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AFFINITY OF THE YEAST MEMBRANE CARRIER FOR GLUCOSE AND ITS ROLE IN THE PASTEUR EFFECT

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SUMMARY

The reduced intracellular level of various pentoses in the presence of glucose served as a basis for calculating the apparent affinity of baker's yeast for glucose uptake under aerobic and anaerobic conditions, as well as in the presence of 2,4-dinitrophenol. It was found that the apparent Michaelis constant for glucose entry was higher aerobically (17.4 mM) than anaerobically (6.7 mM) or aerobically in the presence of $5 \cdot 10^{-4}$ M 2,4-dinitrophenol (5.6 mM). Implications for the regulation of sugar uptake associated with the Pasteur effect are discussed.

INTRODUCTION

As no expenditure of metabolic energy is required for monosaccharide transport in baker's yeast the steady-state intracellular concentration of monosaccharides is expected to equal that in the medium; in other words, after sufficiently long incubation the $[S_i]/[S_o]$ should be unity (S being the sugar in question and the subscripts i and o referring to the inside and outside solution, respectively). If a $[S_i]/[S_o]$ ratio higher than unity is found a metabolic pump is most likely to be involved. If a $[S_i]/[S_o]$ ratio less than unity is obtained the causes may be several:

(1) Not all forms of cell water are available for the given sugar (water for solution, water for hydration *etc.*);

(2) there may be compartments within the cell that are not accessible to the sugar by virtue of impermeable barriers (*cf.* ref. 1);

(3) the sugar in question is metabolized (*cf.* ref. 2);

(4) two sugars are present simultaneously, one of them being metabolized (*cf.* ref. 3).

The last-named case deserves some attention and a description will be given here how it can be used experimentally for establishing the affinity of the sugar-transporting system for metabolizable sugars which is ordinarily not amenable to straightforward estimation.

THEORETICAL

The carrier hypothesis of monosaccharide transport in its simple form[4-6] states that the rate of transport of sugar S in the presence of another sugar R sharing the same carrier is given by

$$d[S_i]/dt \equiv v_S = V\left(\frac{S_o'}{S_o' + R_o' + 1} - \frac{S_i'}{S_i' + R_i' + 1}\right) \quad (1)$$

where V is the maximum rate of uptake, the subscript o stands for extracellular and the subscript i for intracellular concentration. Also S' is the reduced concentration of substrate S equal to $[S]/K_{CS}$, where K_{CS} is the dissociation constant of the sugar–carrier (CS) complex and, similarly, $R' = [R]/K_{CR}$, pertaining to sugar R. In a steady state, when $d[S_i]/dt = 0$, the ratio of concentrations of sugar S across the membrane is given by

$$\frac{[S_i]}{[S_o]} = \frac{K_{CR} + [R_i]}{K_{CR} + [R_o]} \quad (2)$$

The ratio expressed by Eqn. 2 can have different values (it should be borne in mind that no metabolic driving force is involved): (a) it can be equal to 1 after theoretically infinite time if neither of the sugars is metabolized (then, moreover, $[R_i] = [R_o]$); (b) it can be different from 1 at a time when countertransport of sugar S due to transport of R reverses to "downhill" transport[7]; (c) it can be different from 1 at any time in the process if R is metabolized. In this last-named case it can be readily shown that $[R_i] < [R_o]$ because

$$d[R_i]/dt = k_1 \frac{[R_o]}{[R_o] + K_{CR}} - k_2 \frac{[R_i]}{[R_i] + K_{CR}} - k_3 \frac{[R_i]}{[R_i] + K_M} \quad (3)$$

where k_1 and k_2 are the rate constants of transport into and out of the cell, respectively, and are assumed to be equal; k_3 is the metabolic rate constant, K_{CR} is the dissociation constant of the sugar–carrier complex with sugar R (here in the function of a Michaelis constant), K_m is the overall Michaelis constant of the metabolism of the sugar. Setting $d[R_i]/dt = 0$ we have

$$k_1 \frac{[R_o]}{[R_o] + K_{CR}} = k_2 \frac{[R_i]}{[R_i] + K_{CR}} + k_3 \frac{[R_i]}{[R_i] + K_m}$$

so that, unless $k_3 = 0$, $[R_i] < [R_o]$.

Now, since the two sugars are assumed to compete for the same carrier, in a state where $[R_i] < [R_o]$, less R competes in the outward direction than in the inward one and, consequently, in a steady state less S is required intracellularly to balance the entry of S_o so that $[S_i] < [S_o]$.

It is readily seen from Eqn. 2 that K_{CR} can be determined if all the four concentrations are known, thus

$$K_{CR} = \frac{[S_i]\,[R_o] - [S_o]\,[R_i]}{[S_o] - [S_i]} \quad (4)$$

This calculation has the advantage of not being affected by the dissociation constant of the carrier complex with sugar S and hence can be used for the independent determination of K_{CR} even under conditions when K_{CS} may itself be changed.

It is shown in the APPENDIX that even in the non-simplified carrier theory the K_{CS} and the mobility of the carrier with sugar S (D_{CS}) across the membrane play no role in the calculation. Consequently, the different non-metabolizable sugars used in this experimental approach (all of them sharing the glucose carrier) should yield identical results.

Nevertheless, it should be noted that the K_{CR} determined by this procedure actually includes the mobilities of the loaded and the free carrier and it will be designated K'_{CR} subsequently.

METHODS

Yeast. Fresh baker's yeast from the Kolín (Czechoslovakia) distillery was used here as it was found to contain appreciable amounts of intracellular free glucose under all conditions even in the resting state. The yeast was examined at the Distillery Research Institute in Prague and found to contain only negligible contamination with other yeast species or with bacteria. In its qualitative sugar-transport properties it was identical with the collection strain *Saccharomyces cerevisiae* R XII. The cells were washed in distilled water, aerated for 3 h at room temperature and again washed.

Incubation was carried out in a Dubnoff incubator at 30°, using mostly a 1% yeast suspension in distilled water. After adding a mixture of D-glucose and of a non-metabolized sugar to final concentrations of 0.03–0.06 M, samples were withdrawn after 10, 20 and 30 min; 0.6 ml of suspension was filtered through a membrane filter of pore diameter 0.3–0.5 μ, followed by two washings with 0.5 ml of ice-cold water, and 0.6 ml was rapidly centrifuged. The filter with cells was boiled for 20 min in 2 ml distilled water, deproteinized with $ZnSO_4$ and $Ba(OH)_2$ and centrifuged, the supernatant being used for sugar estimation. An aliquot of the supernatant after centrifuging the suspension (*i.e.* the external medium) was also deproteinized and suitably diluted before estimation of the sugar concentrations.

Estimation of sugars. Pentoses were estimated with orcinol according to MEIJBAUM[9], glucose by means of the glucose oxidase reagent with *o*-dianisidine, adding concentrated sulphuric acid to a final concentration of 6 M, the sensitivity of this modification equalling that of the reducing-sugar method. The absorbances were read at 650 nm for pentoses and at 525 nm for glucose.

Reagents. D-Xylose was a product of Fluka, Buchs, Switzerland; D-arabinose of C. Erbo, Milan, Italy; D-lyxose and D-glucose of Lachema, Czechoslovakia. The various reagents were commercial products; the glucose oxidase kit was a kind gift from Dr. W. AUGUSTIN of the Medizinische Akademie in Magdeburg, Germany.

RESULTS AND DISCUSSION

In view of the complexity of the reactions controlling the Pasteur effect in baker's yeast and of indirect indications that the actual transport of glucose may play a role[10], it was considered to be of interest to establish whether the affinity of the sugar carrier (or its membrane equivalent) for glucose is affected by aerobic conditions. It has been suggested by KLEINZELLER AND KOTYK[11] that the different metabolic patterns of glucose under aerobic and anaerobic conditions might affect the activity of the sugar carrier (*i.e.* the transport step) in addition to the complex feedback

TABLE I

APPARENT MICHAELIS CONSTANTS OF GLUCOSE TRANSPORT (K'_{CR}) IN BAKER'S YEAST UNDER VARIOUS CONDITIONS OF INCUBATION

Glucose (R) was added together with the pentose (S) and incubated at 30°. The concentration of glucose did not change appreciably during the incubation so that steady state could be assumed to exist.

Pentose used	*Gaseous phase*	*Inhibitor*	$[R_i]$	$[R_o]$	$[S_i]$	$[S_o]$	K'_{CR} *(mM)*
			(mg/ml)				
D-Xylose	Oxygen	—	1.01	8.7	1.50	4.50	15.8
			0.63	8.2	1.55	4.52	18.4
	Argon	—	0.98	8.15	1.12	4.48	6.0
			0.53	5.44	1.17	4.36	7.1
	Oxygen	$5 \cdot 10^{-4}$ M 2,4-dinitrophenol	1.62	8.15	2.70	9.80	4.9
			1.35	7.71	2.82	10.0	6.1
			1.18	6.75	2.70	9.44	5.9
D-Arabinose	Oxygen	—	0.63	7.72	1.65	4.44	19.7
	Argon	—	0.33	5.72	1.09	5.06	6.4
D-Lyxose	Oxygen	—	1.07	13.2	1.29	5.53	14.6
			1.24	11.5	1.67	5.40	18.6
	Argon	—	0.97	12.8	0.92	5.53	7.7
			1.08	10.4	0.96	5.05	6.2
Mean for aerobic conditions							17.4
Mean for anaerobic conditions							6.7
Mean for aerobic conditions with added 2,4-dinitrophenol							5.6

inhibitions now believed to take place in the regulation of glucose metabolism[12].

The results obtained with three different non-metabolizable sugars studied here are shown in Table I. The difference in the mean values for K'_{CR} under aerobic and under anaerobic conditions, as well as between aerobic conditions with and without 2,4-dinitrophenol, are highly significant ($P<0.001$); the difference between the values for anaerobic conditions and aerobic conditions with dinitrophenol is not significant. The average anaerobic value is in good agreement with the value reported by SCHARFF AND KREMER[13], *viz.* 5.3 mM, the aerobic values with and without dinitrophenol not having been reported in the paper quoted. WILKINS AND CIRILLO[3] computed the K_m for glucose uptake by baker's yeast from the competitive inhibition between D-glucose and L-sorbose and obtained a similar value both for aerobic and anaerobic conditions, *viz.* 2.8 mM. This value is lower than any found here but that can be easily due to the yeast strain used. It is not surprising, however, that the authors found the same value for the Michaelis constant for glucose uptake aerobically and anaerobically since, if the carrier is indeed affected by aerobiosis in the presence of glucose, this would be reflected in equal proportion in the uptake of glucose and sorbose and hence any change in affinity of the carrier for glucose would be concealed by a proportional change in affinity for sorbose. The advantage of the present approach lies in the fact that no kinetic constants of the non-metabolizable sugar enter into the picture.

An objection might be raised to the reliability of glucose estimation by the technique used here since glucose present intracellularly might be consumed during the period of washing[3]. We believe, however, that the danger was negligible in our experimental arrangement. The maximum rate of glucose utilization by the yeast used here was 25 mg glucose per g dry wt. per min at 30° but only 0.5 mg glucose per g

dry wt. per min at 3° which was the maximum temperature attained by the filtered pellet. The values generally found for intracellular glucose were about 2–3 mg per g dry weight and the time the cells spent from the beginning of filtration to their transfer into boiling water never exceeded 15 sec. Hence even under the most unfavourable conditions, the loss due to metabolism would amount to some 10% of the value found. Moreover, the metabolic consumption being greater anaerobically, if the $[R_i]$ values found are too low, any correction upward would only increase the difference between aerobic and anaerobic K'_{CR}.

It thus appears that one of the factors involved in the mechanism of the Pasteur effect is the altered affinity of the glucose-transporting system in the yeast although it is difficult at this stage to explain how the change is effected. What with allosteric inhibitions being demonstrated at various points of metabolic pathways (*cf.* MONOD, WYMAN AND CHANGEUX[14]) it is tempting to envisage an intermediate of glucose metabolism which is more abundant aerobically bringing about an allosteric change of the carrier, thereby reducing its affinity for sugars.

As it has been reported that phosphofructokinase activity is impaired under aerobic conditions through the joint action of ATP and citrate (SALAS *et al.*[15]) one would expect the levels of the preceding intermediates (particularly of glucose 6-phosphate) to be increased, which has once been shown to be the case in LYNEN's laboratory[16] and confirmed in this laboratory, although the level of glucose 6-phosphate apparently undergoes oscillations on switching from anaerobic to aerobic conditions*. Glucose 6-phosphate thus might be responsible for the change in affinity of the monosaccharide carrier.

For all we know, the apparent change in affinity due to aerobiosis may involve a sharp reduction of the rate of movement of the glucose-loaded carrier or, alternatively, an increase in the rate of movement of the free carrier (*cf.* the definition of K'_{CR} in the APPENDIX).

A direct effect of oxygen on the carrier appears to be excluded by the fact that 2,4-dinitrophenol aerobically reduces the K'_{CR} to an anaerobic value, thus indicating that a metabolic intermediate rather than a membrane effect of oxygen is involved in the change.

The decreased affinity of the carrier for glucose under aerobic conditions might in some cases result in an actual limitation of glucose metabolism by the transport step. This is suggested by findings on lactate-grown yeast cells[17] where it appears that intracellular conditions resembling those obtaining in aerobic glucose-grown cells persist under all circumstances. It was actually found there that both the capacity (V) and the affinity were decreased as compared with anaerobic glucose-grown cells. It has not been possible so far to demonstrate the limiting property of the transport step in the yeast directly, *i.e.* by finding higher rates of anaerobic metabolism in a cell-free extract than in intact cells. This might be caused by some of the enzymes involved being bound to cell structures in the yeast and by high requirements for the composition of the milieu.

With Ehrlich ascites cells, on the other hand, the rate of glycolysis is much higher in a cell-free extract than in intact cells[18], this emphasizing the possible role of a transport-step regulation even in other cell types.

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APPENDIX

It has been shown elsewhere[8] that it is not justified to assume equal mobilities for the free (D_C) and for the loaded (D_{CS}) sugar carrier in baker's yeast. The non-simplified equation for uptake of substrate S states that

$$v_S = 2D_{CS}C_t \frac{x_2S_o' - x_1S_i'}{x_1y_2 + x_2y_1}$$

where $2C_t$ is the total carrier concentration in the membrane and x_1, x_2, y_1 and y_2 are functions of D_C, D_{CS}, D_{CR}, S_o', S_i', R_o' and R_i'. Since we are interested in a steady state where $v_S = 0$ we can write that $x_2S_o' = x_1S_i'$ or $(D_C + D_{CS}\,S_i' + D_{CR}\,R'_i)\,S_o' = (D_C + D_{CS}S_o' + D_{CR}R_o')S_i'$. This expression can be simplified to

$$K_{CR}D_C/D_{CR} \equiv K'_{CR} = \frac{[S_i]\,[R_o] - [S_o]\,[R_i]}{[S_o] - [S_i]}$$

Hence, although the K_{CR} determined here involves the ratio of the two carrier mobilities for sugar R it is not affected by either K_{CS} or D_{CS}.

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