Expression of Functional \textit{Streptomyces coelicolor} H+-Pyrophosphatase and Characterization of Its Molecular Properties

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Received April 20, 2005; accepted May 24, 2005

H+-translocating pyrophosphatases (H+-PPases) are proton pumps that are found in many organisms, including plants, bacteria and protozoa. \textit{Streptomyces coelicolor} is a soil bacterium that produces several useful antibiotics. Here we investigated the properties of the H+-PPase of \textit{S. coelicolor} by expressing a synthetic DNA encoding the amino-acid sequence of the H+-PPase in \textit{Escherichia coli}. The H+-PPase from \textit{E. coli} membranes was active at a relatively high pH, stable up to 50°C, and sensitive to N-ethylmaleimide, N,N'-dicyclohexylcarbodiimide and acylspermidine. Enzyme activity increased by 60% in the presence of 120 mM K⁺, which was less than the stimulation observed with plant vacuolar H+-PPases (type I). Substitutions of Lys-507 in the Gly-Gln-x-x-(Ala/Lys)-Ala motif, which is thought to determine the K⁺ requirement of H+-PPases, did not alter its K⁺ dependence, suggesting that other residues control this feature of the \textit{S. coelicolor} enzyme. The H+-PPase was detected during early growth and was present mainly on the plasma membrane and to a lesser extent on intracellular membranous structures.

Key words: H+-pyrophosphatase, proton pump, \textit{Streptomyces coelicolor}.

Proton pumps have two physiological roles: pH regulation and the formation of proton-motive forces across membranes. They convert the energy of chemical bonds into the active transport of H⁺. The simplest of these pumps is the H⁺-translocating inorganic pyrophosphatase (H⁺-PPase), which consists of a single polypeptide of about 80 kDa (1). H⁺-PPases are found in plants, parasitic and free-living protozoa, and some eubacteria and archaeaacteria (2–8). Recently, an H⁺-PPase was detected in membranes of the ovary and egg yolk granules of the insect \textit{Rhodnius prolixus} (9). In prokaryotes such as \textit{Rhodospirillum rubrum} (10) and \textit{Agrobacterium tumefaciens} (11), the enzyme generates a proton gradient across the plasma membrane and the membranes of acidocalcisomes.

H⁺-PPases are excellent models for research on the coupling between PPi hydrolysis and active H⁺ transport, and their structure–function relationships have been investigated extensively (4, 12–17). Studies using heterologous-expression systems to analyze mutant forms of these enzymes have revealed their membrane topology and identified several functional motifs. They are divided into two groups according to their K⁺ requirements: one requires K⁺ for maximal activity (1), whereas the other does not (4, 5). The former is designated type I and the latter is known as type II (4, 5).

\textit{Streptomyces coelicolor} is a bacterium (Actinomycetes) that produces most of the natural antibiotics used in human and veterinary medicine. Its H⁺-PPase (ScPP) was identified by genome sequencing (18). \textit{S. coelicolor} is phylogenetically distant from other organisms with H⁺-PPases, and the physiological role of its H⁺-PPase is not known. In the present study, we detected ScPP in \textit{S. coelicolor} cells, expressed it in \textit{E. coli} and determined its biochemical properties. We focused particularly on its K⁺ dependence, as this property distinguishes the two types of H⁺-PPase (4, 5). We also examined the K⁺ dependence of mutant forms of ScPP.

MATERIALS AND METHODS

Synthesis of DNA Encoding ScPP and Expression in \textit{E. coli}—A laboratory strain of \textit{S. coelicolor} cells was cultivated on agar plates containing 0.1% yeast extract, 0.1% beef extract, 0.2% Nzamine, 1% glucose and 2% agar at 30°C. DNA was isolated according to standard procedures, and was used as a template to amplify the DNA encoding the H⁺-PPase by PCR with a pair of forward and reverse primers. We found that the nucleotide sequence of the native gene for H⁺-PPase (DDBJ/GenBank™/EBI Data Bank accession no. AB180905) differed by eight bases from the reported sequence: T/C845, C/G921, G/A1149, T/C1593, G/A1803, G/A1833, G/A1884 and T/C2049 (reported sequence/results of the present study; numbered from the open reading frame specifying 794 amino acids). The deduced amino-acid sequence agreed with that in the database (18) (DDBJ/GenBank™/EBI accession no. AL645882), with the exception of Phe282 (Ser in the database). We designed and synthesized an artificial ScPP DNA (sScPP) with a gene synthesizer program (MacPerl Script) coding for the same amino-acid sequence as the native H⁺-PPase. A plasmid, pYN309,
derived from pET23b (Novagen) by modifying the PstI site, was used for expressing the ScPP proteins. A His6 tag was added to the carboxyl (C)-terminus to facilitate purification of the ScPP protein. The ScPP DNA construct was introduced into E. coli strain BL21(DE3)pLysS (Novagen), and transformants were selected for antibiotic resistance on Luria-Bertani plates containing 50 mg liter−1 of spectinomycin and 30 mg liter−1 of kanamycin. The constructs for generating the mutant ScPPs were prepared as described previously (15).

Preparation of E. coli Membranes—E. coli cells expressing ScPP were grown with antibiotics on Luria-Bertani plates. After 4 h at 37°C (OD600 = 0.7), isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.4 mM. The cells were incubated for a further 2 h, harvested by centrifugation at 3,500 × g for 10 min, and suspended in 50 mM Tris-Mes (pH 7.3), 1 mM EDTA, 2 mM DTT, 75 mM KCl, 0.15 M sucrose and 1 mM PMSF. They were incubated with 40 mg ml−1 DTT, 75 mM KCl, 0.15 M sucrose, 2 mM MgCl2, 1% sucrose monocholate (Dojin chemicals), 100 mM KCl and 0.2 mg ml−1 lysozyme at 4°C for 10 min, then thoroughly disrupted with a bath-type sonicator (Cosmobio Co., Japan). The homogenate was centrifuged at 2,000 × g for 10 min, and the supernatant was further centrifuged at 150,000 × g for 20 min. The resulting pellet was suspended in 10 mM Tris-Mes (pH 7.3), 1 mM MgCl2, 1 mM DTT, 100 mM KCl, 0.15 M sucrose and 0.5 mM PMSF and used as the E. coli membrane fraction.

Solubilization and Purification of ScPP—Sucrose monocholate at a final concentration of 2% (w/v) was added to the E. coli membrane fraction (2.0 g liter−1), and the suspension was stirred at 25°C for 30 min, sonicated for 2 min and centrifuged at 100,000 × g for 30 min. The supernatant was mixed with E. coli total lipid extract to a final concentration of 0.20 mg ml−1 and applied to a column (bed volume = 4 ml) of Q-Sepharose (Amersham Biosciences) equilibrated with 20 mM Tris-HCl (pH 7.5), 0.15 M sucrose, 2 mM MgCl2, 1% sucrose monocholate (Dojin Chemicals), 100 mM KCl and 0.2 mg ml−1 E. coli total lipid extract (buffer B). ScPP was eluted from the column with 10 ml of buffer B containing 200 mM NaCl (buffer C) and applied to a column (bed volume = 2 ml) of Ni-Sepharose (Amersham Biosciences) pre-equilibrated with buffer C. The column was washed with 10 ml of buffer C containing 30 mM imidazole, and ScPP was eluted with 5 ml of buffer C containing 150 mM imidazole and stored at −80°C. All purification steps were performed at 0–4°C.

Enzyme Assays—PPi hydrolysis was assayed as described previously (7) with a few modifications. The standard reaction medium contained 20 mM Bicine-NaOH (pH 8.0), 100 mM KCl, 1 mM MgCl2, 0.15 M sucrose, 0.4 mM Na2PPI, 1 mM sodium molybdate and 0.5 mM NaF. The reaction was carried out at 30°C. PPI-dependent H+-transport activity was measured at 25°C as the rate of fluorescence quenching of fluorescent monoamine acridine orange (7). Membrane preparations were preincubated with the standard reaction buffer containing 20 mM Bicine-NaOH (pH 8.0), 100 mM KCl, 1 mM MgCl2, 0.15 M sucrose, and 0.75 µM acridine orange, and the reaction was initiated by the addition of 0.4 mM Na2PPI at 25°C.

Expression of ScPP in E. coli and Its Purification—As shown in the phylogenetic tree of H+-PPases (Fig. 1), ScPP is relatively distant from the well-characterized H+-
PPases of other organisms, including plants (1, 5), *R. rubrum* (3, 10), *Trypanosoma cruzi* (8), *Trypanosoma brucei* (19) and *A. tumefaciens* (11). It contains 794 amino-acid residues and a characteristic long additional carboxyl-terminal sequence that includes a transmembrane domain (15).

Expression of ScPP in *E. coli* was confirmed by immunoblotting with an anti-H⁺-PPase antibody raised against the conserved motif of H⁺-PPase (Fig. 2) (20). The expressed ScPP constituted only a few percent of the total membrane protein of the transformants, as judged by SDS-PAGE (Fig. 2A, lane 2). The membrane-associated PPI-hydrolyzing activity was approximately 100 nmol PPI·min⁻¹·mg⁻¹ at 30°C in an assay medium containing sodium fluoride, which inhibited soluble PPases. PPI-dependent H⁺-transport was also observed (see below).

Control membranes had little hydrolyzing activity and showed no PPI-dependent H⁺-transport.

To solubilize the membrane-bound ScPP, we tested various concentrations of 20 different detergents includ-
ing n-dodecyl-β-D-maltoside, sucrose monolaurate, N-
nonanoyl-N-methylglucamine (MEGA-9), lysolecithin and
zwittergent. Most detergents inactivated the enzyme,
and only sucrose monocholate solubilized it in an active
form with good recovery. Thus, the enzyme was routinely
solubilized with 1% sucrose monocholate in the presence
of 2 mM MgCl₂ and 100 mM KCl, and the recovery was
20%. The solubilized fraction was further purified by Q-
Sepharose and Ni-Sepharose chromatography, and ScPP
was detected by immunoblotting (Fig. 2B). The recombi-
ant enzyme with a His₈ tag was partially purified, and its
specific activity was several-fold greater than that of the
original membrane preparation. Addition of a His₈ tag
had no effect on the enzyme activity, as described previ-
ously (15). The PPᵢ-hydrolysis activity was approximately
450 nmol PPᵢ⋅min⁻¹⋅mg⁻¹ at 30°C and required phospholi-
pid micelles (data not shown), similarly to plant vacuolar
H⁺-PPase (7).

Effects of pH and Temperature on the Activity and Sta-
bility of ScPP—Figure 3A shows the pH dependence of
PPᵢ hydrolysis. As the crude E. coli membrane prepara-
tion contained a relatively high level of soluble PPase
activity, the membranes were assayed in the presence
of NaF, which is a potent inhibitor of soluble PPases. ScPP
had maximal activity at pH 7.5–8.0. Purified mung bean
vacuolar H⁺-PPase has an optimum pH of 7.0 and
expresses only 20% of the maximum activity at pH 6.0
and 8.0 (data not shown). The apparent Kₘ values for PPᵢ
of the membrane-bound ScPP and the partially purified
preparation were 110 µM and 120 µM, respectively. The
calculated Kₘ of ScPP for its actual substrate, Mg₂PPᵢ,
was about 3.5 µM; this was comparable to that of the
mung bean enzyme (21).

Membrane-bound ScPP retained full activity after
incubation at 50°C for 30 min, and the purified enzyme
retained 70% of its activity under the same conditions
(Fig. 3B). It should be noted that ScPP activity was only
stable in the presence of 1 mM Mg²⁺. The optimum tem-
perature for PPᵢ-hydrolysis was 40–45°C for both the
membranous and purified preparations (Fig. 3C).

Requirement of K⁺ for ScPP Activity—Figure 4A shows
the substrate-activity curves at various concentrations
of Mg²⁺. The enzyme had a strict requirement for Mg²⁺,
and maximal activity was obtained in the presence of 1 mM
Mg²⁺ and more than 0.4 mM PPᵢ. This is also true of H⁺-
PPases in general (7, 22). As shown in Fig. 4B, the most
effective metal ion was Mg²⁺, although Mn²⁺ could act as
a partial substitute. The enzyme was strongly inhibited
by Ca²⁺ at 0.5 mM (Fig. 4C), and Mn²⁺, Zn²⁺ and Al³⁺ were
partially inhibitory at 0.5 mM. Although Ni²⁺ did not
inhibit the enzyme, it could not substitute for Mg²⁺.

The requirement of K⁺ for activity is thought to be a
key property distinguishing type I and II H⁺-PPases (4).
KCl at relatively high concentrations (120 mM) enhanced
PPᵢ-hydrolysis in both the membrane and the partially
purified ScPP fractions (Fig. 5, A and D). A similar effect
was observed with CH₃COOK (Fig. 5B), but not with
NaCl (Fig. 5C). Thus, the stimulation was due to K⁺ itself.
However, the extent of the stimulation was low (60%)
compared with the plant vacuolar H⁺-PPases, the activity
of which was increased by more than three-fold (7, 23). As
shown in Fig. 5E, the rubidium ion, which is substituted
for K⁺ in experiments on K⁺ channels, stimulated the
activity as effectively as K⁺.

Tris has been reported to act as a competitive inhibitor
of K⁺ that represses plant vacuolar H⁺-PPases at more

Fig. 4. Effect of metal ions on the PPase activity of ScPP. (A) The PPase activity of the membrane fraction was measured in a
standard assay mixture containing the indicated concentrations of Mg²⁺ and PPᵢ. Values are expressed relative to activity at 1 mM
Mg²⁺ and 0.5 mM PPᵢ. (B) The activity of the membrane fraction was assayed in the presence of Mg²⁺ and other metal ions at 0.5 mM
(light-shaded bars) or 1 mM (shaded bars). Activity is expressed relative to that with 1 mM Mg²⁺. (C) Activity was assayed in the
presence of 0.5 mM Mg²⁺ plus the indicated metal ion at 0.5 mM (light-
shaded bars) or 1 mM (shaded bars). Activity is expressed relative
to the control activity in the presence of 0.5 mM Mg²⁺ (closed bar).
Activity is expressed as a percentage of the maximal activity in the
presence of 1 mM Mg²⁺ (approximately 120 nmol PPᵢ⋅min⁻¹⋅mg⁻¹) in
each experiment.
than 25 mM (24). Indeed, Tris markedly inhibited mung bean vacuolar H\(^+\)-PPase in the absence of KCl, and the effect was reversed by KCl (Fig. 5F). By contrast, the activity of ScPP was not inhibited by Tris in either the presence or absence of KCl. This appears to be a characteristic of ScPP.

The stimulation of PP\(_i\)-dependent H\(^+\)-transport activity was also examined using an acridine orange fluorescence-quenching assay (Fig. 6A). The initial rate of fluorescence quenching was stimulated six-fold by adding 100 mM KCl (Fig. 6B), and RbCl at 100 mM had a similar effect (Fig. 6C). However, NaCl also stimulated pump activity (Fig. 6B).

H\(^+\)-PPases that do not require K\(^+\) for their activity are classified as type II and are thought to depend on the GNxxKA motif (4) in the conserved cytoplasmic loop between the eleventh and twelfth transmembrane domains. The limited dependence of ScPP on K\(^+\) suggests that it is a type II enzyme. We therefore generated mutant enzymes with Lys-507 substituted by Ala (K507A) or Arg (K507R). The mutant enzymes were expressed well in E. coli (Fig. 7A), and the amino-acid substitutions resulted in reduced activity, suggesting that this motif is, indeed, involved in catalysis (Fig. 7B). It should be noted that these substitutions of the lysine residue did not affect the K\(^+\)-dependence of ScPP. It has been reported that the threonine residue close to this motif is also essential for K\(^+\)-independence (4, 5). However, neither a single (T510G) nor a double (K507A/T510G) mutation involving this site altered the K\(^+\) dependence.

Sensitivity of ScPP to Inhibitors—ScPP was sensitive to the substrate analogue imidodiphosphate, the proton-transport blocker N,N\(^{\prime}\)-dicyclohexylcarbodiimide (DCCD) (7), the sulfhydryl agent N-ethylmaleimide (NEM) and acylspermidine D (Fig. 8). The \(K_i\) of imidodiphosphate was 50 \(\mu\)M (Fig. 8A), which was comparable to the reported value for mung bean H\(^+\)-PPase (apparent \(K_i = 12 \mu\)M) (25). DCCD, which reacts with carboxyl groups in hydrophobic regions, has been reported to bind and inhibit H\(^+\)-PPases (7, 26, 27). The \(K_i\) of DCCD for ScPP (0.2 mM) (Fig. 8B) was similar to that for plant H\(^+\)-PPase (0.5 mM) (7). The \(K_i\) of NEM was 3 \(\mu\)M (Fig. 8C), which was similar to that of the Arabidopsis enzyme (13). The \(K_i\) of acylspermidine D from soft coral (28) was 3 \(\mu\)g ml\(^{-1}\) (Fig. 8D), which was comparable to that for mung bean H\(^+\)-PPase (6 mg ml\(^{-1}\)) (28). These results indicate that the fundamental structural and functional characteristics of H\(^+\)-PPases are conserved in ScPP.

Detection of ScPP in S. coelicolor Cells—To determine whether ScPP protein could be detected in S. coelicolor cells, we obtained crude membrane preparations from...
relatively young cells and subjected them to immuno-
blotting. ScPP protein was clearly detected at approxi-
mately 70 kDa in membranes prepared from 2-day-old
cultures. The protein had decreased by day 4 and had dis-
appeared by day 6 (Fig. 9, A and B). The 70-kDa protein
was not a major component of the crude membrane frac-
tion, as shown by a Coomassie Blue–stained SDS-PAGE
gel. Therefore, the H+-PPase might be a minor component
of *S. coelicolor* membranes. Electron-microscopic images
revealed membranous structures in the cells cultured for
two days (Fig. 9, C and D). We carried out an immuno-
cytochemical analysis to determine the intracellular loca-
tion of the ScPP. Numerous protein A-gold particles that
reacted with the antibody were located in the plasma
membrane (Fig. 10). There were also some particles in
the cell interiors.

**DISCUSSION**

In this study, we characterized the ScPP of *S. coelicolor*,
the primary structure of which is phylogenetically
distant from the H+-PPases of other organisms (Fig. 1).
The growth rate of *S. coelicolor* is relatively low, and
the H+-PPase activity of the crude membrane fraction
(approximately 7.0 nmol min⁻¹ mg⁻¹) was much lower than
in the vacuolar membranes of young plants (1,100
nmol min⁻¹ mg⁻¹) (7). We therefore expressed ScPP in *E.

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**Fig. 6. Metal ion dependence of the proton-pump activity of ScPP**. Membrane vesicles were prepared from ScPP-expressing *E. coli* cells and assayed for PPi-dependent proton pumping in the presence of various concentrations of KCl. (A) Fluorescence quenching of acridine orange recorded with a fluorescence spectrophotometer. Ammonium chloride was added to collapse the proton gradient generated by the H+-PPase at the indicated time points (arrows). (B) Initial rates of fluorescence quenching in the presence of various metal ion concentrations. (C) PPi-dependent proton-pump activity in the presence of 100 mM KCl or RbCl under the same conditions.

**Fig. 7. Dependence of ScPP mutants on K⁺**. (A) Wild-type and mutant enzymes (K507A, K507R, T510G and K507A/T510G) were expressed in *E. coli*, and gels of the membrane fractions (10 µg) were immunoblotted with anti–H+-PPase antibody. ScPP was located at a position corresponding to 80 kDa. (B) *E. coli* membranes containing WT and mutant enzymes were assayed for PPase activity in the presence (shaded bars) or absence (light-shaded bars) of 120 mM KCl. The data are presented as the mean of five experiments.
coli, where it could be partially purified from membranes (Figs. 2, 3 and 6).

**Biochemical Characteristics of ScPP**—Several parameters of ScPP—such as its requirement for phospholipids and Mg\(^{2+}\), inhibition by Ca\(^{2+}\), and the apparent \(K_m\) values for PP\(_i\) and Mg\(^{2+}\)PP\(_i\) (Figs. 4 and 8)—are similar to those of other H\(^+\)-PPases (1). Its thermal stability is higher than that of a purified plant enzyme that was completely inactivated at 40°C (29), but lower than that of the enzyme of *Pyrobaculum aerophilum*, which had maximal activity at 80°C when expressed in yeast (30). The thermal stability of H\(^+\)-PPases is likely to depend on the structure of both the transmembrane domains and the catalytic domain formed by the cytoplasmic loops. Future studies of the three-dimensional structures of H\(^+\)-PPases should provide insight into the basis of their thermal stabilities.

H\(^+\)-PPases are divided into types I and II according to their requirement for K\(^+\) (5). It has been proposed that the presence of a lysine versus an alanine residue in the conserved GNxx(A/K)A motif of one of the cytoplasmic segments distinguishes the two types (4). This motif is located in the cytoplasmic loop between the eleventh and twelfth transmembrane domains of ScPP. According to this criterion, ScPP is a type II enzyme, as it has the sequence GNTTKA (residues 503-508) (15).

In general, type I plant vacuolar H\(^+\)-PPases are stimulated more than four-fold by K\(^+\) (23, 31), whereas ScPP was only slightly stimulated (Fig. 5). Therefore, both its relative lack of dependence on K\(^+\) and the presence of a lysine residue (Lys-507) in the critical motif indicate that it is a type II H\(^+\)-PPase. Although its H\(^+\)-pump activity, unlike PP\(_i\)-hydrolysis, was strongly stimulated by 100 mM KCl, similar stimulation was obtained with NaCl or RbCl. This was probably due to the Cl\(^-\) ions, which act as counter ions during H\(^+\) transport and can be replaced by other anions, such as sulphate (32). The transport of chloride ions might take place through a chloride channel, rather than via the H\(^+\)-PPase itself.

The lack of dependence of ScPP on K\(^+\) was further examined by making amino-acid substitutions (Fig. 7). The substitution of Lys-507, which is reported to be a key residue affecting K\(^+\) dependence, did not alter this activity of ScPP. Similarly, the replacement of another potentially key residue, Thr-510 (T510G and K507A/T510G), did not increase the K\(^+\) dependence. Therefore, the lysine and threonine residues in the conserved motif (GNTTKAIT; 503–510 in ScPP) do not control the K\(^+\) dependence of ScPP. The other residues may determine this property with Lys-507 in the tertiary structure of the catalytic domain.

ScPP was inhibited by imidodiphosphate, DCCD, NEM and acylspermidine (Fig. 8). Its sensitivity to these inhibitors was similar to that of other H\(^+\)-PPases, such as the *R. rubrum* enzyme (10). DCCD, which is an inhibitor of proton conductance, has been reported to react with Glu-
305 and Asp-504 of Arabidopsis H+-PPase (27). These residues correspond to Glu-262 and Asp-469 of ScPP, which are located in the sixth transmembrane domain and on the cytoplasmic side of the eleventh transmembrane domain, respectively (15). Therefore, these two residues, or the neighboring transmembrane domains (the sixth and eleventh), might be involved in H+ translocation.

The sulfhydryl reagent NEM reacts with Cys-634 of the Arabidopsis enzyme, which corresponds to Cys-621 of ScPP (15), and inhibits the enzyme (25). The amino-acid sequence of loop m, which includes Cys-621, is not conserved in H+-PPases. Acylspermidine derivatives isolated from a soft coral are novel inhibitors of plant vacuolar H+-PPase (28). They do not inhibit soluble-type PPase or the other proton pumps. Our findings support the theory that these compounds are useful for identifying type I and II H+-PPases, although their target site is unknown.

Presence of H+-PPase in S. coelicolor—ScPP protein could be detected by immunological methods, at least in the early stages of S. coelicolor growth (Figs. 9 and 10). Over a period of two days, germ tubes emerge from the spores and grow by tip extension and branch formation to form a mycelium extending into the medium. ScPP was observed mainly in the plasma membrane, but was also detected in intracellular membranous structures. During growth, S. coelicolor cells synthesize many kinds of macromolecules, including proteins, RNAs and cell wall components, and generate a large amount of PPi as a byproduct, as do growing plant cells (33). ScPP might scavenge cytosolic PPi to stimulate macromolecular biosyntheses. It might also generate a pH gradient across the plasma membrane to provide a driving force for secondary active-transport systems for the uptake of nutrients, the secretion of secondary metabolites and the release of waste products.

It should be noted that some membranous structures were observed in two-day-old cells of S. coelicolor (Fig. 9), and immunocytochemical analysis suggests that they contained ScPP (Fig. 10). Recently, Docampo and colleagues have demonstrated that H+-PPase is located in acidocalcisomes and related organelles in certain microorganisms (8, 10, 11, 34-36). In the unicellular eukaryote T. cruzi, H+-PPase is located in the acidocalcisomes and contractile vacuoles and is thought to be involved in osmoregulation (37). H+-PPase was first described in the photosynthetic bacterium R. rubrum (2, 38), where it was found on the chromatophore membrane that is continuous with the plasma membrane, and functions in the photophosphorylation of inorganic orthophosphate to pyrophosphate (3). However, this enzyme also exists in the membranes of the acidocalcisomes of R. rubrum, where it functions in the acidification of the intracellular compartment (10). The H+-PPase of the soil bacterium A. tumefaciens is also present in volutin granules, which resemble acidocalcisomes (11). In future studies, we intend to determine whether S. coelicolor possesses acidocalcisomes or similar structures. If it does, we will assess the relationship between these organelles and the intracellular membranous structures that were observed in young cells of S. coelicolor (Fig. 9). The function of these structures and the physiological role of ScPP remain to be determined.

This work was supported, in part, by Grants-in-Aid for Scientific Research from the Ministry of Education, Sports, Culture, Science and Technology of Japan to M.M. (nos. 16380068, 13CE2005 and 14COEA2). M.H. is a recipient of the Japan Society for the Promotion of Science for Young Scientists Research Fellowship 16-5967.

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