood.[10]

Caenorhabditis elegans, consisting of 959 somatic cells, shows a very short life cycle and has the ability to change its locomotor behavior, reproductive rate, and lifespan.[11] *Caenorhabditis elegans* conserves many molecular processes and cellular metabolisms of mammals, and many genes responsible for human disease are also present in the worm.[12] Therefore, *C. elegans* has been extensively used as a model animal for understanding various biological phenomena.[11]

Recently, *C. elegans* has been used to gain a new understanding between physiological events and diets, which contain nutrients and other bioactive compounds.[13,14] Different diets significantly affect various gene expressions and metabolic pathways in worms.[15] Dietary B_{12} is identified as a potent modulator in *C. elegans*.[13] Our study[16] indicates that *C. elegans* essentially requires B_{12} for growth, and dietary B_{12} deficiency results in infertility, retardation of growth, and reduced lifespan. Such B_{12}-deficient phenotypes are reported in mammals. Thus, the worm appears to be a highly suitable model animal for elucidating the mechanisms of nutrient-related diseases.

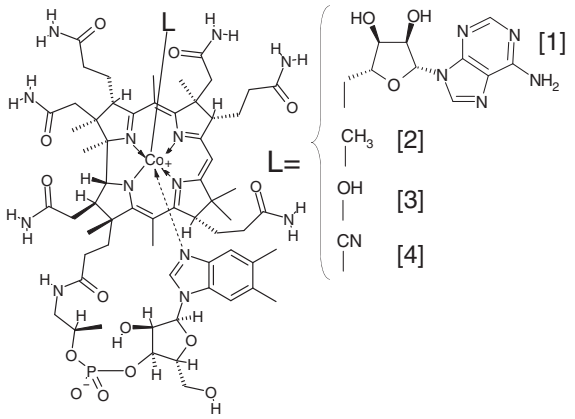

Figure 1 Structural formula of vitamin B_{12} and partial structures of vitamin B_{12} compounds. The partial structures of vitamin B_{12} compounds show only the portions of the molecules that differ from vitamin B_{12}. 1: 5′-deoxyadenosylcobalamin; 2: methylcobalamin; 3: hydroxocobalamin; 4: cyanocobalamin or vitamin B_{12}

In the minireview, we describe up-to-date information on the physiological functions of B_{12} and on the disordered biological processes due to B_{12} deficiency in *C. elegans*.

Vitamin B_{12} requirements

Like all animals, *C. elegans* has to eat to obtain nutrients for development, growth, and reproduction. Although *Escherichia coli* OP50 is considered best suitable as a bacterial diet, *C. elegans* has been grown using many other bacteria.[15] Although *C. elegans* can be grown well by feeding different bacterial diets, its physiological properties are significantly affected by different diets.[15] Although *E. coli* OP50 does not differ significantly from *Comamonas* sp. DA1877 in macronutrient levels (carbohydrates, protein, and fat), feeding with *Comamonas* sp. DA1877 shows faster development in worms than with *E. coli* OP50.[14] When Watson *et al.*[13] compared *E. coli* OP50 and *Comamonas* sp. DA1877 diets to identify biologically active compounds affecting the worm's gene expression and physiology, B_{12} was identified as the major compound provided by *Comamonas* DA1877. The B_{12} level in the *Comamonas* DA1877 diet is higher than that in the *E. coli* OP50 diet. Our unpublished data showed that *E. coli* OP50 contained 11.1 μg of B_{12} per gram of wet weight of cells when the bacterium was grown in Luria-Bertani medium. To clarify B_{12} requirements for the growth of *C. elegans*, the worm was grown on B_{12}-sufficient M9 agar plate and fed with B_{12}-deficient *E. coli* OP50 (0.9 μg of B_{12} per gram of wet weight of cells). Worms absorbed a sufficient amount of free B_{12} from the B_{12}-supplemented agar medium.[16] Control worm eggs were transferred onto the B_{12}-deficient M9 agar plate and fed with the B_{12}-deficient *E. coli* OP50. The eggs hatched and then worms were grown until adults (these worms represent the F1 generation). When this process was repeated for five generations, the cellular B_{12} level of the worms was significantly reduced over four generations. The B_{12} level of the fifth generation worms is only 4% of that in the B_{12}-sufficient worms (138 ± 51 ng of B_{12} per gram of wet weight).[16] The methylmalonic acid (MMA) and homocysteine (Hcy), which are usually used as the indices of B_{12} deficiency, are significantly increased because holo-MCM and -MTR activities are significantly decreased in the worms of the fifth generation grown under B_{12} deficiency conditions.[16] These results demonstrate that *C. elegans* absolutely requires B_{12} for growth and develops severe B_{12} deficiency when it is grown under B_{12} deficiency conditions for five generations. Such changes in B_{12}-dependent enzyme activities are reported to occur in mammals[17,18]; a B_{12}-deficient animal can be produced in only 15 days (five generations) using the nematode model.

Intestinal absorption and body circulation

Humans have a complicated system for intestinal absorption of B_{12}.[19] Dietary B_{12} reaches the stomach and is liberated from food protein by stomach acid and protease. Salivary protein haptocorrin binds with B_{12} and transports it through the stomach and into the small intestine. The protein moiety of the B_{12} complex is digested by pancreatic proteases and then free B_{12} is formed. The liberated B_{12} attached to the gastric protein intrinsic factor (IF). The B_{12}–IF complex is internalized into mucosal cells of the terminal ileum by receptor-mediated endocytosis. After the protein moiety of the B_{12}–IF complex is digested in the mucosal cells, released B_{12} binds to transcobalamin II (TC) and moves into the blood circulation.

Caenorhabditis elegans is not a suitable model animal to elucidate the mechanisms of megaloblastic anemia and disorders of B_{12} absorption and transport as human diseases caused by B_{12} deficiency because the blood corpuscle systems and the three B_{12} transporters (haptocorrin, IF, and TC) are not found in this worm.

Intracellular metabolisms and functions

In human cells, the B_{12}–TC complex is internalized by the receptor-mediated endocytosis.[20] The protein moiety of the complex is digested by the action of lysosomal proteases and then B_{12} is released from the complex.[21] The released B_{12} is transferred to the cytosol by the actions of LMBD1, a lipocalin-interacting membrane receptor,[22] and ABCD4, an ATP-binding cassette transporter protein.[23] In the cytosol, the cobalt atom of B_{12} is reduced by methylmalonic aciduria and homocystinuria type C protein (MMACHC or CblC), which catalyzes the reductive decyanation of CN-B_{12} as dietary B_{12}[24] and intracellular delivery of B_{12}[25] is converted into CH_3-B_{12}, a coenzyme for MTR in the cytosol, or transferred into the mitochondria, and then $AdoB_{12}$ is synthesized and functions as the coenzyme for MCM. In the enzymatic reaction of MTR, CH_3–B_{12} serves as acceptor of the methyl group in the catalytic cycle between cob(I)alamin and CH_3–B_{12}. Methionine synthase reductase (MTRR) catalyzes the reductive reactivation of inactive MTR bound to oxidized cob(II)alamin to maintain its active form using S-adenosylmethionine (SAM) as a methyl donor.[26] In the mitochondria, the methylmalonic aciduria type A protein (MMAA) binds to the MCM protein

to prevent MCM inactivation as a chaperone.[27] ATP:cob(I)alamin adenosyltransferase (MMAB) catalyzes the adenosyl transfer reaction from ATP to cob(I)alamin in the $AdoB_{12}$ biosynthesis.[28] The MMAB protein also functions as a chaperone involved in the transfer of the formed $AdoB_{12}$ to the apo-MCM protein.[29]

Using bioinformatics, a full complement of homologs of the mammalian B_{12}-related metabolic pathways has been identified in the genome of *C. elegans*, including propionyl-CoA carboxylase (*pcca-1*, *pccb-1*), methylmalonyl-CoA epimerase (*mce-1*), MMACHC (*cblc-1*), MMAA (*mmaa-1*), MMAB (*mmab-1*), MCM (*mmcm-1*), MTR (*metr-1*), MTRR (*mtrr-1*), and SAM synthase (*sams-1*) (Table 1).

Chandler *et al.*[33] characterized the $AdoB_{12}$-dependent methymalonlyl-CoA pathway in the tricarboxylic acid (TCA) cycle. RNA interference (RNAi) experiments indicate that the decreased *mmcm-1*, *mmab-1*, and *mmaa-1* gene expressions result in increased MMA. Worm deletion mutants *mmab-1, mmcm-1*, and *mce-1* decrease the incorporation of propionate into macromolecular compounds. These results show occurrence of $AdoB_{12}$-dependent methymalonlyl-CoA metabolic pathway in worms. The worm *mce-1* deletion mutant demonstrates that the flux through the methylmalonyl-CoA pathway is significantly reduced because of the defect in the epimerase reaction but the *mce-1* deletion shows no significantly phenotypic change.

The worm's methylmalonyl-CoA epimerase, a 15 kDa protein, was shown to be significantly expressed in body-wall muscles, and its subcellular pattern of expression suggests the mitochondrial localization of the enzyme.[38]

The methionine synthetic pathway is localized in the cytosolic fraction of mammalian cells.[39] In *C. elegans*, MTR, MTRR, and SAM synthase are likely to be cytosolic enzymes, considering their involvement in methionine metabolism. Human MMACHC (or CblC) exhibits reduced glutathione-dependent alkyltransferase activity and flavin-dependent reductive decyanation activity with CN-B_{12}.[25,40] The *C. elegans* MMACHC, which shares 36% identity and 53% similarity with the human enzyme, catalyzes the decyanation reaction of CN-B_{12} by the addition of reduced glutathione, an activity that has been overlooked previously with human MMACHC.[35] The decyanation product, cob(II)alamin, is stabilized by worm MMACHC. Worm and human MMACHC activities show different susceptibilities to oxygen.

Detailed properties of the remaining enzymes and proteins that are responsible for the intracellular metabolic processes of B_{12} have not been characterized in *C. elegans*. The intracellular metabolisms and functions of B_{12} in *C. elegans* are postulated in Figure 2 based on the above information.

Vitamin B_{12} deficiency

The effects of B_{12} deficiency on MCM and MTR activities in *C. elegans* have been evaluated. Holo-MTR activity rapidly decreased in a cell homogenate of worms treated with the B_{12} deficient medium for one generation, but holo-MCM activity was not altered until the fourth generation. The result suggests that MTR is more susceptible to cellular B_{12} levels than MCM.[16] Hepatic MTR activity is mostly derived from the holo-enzyme in B_{12}-sufficient or -deficient rats.[17] Although MTR protein levels in liver homogenates of B_{12}-deficient rats are significantly reduced compared with those of B_{12}-sufficient rats, the hepatic B_{12} level does not affect the level of MTR-encoding mRNA.[17] Thus, the apo-MTR is very unstable. Determination of the changes in MTR protein and mRNA (*metr-1*) levels in B_{12}-deficient worms has shown that the level of mRNA was not altered; however, the level of MTR protein in B_{12}-deficient worms was decreased to approximately 32% compared with that in B_{12}-sufficient worms.[41] Considering that a trace band of MTR was detected in the liver homogenates of B_{12}-deficient rats, this result suggests that the worm apo-enzyme is considerably stable. MTR is important for resynthesizing methionine and metabolizing *5*-methyltetrahydrofolate. Tetrahydrofolate generated by the action of MTR is converted to *5,10*-methylenetetrahydrofolate, which is crucial for the biosynthesis of thymidine that occurs exclusively in DNA.[42] Moreover, methionine is converted to SAM, which donates a methyl group to certain compounds in various methyl transfer reactions. Hcy acquires a methyl group from either *5*-methyltetrahydrofolate or betaine to form methionine. Both synthetic pathways of methionine play important roles in mammals.[43] Betaine acts as a methyl group donor for reactions catalyzed by betaine homocysteine methyltransferase (EC 2.1.1.5) to form dimethylglycine.[44] Administration of betaine leads to the normalization of homocystinuria and clinical improvement of B_{12} deficiency symptom.[45] However, bioinformatics indicate that *C. elegans* has no ortholog of betaine homocysteine methyltransferase. Moreover, a high dose of betaine arrests larval development of the nematode.[46] MCM activity of the apo-enzyme was significantly increased during B_{12} deficiency. Even in B_{12}-sufficient rats, more than 95% of MCM activity was derived from the apo-enzyme.[18] Thus, holo-MCM activity was very low during B_{12} deficiency and then significant accumulation of MMA occurs in the cells.[47] MMA is a potent inhibitor of succinate dehydrogenase (EC 1.3.99.1) that participates in both the TCA cycle and respiratory chain.[48] Thus, MMA increase due to B_{12} deficiency blocks the mitochondrial respiration and consequently disrupts various metabolic pathways.[48]

However, B_{12} deficiency did not alter the levels of mRNAs encoding proteins that participate in worm intracellular B_{12} metabolism, including MCM (*mmcm-1*), MMAA (*mmaa-1*), MMACHC (*cblc-1*), and MTRR (*mtrr-1*). However, the level of the mRNAs encoding MMAB (*mmab-1*) increased significantly.[41]

Our results indicate that *C. elegans* essentially required B_{12} for growth. A B_{12}-deficient animal was readily produced in only 15 days using this nematode. Various phenotypes, such as infertility, delayed growth, and shorter lifespan, are observed during B_{12} deficiency. The nematode has been used as a model animal for elucidating the biological phenomena of fertilization,[49] embryogenesis,[50] nervous system,[51] and learning and memory.[52] In addition, *C. elegans* may become a new tool for human B_{12} deficiency diseases, such as fertility disorder[53,54] and nerve[55] and cognitive dysfunctions.[56]

Table 1 Mammalian homologs of members of the *Caenorhabditis elegans* B_{12}-related metabolic pathways deduced from the genome sequence

Protein name	*Homo sapiens*	*Caenorhabditis elegans*	BLAST score	Reference
Propionyl-CoA carboxylase alpha chain	PCCA	*pcca-1*	1.20 E-214	30
Propionyl-CoA carboxylase beta chain	PCCB	*pccb-1*	4.50 E-213	31
Methylmalonyl-CoA epimerase	MCE	*mce-1*	2.50 E-45	32
Methylmalonyl-CoA mutase	MCM	*mmcm-1*	9.91 E-280	33
Methylmalonic aciduria type A protein	MMAA	*mmaa-1*	2.60 E-89	34
Methylmalonic aciduria type B protein	MMAB	*mmab-1*	4.80 E-35	33
Methylmalonic aciduria and homocystinuria type C protein	MMACHC	*cblc-1*	6.20 E-26	35
Methionine synthase	MTR	*metr-1*	0	36
Methionine synthase reductase	MTRR	*mtrr-1*	1.40 E-74	30
S-adenosylmethionine synthase	MAT	*sams-1*	1.50 E-148	37

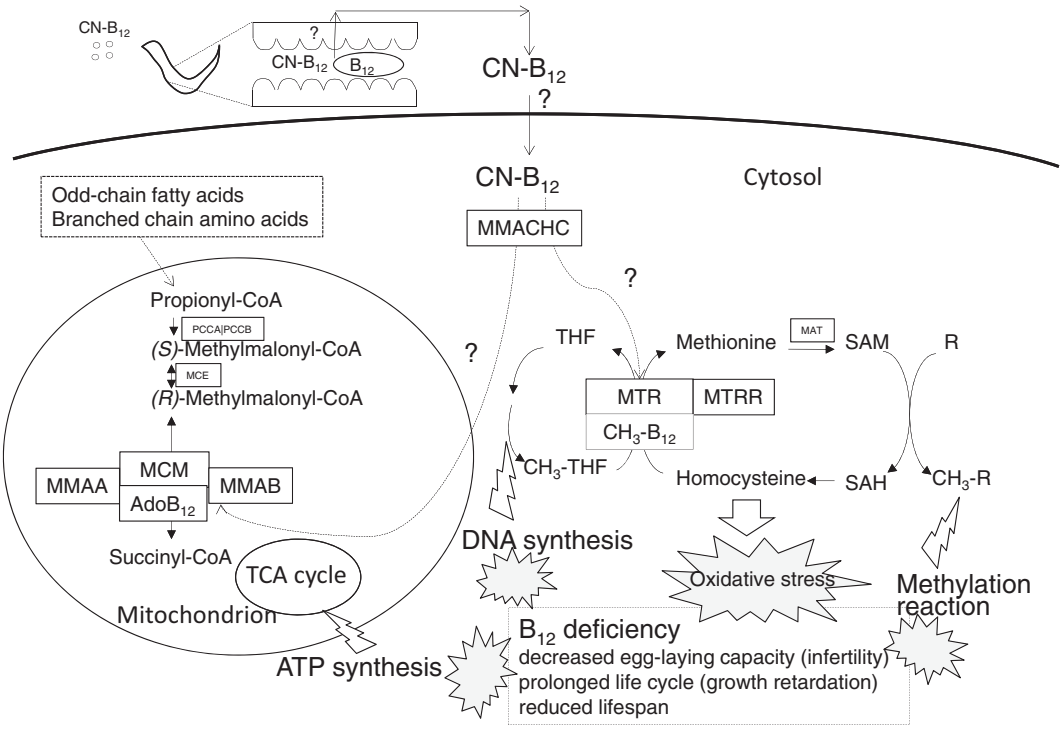

Figure 2 Postulated intracellular metabolism and function of vitamin B_{12}. SAM: *S*-adenosylmethionine; SAH: *S*-adenosylhomocysteine; THF: tetrahydrofolate; TCA: tricarboxylic acid. The other abbreviations used are as shown in Table 1

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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