Phytomonas: Transport of amino acids, hexoses and polyamines

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Abstract

Phytomonas cells (Phytomonas Jma) isolated from the latex of Jatropha macrantha were assayed for amino acid, hexose and polyamine transport. Results showed high transport rates for glucose and fructose (193 and 128 pmol min−1 107 cells, respectively) and lower, but significant rates, for proline, arginine, cysteine and glutamate (between 1.7 and 5.8 pmol min−1 107 cells). Minor transport activities were observed for serine, glycine and aspartate (<1 pmol min−1 107 cells). Amino acid transport processes do not seem to be regulated by starvation or during the growth phases. Polyamine transport was also evaluated showing a clear preference for spermidine over putrescine (3.4 and 0.4 pmol min−1 107 cells, respectively). This work represents the first report on metabolite transport in phytomonads.

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Index Descriptors and Abbreviations: Phytomonas; Latex; Amino acid transport; Hexose transport; Polyamine transport; EST, expressed sequence tag; Jma, Jatropha macrantha

1. Introduction

The Phytomonas genus comprises unicellular flagellates which infect a wide variety of plants. Although being phylogenetically close to parasites that cause several human diseases, until 1970 these protozoan organisms were of little interest to researchers (Dollet, 2001). However, economic losses due to coconut and palm oil plants diseases has led to increased interest in these pathogens. Thus far, the life cycle of these flagellated protozoa is not completely understood, but probably involves phytophagous insects which would act as vectors in the transmission of the procyclic form (Jankевичius et al., 1989).

Historically, organisms of at least three different groups and probably different genera have been classified as Phytomonas. One group includes organisms found in the latex vessels of some laticiferous plants, another group is formed by organisms found only in fruits and seeds, and a third group is found only in the phloem sap of certain Latin American plant species (Dollet, 2001).

Since phytomonads have no cytochromes and lack a functional Krebs cycle they depend on glycolysis to obtain energy (Sanchez-Moreno et al., 1992) similar to bloodstream forms of Trypanosoma brucei. In a recent study a large number of ESTs (Expressed Sequence Tags) from Phytomonas serpens, corresponding to a glucose transporter family, were found (Pappas et al., 2005). This high redundancy would be related to the phytomonad’s particular metabolism, mostly dependent on substrate level phosphorylation. High levels of hexose transporters could be necessary to maintain glycolysis. Supporting this hypothesis, phytomonads have a very large number of glycosomes in comparison to other trypanosomatids (Sanchez-Moreno et al., 1992). This is in agreement with the rich carbohydrate environment provided by plant hosts. However, phytomonad metabolism in the insect vector stage remains...
unclear. It has been proposed that amino acids are the main energy source, as occurs in procyclic forms of *T. brucei*. On the other hand, polyamines are involved in crucial cellular processes including the synthesis of the antioxidant compound trypanothione (bis-glutathionyl spermidine) which has been found exclusively in trypanosomatids (Oza et al., 2002). In *Phytomonas* (*P. serpens*) the unique evidence of a trypanothione-based detoxification pathway is the presence of a putative trypanothione reductase (EC 1.6.4.8, GenBank Accession No. CO724369, Table 1, Pappas et al., 2005).

Here, we present data on the ability of *Phytomonas Jma* to transport different metabolites from extracellular media. This work represents the first step to further characterize permease systems from phytomonads and better understand their metabolic requirements and biochemical pathways.

2. Materials and methods

2.1. Cell cultures

*Phytomonas Jma* cells (Serrano et al., 1999) isolated from the latex of *Jatropha macrantha* (Euphorbiaceae) were kindly provided by Prof. Israel Algranati (Fundación Instituto Leloir, Argentina), and originally obtained from the “Trypanosomatid Culture Collection”, University of São Paulo (TCC-USP). Cells were cultured at 28 °C in plastic flasks (25 cm²), containing 5 ml of Liver Infusion Tryptose (LIT) medium (started with 10⁶ cells/ml) supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Camargo, 1964). Cells were subcultured every seven days, unless otherwise indicated, and counted using a hemocytometer.

2.2. Transport assays

Aliquots of *Phytomonas Jma* cultures (2 × 10⁷ parasites), grown for four days, were centrifuged at 1500 g for 5 min, washed twice with phosphate buffered saline (PBS), resuspended in 0.1 ml PBS and then added to 0.1 ml of the transport mixture containing the appropriate radiolabeled metabolite ([l-2,3,3H]Proline, [l-2,3,4,5-3H]Arginine,l-[35S]Cysteine, [l-[G-3H]Glutamic acid, [3H]Glycine, l-[3-3H]Serine, l-[2,3,3H]Aspartic acid, [14C]Spermidine, [1,4-14C]Putrescine, d-[U-14C]Glucose and d-[U-14C]Fructose) at 100 µM final concentration in PBS (specific activity, 10 µCi/ml). Following incubation at 28 °C, cells were centrifuged 10,000 g and washed twice with 1 ml of ice-cold PBS. Pellets were then resuspended in 0.2 ml of a solution containing 1% SDS and 0.2 N NaOH and counted for radioactivity in UltimaGold XR liquid scintillation cocktail (Packard Instrument Co., Meriden CT, USA). Non-specific transport and carry over were measured in transport mixtures containing 100 mM of the appropriate metabolite (Sigma–Aldrich Co). Assays were run in triplicate. Cell viability was assessed by direct microscopic examination. All radiochemicals were supplied by GE Healthcare Life Sciences.

2.3. Protein precipitation

Aliquots of *Phytomonas Jma* cultures (4 × 10⁷ cells), were treated as indicated above and incubated 2 h in the presence of an radioactive amino acid mixture in PBS. Cells were washed twice with 1 ml of ice-cold PBS and resuspended in 200 µl of TCA 20% (v/v) in PBS, incubated 1 h at 4 °C and then centrifuged 10 min at 20,000g. Supernatants were removed and protein pellet was washed with cold acetone. Pellet and supernatants were counted for radioactivity.

3. Results

3.1. *Phytomonas* growth kinetics

In order to study the different in vitro growth phases and kinetics, *Phytomonas Jma* cells were grown in LIT medium starting from 10⁶ cells/ml and counted over an eight day period. Cells are in the logarithmic phase of growth up to day 5 with a calculated average doubling time during this phase of approximately 19 h. Maximum cell densities were observed at day 5 (7.75 × 10⁷ cells/ml). After that, cells enter in the stationary and decline phases (Fig. 1). Cultures from the logarithmic phase on day 4 were chosen for further transport assays. Cell morphology was homogeneous during logarithmetic phase of culture; however, cells became highly polymorphic during the stationary and decline phases.

3.2. Amino acid, hexose and polyamine transport

Three groups of essential molecules, amino acids, hexoses and polyamines, have been chosen for a first approach on

<table>
<thead>
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<th>Accession No.</th>
<th>Inferred function</th>
<th>Best hit</th>
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<tbody>
<tr>
<td>CO724362</td>
<td>Possible amino acid transporter</td>
<td>Trypanosoma cruzi strain CL Brener amino acid permease (XM_797446)</td>
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<tr>
<td>CO723919</td>
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<td>Leishmania donovani amino acid permease 7 gene (DQ402427)</td>
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<tr>
<td>CO723750</td>
<td>Possible hexose transporter</td>
<td>Trypanosoma cruzi strain CL Brener hexose transporter (XM_799269)</td>
</tr>
<tr>
<td>CO724369</td>
<td>Possible trypanothione reductase</td>
<td>Crithidia fasciculata gene encoding trypanothione reductase (Z12618)</td>
</tr>
</tbody>
</table>

All the sequences were obtained from the GenBank and correspond to *Phytomonas serpens* (isolated from tomato) plant-stage cDNA library (Pappas et al., 2005).
the study of Phytomonas Jma metabolite transport and requirements. The unique evidence of the molecules involved in amino acid/hexose transport and polyamine metabolism in Phytomonas are summarized in Table 1 and includes ESTs from three different amino acid permeases, one hexose permease and a trypanothione reductase.

Amino acid transport assays showed that proline was transported at highest velocities (5.8 pmol min\(^{-1}\) 10\(^{-7}\) cells). Arginine, cysteine and glutamate transport had velocities in the range 1.7–4.3 pmol min\(^{-1}\) 10\(^{-7}\) cells, while glycine, serine and aspartate transport showed velocities lower than 1 pmol min\(^{-1}\) 10\(^{-7}\) cells (Fig. 2a). Polyamine transport assays revealed a higher transport rate of spermidine (3.4 pmol min\(^{-1}\) 10\(^{-7}\) cells) than putrescine (0.4 pmol min\(^{-1}\) 10\(^{-7}\) cells; Fig. 2b). On the other hand, glucose and fructose were transported at higher rates than amino acids and polyamines (193.4 and 128.1 pmol min\(^{-1}\) 10\(^{-7}\) cells, respectively; Fig. 2c).

In order to study the existence of potential regulatory mechanisms of transport processes, Phytomonas Jma cultures were evaluated during the different growth phases and also subjected to carbon starvation. It was previously demonstrated that these conditions can modulate amino acid transport in other trypanosomatids such as Trypanosoma cruzi (Canepa et al., 2004, 2005). However, in Phytomonas Jma, no difference in transport velocities were observed between starved cells and controls or between cells from the logarithmic (day 4) and stationary phases (day 8) of growth. The proportion of transported amino acids which was incorporated into proteins was also evaluated by transport assays using a radioactive amino acid mixture followed by TCA protein precipitation. Only 9.7% of the transported amino acids were incorporated into proteins after 2 h, indicating that they remain mainly as free amino acids or derived metabolites.

4. Discussion

Trypanosomatid organisms are capable of using carbohydrates and amino acids as carbon sources depending on their availability in extracellular media (Cazzulo, 1994). Although no reserve of polysaccharides has been detected, trypanosomatids store proteins in special organelles called reservosomes and obtain glucose and other metabolites mainly by transport processes (Urbina, 1994). Our results support the hypothesis that Phytomonas transports glucose and fructose more efficiently than amino acids. The calculated velocities of the glucose and the fructose transport in Phytomonas Jma are similar to those reported for other insect-form kinetoplastids (Tetaud et al., 1994). Moreover, it has been shown that in phytomonads isolated from the latex of Euphorbia characias, glucose, fructose and mannose serve as the main energy substrates and all the enzymes from the Embden-Meyerhof pathway were detected (Sanchez-Moreno et al., 1992). Rather than synthesize amino acids, parasitic protozoa rely on transport systems for the acquisition of these macromolecules (Silber et al., 2005). Phytomonas Jma was able to transport arginine, cysteine and proline at a high rate showing similarities with other transport systems in kinetoplastids (Carrillo et al., 2006; Silber et al., 2002, 2005; Canepa et al., 2004; Pereira et al., 1999; L’Hostis et al., 1993). Although arginine kinase activities have not been reported in phytomonads, it is known that T. cruzi and T. brucei use this amino acid as an energy buffer when it is converted to phosphoarginine by arginine kinase (Pereira et al., 1999, 2002). Further-
more, proline is essential for *T. brucei* survival in the insect stage (Lamour et al., 2005) and cysteine plays a crucial role in the synthesis of trypanothon (Muller et al., 2003). Whereas significant glutamate transport was measured, aspartate transport was very low (0.2 pmol min⁻¹ 10⁻⁷ cells), presumably it can also be obtained by other routes. In trypanosomatids such as *T. cruzi*, amino acid transport velocities are in the range of 3 (aspartate) to 100 (arginine) pmol min⁻¹ 10⁻⁷ cells (Canepa et al., 2004, 2005), while hexose transport is significantly greater (two orders of magnitude, Tetaud et al., 1994), thus showing similarities to the values reported here for phytomonads. Since polyamines are important in trypanosomatids for their role as cell growth and differentiation regulators and are involved in synthesis of the antioxidant compound trypanothione (Wallace et al., 2003), the transport of putrescine and spermidine was determined in *Phytomonas* Jma. Rates were similar to values previously obtained in *T. cruzi*, being 3.6 and 6.3 pmol min⁻¹ 10⁻⁷ cells for spermidine and putrescine, respectively (Carrillo et al., 2006; Gonzalez et al., 1992).

One of the common features of parasitic organisms is the evolutionary loss of biosynthetic pathways and gain of transport systems. The relevance of studying the physiological role of these processes relies on transporters being considered as the first step in a wide range of metabolic pathways. In the particular case of the *Phytomonas* genus, it is clear that further investigation in this field is required.

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