Biochimica et Biophysica Acta, 513 (1978) 296—300

BBA Report

BBA 71361

PROPERTIES OF THREE DISTINCT PYRIMIDINE TRANSPORT SYSTEMS IN YEAST

EVIDENCE FOR DISTINCT ENERGY COUPLING

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(Received July 14th, 1978)

Summary

In *Saccharomyces cerevisiae* the uptake of cytosine, uracil and uridine is mediated by three permeases. Using mutants blocked in the metabolic utilization of these three compounds we were able to study their specific uptake. Cytosine and uridine show simple saturation kinetics, whereas uracil uptake is a biphasic process. A comparison of the effects of several inhibitors of energy metabolism on these uptake systems was made. Striking differences were found 2,4-Dinitrophenol (10^{-3} M) and NaN_3 (10^{-2} M) inhibit the entry of the three compounds to similar extent, but chlorhexidine (10^{-5} M) and Dio 9 (50 μg/ml) which are ATPase inhibitors in vitro strongly impaired cytosine and uridine entry and remained without effect on uracil uptake.

We provisionally conclude that these systems may be energized by different mechanisms. In the case of cytosine and uridine permease, a membrane ATPase is possibly involved in the process of energetic coupling whereas this does not seem to be so for uracil.

The plasma membrane controls the cellular uptake and exit of various metabolites. In bacteria it has been shown that the energization of the transport of a large number of solutes can be achieved by oxidation via the respiratory chain, by ATP hydrolysis via the membrane bound (Mg^{2+} - Ca^{2+})-ATPase or by ion gradients [1]. In eukaryotic cells it is generally assumed that the plasma membrane lacks oxidoreductase activity and that ATP is the sole energizing agent. However, some transport systems which do not use ATP as energy source have been described [2—4] and in a recent report Christensen and co-workers propose that redox proton pumps might also energize the eukaryotic plasma membrane [5]. A study of the action of different energy in-

hibitors with known specific modes of action on the active transport of various metabolites should give us more information on the energy coupling of the plasma membrane in eukaryotic cells.

We report here the effect of several energy inhibitors on the uptake of cytosine, uracil and uridine. These three active transport systems have previously been well characterized genetically and physiologically and it was shown that three distinct permeases are involved [6—8]. Our results suggest that the energetic coupling is different for the uracil transport system than for the uridine and cytosine systems in *Saccharomyces cerevisiae*.

Most strains used in this work were derived from *S. cerevisiae* strain FL 100 (haploid, a mating type) or from an isogenic strain, FL 200 (haploid, α mating type) [7]. The strains carrying the *rh* mutation were obtained from crosses of our strains with strain *rh*(*a*), a generous gift from Dr. Grenson (Université Libre de Bruxelles, Bruxelles) [8]. The strains used in this study and their characterization are listed in Table I. Media and technics used in the present work have already been reported [8—10].

The ATPase assay was performed by incubation at 30°C for 20 min in a total volume of 1 ml, a reaction medium containing 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$ and 5—50 μl crude plasmalemma (protein concentration ranging from 30 to 300 μg). The reaction was started by the addition of 2 mM ATP and stopped by the addition of 0.1 ml 50% trichloroacetic acid. Control tubes without ATP and without $MgCl_2$, respectively, were incubated simultaneously as well as a blank with ATP alone to check spontaneous ATP hydrolysis. The appropriate corrections were made to calculate the P_i liberated by the ATPase activity. The P_i was measured in the cold trichloroacetic acid supernatant as described by Lowry and Lopez [11]. The membrane fraction used to test the activity of the ATPase was shown to consist of a mixture of light and heavy membranes from renografin gradients (densities less than 1.10 and 1.18, respectively) [9, 10] which both contain ATPase activities. The membrane fraction could be stored at +4°C for several days with a loss of around 10% specific activity per day.

The kinetics of the cytosine-purine and uracil permeases in *S. cerevisiae* have been described in previous papers [6, 7]. We studied in more detail the uptake of uridine in a mutant strain deficient in the metabolic interconversions of this compound (strain RL 42-1B). The results plotted according to

TABLE I

STRAINS USED

The products of the genes *fcy 1* and *fcy 2* are respectively cytosine deaminase and cytosine permease. Gene products of *fur 1* and *fur 4* are UMP pyrophosphorylase and uracil permease; *rh*, uridine ribohydrolase; *urk*, uridine kinase.

Strains	Genotype	Phenotype
FL 100 (*a*)	Wild type	Resistance to 5 FC 10^{-6} M
FL 200 (α)	Wild type	5 FU 10^{-5} M
		5 FUine $3 \cdot 10^{-5}$ M
FL 442-2D (*a*)	*fcy 1-1*	Resistance to 5 FC $3 \cdot 10^{-5}$ M [7]
FL 442-4B (α)		
RJ 22 (*a*)	*fur 1-8, rh*	Resistance to 5 FU $> 10^{-3}$ M
		5 FUine 10^{-3} M [8]
RL 42-1B (*a*)	*urk, rh*	Resistance to 5 FUine $> 3 \cdot 10^{-3}$ M [6, 8]

TABLE II

KINETIC CONSTANTS FOR CYTOSINE, URACIL AND URIDINE TRANSPORT SYSTEMS

V is given in mmol/l intracellular fluid per min. See ref. 7 for data concerning cytosine. The strains used were *fcy 1-1*, *fur 1-8 rh* and *urk rh*, respectively.

	K_m (M)	*V*
Cytosine	$2.5\ (\pm 0.80)\cdot 10^{-6}$	1.72 (± 0.42)
Uracil		
High affinity system	$0.22\ (\pm 0.06)\cdot 10^{-4}$	0.73 (± 0.10)
Low affinity system	$1.51\ (\pm 0.56)\cdot 10^{-4}$	1.60 (± 0.41)
Uridine	$1.53\ (\pm 0.10)\cdot 10^{-4}$	0.60 (± 0.11)

Lineweaver and Burk, show that uridine transport in yeast cells, like cytosine transport, has typical Michaelis-Menten saturation kinetics (see Table II). For uracil uptake, the double reciprocal plots give a curved line, concave downwards, with a break at substrate concentration of 0.2 mM (Fig. 1).

The effects of several inhibitors on uracil, uridine and cytosine uptake were studied. We used 2,4-dinitrophenol. a classical uncoupler of oxidative phosphorylation and proton conductor [13], NaN_3, an inhibitor of energy metabolism acting on ATPase [13, 14] but also considered as a proton conductor [13], and chlorhexidine and Dio 9, both agents known as ATPase inhibitors [15—17]. Typical results are outlined in Table III and were reproducib ly found. The high affinity component of uracil uptake shows differential sensitivity. It is resistant to Dio 9 and chlorhexidine at concentrations which clearly inhibit the other two systems.

The inhibitory action of DNP, NaN_3, chlorhexidine and Dio 9 on the ATPase activity of the membrane fraction was tested at the same concentrations as those used to measure the uptake inhibition. Table IV shows that

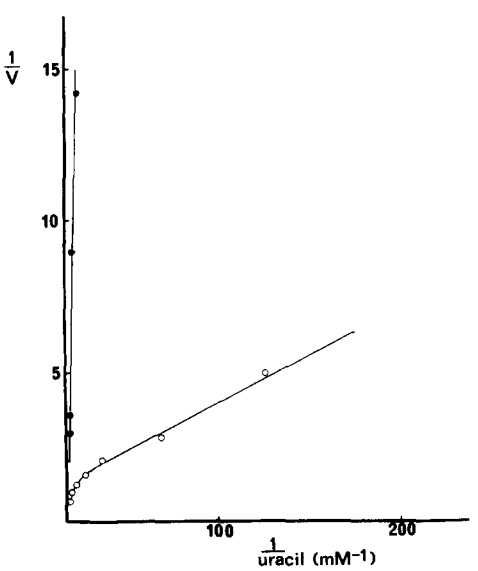

Fig. 1. Uracil uptake by the mutant strains *fur 1-8 rh* (○) and *fur 4-6* (●). Initial velocities (*V*) are expressed as mmol/l cell water per min.

TABLE III

ACTION OF SEVERAL INHIBITORS ON INITIAL VELOCITY OF UPTAKE OF URACIL, URIDINE AND CYTOSINE

To measure the initial velocity of uptake the radioactive compounds were added to test tubes with or without inhibitor. 1 ml culture was added, the tubes were shaken manually and after 30 s of incubation, the samples were filtered and the filters washed with 2 X 10 ml cold water, dried and the radioactivity counted. All experiments were carried out at 30°C.

	Inhibition (%)			
	Dinitrophenol (10^{-3} M)	NaN_3 (10^{-2} M)	Dio 9 (25 μg/ml)	Chlorhexidine ($1.6 \cdot 10^{-4}$ M)
Uracil	95	96	0	0
Uridine	82	100	42	66
Cytosine	95	97	80	88

TABLE IV

EFFECT OF INHIBITORS OF UPTAKE ON THE ATPase ACTIVITY OF THE MEMBRANE FRACTION

Incubation of membrane fraction was carried out as indicated in the text. The inhibitors were added to the test tubes kept in iced water and already containing buffer, $MgCl_2$ and membrane proteins. ATP was then added and the incubation at 30°C started. Specific activity of the ATPase was 0.37 μmol/mg protein per min at 30°C.

Addition to the incubation	Relative ATPase activity
None	100
Dio 9	
12.5 μg/ml	25
25 μg/ml	19
50 μg/ml	9
Chlorhexidine	
$8.0 \cdot 10^{-5}$ M	3
$1.6 \cdot 10^{-4}$ M	6
NaN_3	
$2 \cdot 10^{-2}$ M	9
10^{-1} M	5
Dinitrophenol	
$5 \cdot 10^{-4}$ M	92
10^{-3} M	102

among the compounds tested only dinitrophenol is without effect on the ATPase activity, whereas NaN_3, chlorhexidine and Dio 9 are strongly inhibitory.

Among the three distinct transport systems which were investigated in these studies, one can distinguish two types: (1) the uracil transport system which is characterized by biphasic entry kinetics and by its inhibitor sensitivity. This uptake system is sensitive to dinitrophenol but highly resistant to Dio 9 and chlorhexidine. (2) The cytosine and uridine transport systems which have single phase uptake kinetics and are sensitive to dinitrophenol as well as to Dio 9 and chlorhexidine. One main feature common to the three systems is their inhibition by dinitrophenol. In the case of cytosine and of uracil uptake it was shown that this inhibition occurs whether the strains are ρ^+ or ρ^- [7]. This indicates that dinitrophenol acts directly on the plasma membrane. The proton conductor property of dinitrophenol best explains the fast inhibitory effect on uptake and this implies that proton gradients are involved in the three transport systems studied. Indeed, it has recently been reported that uptake of hypoxanthine (another substrate of the cytosine-purine

permease) is accompanied by uptake of one equivalent of protons [18]. The differential action of Dio 9 and chlorhexidine on uracil as opposed to cytosine and uridine uptake remains to be explained. These compounds have been reported to inhibit ATPase [15—17] and we found that our membrane fraction ATPase is highly sensitive to both inhibitors (Table IV). Therefore, we suggest that this inhibitory action on cytosine and uridine uptake is linked to an effect on the plasma membrane ATPase rather than to differential sensitivities of the carrier, even if several lines of evidence suggest that inhibitor action is complex (refs. 19—22 and Chevallier, M.R., unpublished results). It would be of interest to check other inhibitors acting specifically on yeast plasma membrane ATPase to determine whether the parallelism found in this study can be extended. A search for such inhibitors is presently undertaken.

We provisionally conclude that the three systems we have studied are energized by different mechanisms. In the case of uridine and cytosine permeases, which share similar kinetic properties and inhibitor's sensitivities, a membrane-bound ATPase is possibly involved in the process of energetic coupling whereas it does not seem implied in the case of uracil.

A preliminary abstract of this work has been published [23].

We thank B. Winsor for help in editing the manuscript. This work was supported by a grant from the French Délégation Générale à la Recherche Scientifique et Technique (D.G.R.S.T. No. 75 7 1337).

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