THE POLYAMINE TRANSPORT SYSTEM IN MAMMALIAN MITOCHONDRIA

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INTRODUCTION

The natural polyamines, putrescine, spermidine and spermine are amino-aliphatic molecules almost completely protonated at physiological pH and so behaving as polycation in their interactions with biological structures. It follows that their most important targets are the polyanionic groups present on nucleic acids, proteins and membranes.

Polyamines are ubiquitous metabolites in prokaryotic and eukaryotic cells and are known to be essential for a variety of physiological processes as cell growth and cell differentiation [1-5]. The study in polyamine metabolism has drawn the interest towards a their possible correlation with diseases of neoplastic origins, [6], particularly in considering the polyamine biosynthetic pathway as a therapeutic target. In this regard several investigations were concerned with the effect of inhibitors on ornithine decarboxylase and S-adenosylmethionine decarboxylase, two key enzymes of polyamine synthesis [7-10].

The molecular mechanisms of action of polyamines and their specific physiological function remain to be completely understood despite of the large number of investigations carried out worldwide by qualified research groups.

Polyamines are synthesized and catabolized at intracellular level [3], and their concentration is regulated by a bi-directional transport across the plasma membrane [11]. External polyamine uptake can assume a specific role when the cell requires an increase of concentrations of their molecules [4-5]. The unusual requirement of polyamines by the cell and its organelles can account for the presence of a polyamine transport system in the plasma membrane. Among subcellular organelles, mitochondria apparently lack of a biosynthetic pathway. However substantial amounts of spermidine and spermine are present in liver, heart and brain mitochondria [12-14], suggesting the presence of a transport system for these molecules in mitochondrial membranes.

POLYAMINE BINDING TO MITOCHONDRIAL MEMBRANES

Studies of non-equilibrium binding of spermine to liver mitochondria have demonstrated the presence of two specific binding sites indicated as S_1 and S_2, both exhibiting low affinity, high binding capacity, monocoordination and a negligible cooperation effect [15]. These sites are most likely located at the level of the so-called gap-junctions, the contact sites between outer and inner membrane [16, 17], that would account for the involvement of the above mentioned sites in the transport process.

Taking into account the total free-energy binding value, spermine binds to mitochondrial sites by weak interactions [15], excluding the possibility of strong electrostatic binding. Spermidine binds in a way comparable to that of spermine [18] suggesting that the two polyamines might share the same sites. Putrescine binding, instead, is different. In fact, comparison of the calculated binding constants indicate that it binds only to S_2 site of spermine and spermidine [18].

The investigations concerning the functions of S_1 and S_2 binding sites [15, 18] have demonstrated that the former is mainly involved in spermine and spermidine transport and prevention by these polyamines of mitochondrial permeability transition (MPT). The S_2 site, beside putrescine transport may also participate in spermidine transport and in the inhibition by these polyamines, as S1 site, in the MPT induction. Site S_2, indeed, seems to be also involved in the protein transport and other mitochondrial effects induced by polyamines [19-22].

Polyamine binding sites have a different flexible structure that can be modified during the interaction with these polycations, as a consequence they can exhibit different binding constant.

Indeed, differences in the flexibility and hydration of the polyamine molecules may explain their different capacity to bind to the two sites. In this regard it is noteworthy that spermine is much more flexible than spermidine, and putrescine is completely rigid. Their degree of hydration follows the same trend, spermine is the most hydrated while putrescine is the least hydrated [23]. Taking into account these consideration one can

suggest that S_1 is a rigid and hydrophilic site, able to bind the flexible, highly hydrated spermine. The less flexible spermidine would bind only partially, while the completely rigid putrescine would not bind. The site S_2, instead, should be a flexible and less hydrophilic site able to bind the large part of spermidine and the total amount of putrescine.

Most likely the conformational changes taking place during spermidine and putrescine binding transform S_2 in a site having similar characteristic as S_1, in particular in its ability to accomplish their transport.

The use of a thermodynamic model for ligand receptor interactions [24], and the free-energy profile subsequently individualized during the investigation regarding the transport mechanism [25] have provided evidences about the precise position of S1 site along the polyamine transport channel. The energy trough resulting from the interaction of spermine with this site has been individualized at 1/8 of the width of the inner membrane (or better the gap-junction), towards the external side [25]. In other terms the S_1 site should be located inwardly in the membrane in the first part of the polyamine channel. The position of S_2 site is not known. However, since S_2 site can substitute for S_1 in the transport of spermidine and putrescine, it can be suggested that it is strictly closed to S_1.

POLYAMINE TRANSPORT IN MAMMALIAN MITOCHONDRIA

The first report demonstrating the existence of a polyamine transport system in liver mitochondria was published in 1985 [26]. This paper evidenced that in the presence of phosphate spermine can be consistently taken up by mitochondria and the translocation is sensitive to Mg^{2+} and mersalyl, an inhibitor of phosphate, against the general opinion that this process was not possible, due to the theoretically very high Born charging energy barrier to be overcome [27]. Furthermore, addition of antimycin A or FCCP after spermine uptake induced a rapid efflux of the accumulated polycation [26]. A first deduction of these results was that spermine is taken up by an electroneutral mechanism in co-transport with phosphate and that this mechanism is energy dependent. The observation that also the uptake of phosphate was enhanced by spermine to an extent dependent on its concentration in the medium led to the conclusion that the transport of spermine and phosphate are mutually related processes [26]. The necessity of a counter-ion for spermine transport was subsequently strengthened with the observation that also acetate favors the process although to a lesser extent than phosphate [28].

However, other results reported in the above paper raised serious doubts about the proposal for an electroneutral transport of spermine. Infact it has been shown that in the presence of nigericin, a ionophore which exchanges internal K^+ with external H^+, with the result of collapsing ΔpH and increasing $\Delta\Psi$, a strong increase in the rate and total extent of spermine is induced [28]. Again, if mitochondria are incubated in the presence of another ionophore, valinomycin, which induces an electrophoretic uptake of K^+, with the result of a $\Delta\Psi$collapse and a strong increase of ΔpH, spermine transport is completely abolished [28].

These results clearly demonstrate that spermine is transported by an electrophoretic mechanism dependent on $\Delta\Psi$ value, and the effects of phosphate and acetate are not attributable to the triggering of an electroneutral mechanism. In this regard it is to take into account that phosphate and acetate cross the mitochondrial membrane as indissociated acid, then in the matrix they dissociate H^+ which collapse ΔpH and shift $\Delta\Psi$ to much higher values.

In conclusion the presence of an electroneutral anion transport favors the electrophoretic uptake of spermine but the two transport mechanisms are independent. The recognition of a transport system for spermine in liver mitochondria raised an interest in further characterizing this process. A subsequent paper [29] provided evidences that, besides spermine, also spermidine and putrescine are transported by the same specific uniporter system. While the involvement of basic aminoacids and organic amines transporters it should be ruled out. Polyamine transport is a saturable process with apparent K_m values of 0.13 mM for spermine, 0.26 mM for spermidine, 1 mM for putrescine. All these polyamines are reciprocally competitive inhibitors confirming a common transport system which is insensitive to external pH. Spermine, spermidine and putrescine are taken up by mitochondria at rates that increase with increasing valence of the transported polyamines. Polyamines exhibit a nonlinear current-voltage relationship apparently exponential, according to the general behaviour of monovalent cations in mitochondria [30]. Flux-voltage analyses of their transport, in comparison with the organic cations, demonstrated that the intrinsic permeability coefficient of polyamines show minimal differences among them and are very similar to those of the organic cations. Hence their permeabilities are therefore extremely large in consideration of the strong electric charge present in these molecules [29].

The possibility that the energy barrier for polyamine transport is lowered by the availability of a hydrophilic proteinaceous transport system is further strengthened by the enthalpy energy for their uptake. This value per unit charge transported has an average of about 12 KJ/mol [29] and is lower than those of other mitochondrial carriers [31, 32].

Further investigation on the voltage-dependent mechanism of spermine transport in liver mitochondria demonstrated that an increase in $\Delta\Psi$ from 150 to 210 mV favors the process, as reflected by an approximate 4-fold increase in V_{max} and 25% decrease in K_m [25]. Flux-voltage analyses performed at very high and very low spermine concentrations yelded β values of 0.125 and 0.25 for V_{max} and V_{max}/K_m, respectively [25].

The parameter β used for these analyses is the slope of ln(flux) vs $F\Delta\Psi/RT$ plots and the physical significance of these β values was analysed using a theory relating the enzyme reaction rate to the free energy profiles [25]. These analyses together with those concerning spermine binding [15] provided evidences that the polyamine transporter has an asymmetrical energy profile composed of two peaks with the binding site near the external membrane surface followed by a rate determining energy barrier for the movement of spermine towards the internal compartment [25].

Taking into account that most of the investigations on polyamine transport have been performed with energized mitochondria, normally having $\Delta\Psi$ values of about 180 mV, according to the rationale of Nernst for an electrophoretic transport, the ratio inner/outer concentration of spermine should reach the value of 10^{12}. Instead the observed experimental ratio have been calculated in the order of 10^2! This means that besides a mechanism of polyamine accumulation also a mechanism of efflux is operating. The first indications of the existence of an efflux mechanism were evidenced observing the effects of Mg^{2+}, FCCP and antimycin A, added after spermine accumulation [26]. As reported in the above paper a rapid and intense release of the polyamine was observed upon the mentioned addition. Furthermore other indications were subsequently provided. Infact, when energized mitochondria are pre-loaded with labeled spermine and then resuspended in a new medium with cold polyamine, an instantaneous release was observed followed by a slow, long-lasting release of labelled polyamine [28]. It has been suggested that this secondary energy-dependent efflux may reflect an exchange between accumulated and external spermine. The definitive individualization of an efflux mechanism for the polyamines was reported in 1992 [33]. In that paper, it was reported that accumulated polyamine is retained by energized RLM as long as $\Delta\Psi$remains above a certain critical value. A decrease of $\Delta\Psi$ as that caused by de-energizing agents [26] or by the same electrophoretic transport of spermine, causes an immediate outflow of accumulated polyamine. Spermine can also be released from energized mitochondria by inhibiting the influx of phosphate by mersalyl, thus decreasing $\Delta\Psi$ and, concomitantly, increasing ΔpH. Under this condition ΔpH, no longer utilizable for phosphate influx, becomes available for driving spermine efflux.

In conclusion it can be hypothesized that "in vivo" exists a continuous energy-dependent influx-efflux cycling of spermine driven by $\Delta\Psi$ and ΔpH, respectively.

Most of investigations regarding polyamine transport have been carried out on liver mitochondria [26, 28, 29]. However it can be emphasized that the presence of this mechanism has been recognized also in heart [22], brain [14] and Kidney [A. Toninello personal communication] mitochondria.

No particular difference in the characteristic of this mechanism has been observed in the different types of mitochondria. As far as the extent of transport is concerned liver mitochondria are able to accumulate the largest amount of spermine, followed by heart and brain mitochondria [28, 22, 14].

Since the synthesis of polyamines is known to be an extra-mitochondrial process, the existence of a polyamine uptake mechanism implies a physiological role for these molecules within the mitochondrial matrix and several evidences for such a role are accumulating. The loss of cellular spermine resulted in 80% reduction in the content of mitochondrial DNA in a manner indicating cessation of mitochondrial DNA replication and without altering nuclear DNA levels [34]. Indeed it has been reported that spermine is able to stimulate mitochondrial protein phosphatases active towards pyruvate dehydrogenase [35] and spermine has also been shown to activate citrate synthase [36]. These findings suggests that spermine but also the other polyamines may play an important role in modulating energy metabolism and may be essential for mitochondrial gene-expression.

A very recent review reports all the physiological and pathological implications related to the polyamines transport in mammalian mitochondria [37].

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