Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Diversity of membrane transport proteins for vitamins in bacteria and archaea☆,☆☆

Michael Jaehme, Dirk Jan Slotboom *

Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form 30 April 2014
Accepted 3 May 2014
Available online xxxx

Keywords:
membrane transport
bacterial vitamin uptake

ABSTRACT

Background: All organisms use cofactors to extend the catalytic capacities of proteins. Many bacteria and archaea can synthesize cofactors from primary metabolites, but there are also prokaryotes that do not have the complete biosynthetic pathways for all essential cofactors. These organisms are dependent on the uptake of cofactors, or at least their precursors that cannot be synthesized, from the environment. Even in those organisms that contain complete biosynthetic pathways membrane transporters are usually present, because the synthesis of cofactors is more costly than uptake.

Scope of review: Here we give an overview of bacterial and archaeal transport systems for B-type vitamins, which are either cofactors or precursors thereof.

Major conclusions: Prokaryotic vitamin transporters are extremely diverse, and found in many families of transporters. A few of these transport systems have been characterized in detail, but for most of them mechanistic insight is lacking.

General significance: The lack of structural and functional understanding of bacterial vitamin transporters is unfortunate because they may be targets for new antibiotics. This article is part of a Special Issue entitled Structural biochemistry and biophysics of membrane proteins. Guest Editor: Bjorn Pedersen.

1. Introduction

Cofactors greatly extend the catalytic potential of enzymes, and allow complex reactions to take place in living cells [1]. B-type vitamins or derivatives thereof constitute a large group of cofactors. Table 1 lists the eight diverse molecules (or groups of molecules) known as B-type vitamins, and the cofactors that are derived from them. The B-type vitamins are essential nutrients for humans, but can be synthesized by many prokaryotes [1]. However, numerous bacteria lack the complete biosynthetic pathways for one or more of these compounds, and therefore depend on their uptake from the environment by membrane-embedded transport proteins. In addition, even the genomes of organisms that encode complete biosynthetic pathways for the vitamins usually also have genes coding for transporters. These organisms can produce vitamins themselves if needed, but probably prefer to take up the compounds when available in the environment, because synthesis requires usually more metabolic energy than transport. For example, 25 mol of ATP are needed for the synthesis of 1 mol of riboflavin [2,3], whereas transport usually costs two ATP or less, depending on the transport system.

Vitamin transporters are essential proteins in many bacteria with incomplete metabolic pathways [4–6], and specific inhibition of the function of these proteins could be a strategy for new antibiotic development. In the past decade the molecular identities of many bacterial vitamin transporters have been revealed. Computational methodologies (comparative genomics, metabolic reconstruction [7]), in combination with classical microbiological and biochemical experiments (e.g. [5]) have played a major role in the recent discoveries, which we will review here.

1.1. Bacterial solute transport systems

Based on differences in the way solute transport is energized, membrane transporters are classified in three major groups [8]: Primary active transporters, Secondary transporters and Group translocators. In this section we will provide a brief overview of the main characteristics of these three groups.

Primary active transporters comprise many very diverse protein families that use chemical, electrical or solar energy sources to transport

Abbreviations: ABC, ATP-binding cassette; ECF, energy coupling factor; HET, hydroxyethylthiazole; HMP, hydroxymethylpyrimidine; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; MFS, major facilitator superfamily; Na, nicotinate; Nm, nicotinamide; NR, nicotinamide riboside; NaMN, nicotinate mononucleotide; NMN, nicotinamide mononucleotide; NAD, nicotinamide adenine dinucleotide; SSS, sodium–solute symporters; TMP, thiamin monophosphate; TPP, thiamin pyrophosphate

☆ This article is part of a Special Issue entitled Structural biochemistry and biophysics of membrane proteins. Guest Editor: Bjorn Pedersen.

☆☆ Funded by the Netherlands Organisation for Scientific Research (NWO) and the European Research Council (ERC).

* Corresponding author at: Tel.: +31 50 363 4187; fax: +31 50 363 4165.
E-mail address: d.j.slotboom@rug.nl (D.J. Slotboom).

http://dx.doi.org/10.1016/j.bbagen.2014.05.006
0304-4165/

Table 1
Overview of B-type vitamins and related cofactors.

Vitamin	Name	Associated cofactor
B_1	Thiamin	Thiaminpyrophosphate
B_2	Riboflavin	FMN/FAD
B_3	Niacin	$NAD^+/NADP^+$
B_5	Pantothenate	Coenzyme A, phosphopantetheine
B_6	Pyridoxine	Pyridoxal-phosphate
B_7	Biotin	
B_9	Folate	Tetrahydrofolate
B_{12}	Cobalamin	Adenosylcobalamin, methylcobalamin

substrates across the membrane. Vitamin transporters are found in the largest and most widespread family of primary active transporters: the ATP binding cassette (ABC) transporter family [9,10]. ABC transporters couple ATP hydrolysis to substrate transport. All ABC transporters share the same architecture: Two soluble nucleotide binding or ATPase domains or subunits (NBDs) are located on the cytoplasmic side of the membrane, and two transmembrane domains (TMDs) or subunits are embedded in the lipid bilayer and constitute the pathway for substrate translocation [9,10]. The ATPase domains are conserved in structure and sequence, but the transmembrane domains can adopt different, unrelated structures. Based on the structural diversity ABC transporters have been classified in four different types [11]. These types also differ in details of the transport mechanism. Three of the four types are found exclusively in prokaryotes and are involved in the uptake of nutrients: Type I and Type II importers and ECF transporters. Type I and Type II ABC transporters are dependent on periplasmic or extracellular substrate-binding proteins or domains (SBDs) to bind the transported substrate and deliver it to the transmembrane domains. The substrate is then transported along a pathway at the interface between the two TMDs. Despite these global similarities, the mechanism of transport appears to be very different between the two types [10]. ECF transporters do not make use of soluble SBDs, but instead use one of their TMDs (the S-component) for substrate binding [5,6,12]. The other TMD (The T-component, or EcfT subunit) together with the two NBDs form the so-called ECF module. In many cases the ECF module can associate with different S-components (specific for different substrates, often vitamins) to form a variety of four-subunit complexes, each transporting a different substrate. The fourth ABC transporter type is the exporter, which is found both in pro- and eukaryotes [11,13]. The prokaryotic exporters consist of two NBDs and two TMDs and transport substrate out of cells (or from the inner leaflet of the bilayer to the outer leaflet), and therefore are not likely candidates for vitamin uptake. Nonetheless, a recent study suggests that export-type ABC transporters may in some cases be involved in import functions (see below).

Secondary transporters belong to many different families, with different tertiary structures, oligomeric states and transport mechanisms [13–15]. In bacteria secondary transporters often accumulate substrates in – or deplete them from – cells by coupling substrate transport to the co- or counter-transport of a secondary substrate, frequently Na^+ or H^+. Primary active transporters (such as P-type ATPases) maintain the membrane gradients of the secondary substrate. Some secondary transporters do not catalyze coupled transport, but only facilitate the equilibration of the pools of the substrate on either side of the membrane in a process that is named facilitated diffusion. Secondary transporters usually do not depend on soluble domains or subunits (in contrast to ABC transporters).

Group translocators chemically modify the substrate during the transport reaction [7,16]. The phosphotransferase system (PTS) is the prototypical example of a group translocator, and is used by many prokaryotes for the import of carbohydrates. For each sugar there is a specific integral membrane domain (enzyme IIC), which contains the translocation pathway, and two soluble domains (enzymes IIA and IIB), which transfer a phosphate group to the carbohydrate once it has reached the cytoplasmic side of the membrane. Sugar transport and phosphorylation by the PTS are strictly coupled. Phosphoenolpyruvate (PEP) is the ultimate donor of the phosphate group, which is transferred to enzyme IIA via two proteins that are shared by phosphotransferase systems specific for different substrates: HPr and enzyme I. Apart from the sugar PTS several other transport systems have been loosely classified as group translocators. In these systems the phosphorylation of the substrate may not be very tightly coupled to transport, and therefore these systems could also be classified as secondary transporters that catalyze facilitated diffusion. Some bacterial vitamin transporters use such a mechanism of transport (see below). Cytosolic enzymes may then modify the transported substrate, without the need for a strict coupling between transport and modification.

1.2. Bacterial and archaeal vitamin transporters

In this section we will give an overview (Table 2) of the known or predicted prokaryotic transporters for the eight B-type vitamins listed in Table 1. The diversity of these transport systems is also schematically summarized in Fig. 2.

1.3. Vitamin B_1: Thiamin

Thiamin pyrophosphate (TPP) is the cofactor derived from thiamin. TPP containing enzymes are involved in cleavage of bonds adjacent to carbonyl groups, and rearrangements in which an acetaldehyde group is transferred from one carbon to another [17]. Thiamin consists of a hydroxyethylthiazole (HET) and a hydroxymethylpyrimidine (HMP) moiety (Fig. 1). The synthesis of thiamin from these compounds is conserved in archaea, bacteria and eukaryotes, whereas the biosynthetic pathways for the two precursors differ substantially [17,18]. Genes for the biosynthesis and transport of thiamin and its precursors have been identified by the presence of the thiamin regulatory RNA element (*THI* element), which operates as TPP-responsive riboswitch [13,18,19]. Missing parts of the biosynthesis pathways allowed for the prediction of substrates for the putative transporters [13,20].

1.4. Experimentally characterized thiamin transporters

1.4.1. ThiBPQ

ThiBPQ is an ABC transporter for thiamin in *Escherichia coli*. It consists of the substrate-binding protein ThiB, the transmembrane domain ThiP and the NBD ThiQ. Thiamin uptake activity of *E. coli* [20–22] was assigned to this transporter, which is encoded by the *sfuABC* genes in *E. coli* and the *thiBPQ* genes in *Salmonella typhimurium* [18,23]. The structure of the entire ThiBPQ complex is not known, but it is likely to be a Type I ABC importer. The substrate specificity was determined by structural and functional analysis of ThiB, which has a characteristic fold for substrate-binding proteins from ABC transporters, and belongs to cluster D according to the structural classification of Berntsson et al. [20,24–26]. The structure of the protein was solved with thiamin monophosphate (TMP) bound (Fig. 3). ThiB binds TMP, thiamin and thiaminpyrophosphat (TPP) with very similar dissociation constants in the range of 2.3–7.4 nM [13,25]. It must be noted that Hollenbach et al. found a much weaker affinity of ThiB for thiamin (K_D of 0.8 μM) [24,27], which is difficult to reconcile with most of the other available data. Analysis of thiamin transport into *E. coli* revealed a K_M of 15.2 nM. The observed first order rate constant of 1.9×10^{-4} s^{-1} shows that ThiBPQ transports only 1 molecule of a thiamin per 90 min [28,29].

1.4.2. ThiXYZ

ThiXYZ is another ABC transporter related to thiamin uptake consisting of ThiY (the SBD), ThiX (the TMD) and ThiZ (the NBD) [1, 13,28]. The genes *thiXYZ* are found in organisms of several different taxonomic divisions and are always preceded by a *THI* regulatory element, except in *Thermotoga maritima*. Three observations indicate that HMP

Please cite this article as: M. Jaehme, D.J. Slotboom, Diversity of membrane transport proteins for vitamins in bacteria and archaea, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagen.2014.05.006