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On Trehalose Metabolism in Yeast

T. SAVIOJA and J. K. MIETTINEN

Biochemical Research Institute, Helsinki, Finland

At 38° to 48°C trehalose-6-phosphate is accumulated in the food yeast *Candida utilis* and in the baker's yeast *Saccharomyces cerevisiae* in aerobic as well as anaerobic conditions. At 30°C traces of this compound are found only if the cell suspension is agitated by pure O_2. Accumulation is evidently due to an irreversible enzymatic block. Trehalose phosphate was only synthetized when phosphate and sugar were present in the nutrient, but the presence of sucrose was not necessary for the accumulation of the two glycolytic intermediates, α-glycerophosphate and fructose diphosphate, which takes place in *Candida utilis* above 38°C.

Several authors have investigated trehalose metabolism in yeast. After this compound had originally been isolated from yeast "Pressaft" as the fourth sugar phosphate detected by Robinson and Morgan [1] in 1928, its metabolism was soon investigated by Veibel,[2] who in 1931 proved that it is enriched in the yeast macerate some 4 to 32 h after the beginning of fermentation but then decomposed with simultaneous increase of free trehalose. Sato and Tsumura [3] noticed synthesis of trehalose-6-phosphate in fresh baker's yeast, when sugars were fermented at 38°C under toluene. It appeared a few hours after the beginning, but disappeared before the end of fermentation, and the authors believed that the compound can only be formed during fermentation. A mechanism leading to the enzymatic synthesis of trehalose-6-phosphate was demonstrated by Leloir and Cabib [4] in the press juice of yeast. UDPG and G6P reacted irreversibly to trehalose-6-phosphate and UDP. Panek [5] studied the synthesis of trehalose in resting and growing cells of baker's yeast. She found trehalose but no trehalose phosphate in resting cells, and supposed that it is so rapidly dephosphorylated that it could not be detected. Elander [6] made a quantitative study of the changes in the products of glucose fermentation in acetone-treated cells of baker's yeast. 30 min after the onset of fermentation the concentration of trehalose phosphate was about three times as high as that of any other sugar phosphate or the free trehalose. Then the concentration of trehalose continuously increased, while that of trehalose phosphate remained about the same until it almost disappeared — like several other sugar phosphates — towards the end of fermentation (150 min). From these

results the author drew the conclusion that trehalose phosphate is an intermediate of trehalose synthesis.

We found in an earlier study [7] that trehalose-6-phosphate, together with α-glycerophosphate and fructose diphosphate, is accumulated in *Candida utilis* when assimilation of orthophosphate takes place at 38 to 45°C. In this work we have made a closer study of the conditions of this accumulation.

Experiment No. 1

Fresh baker's yeast was agitated at 30°, 38°, and 45°C with air, oxygen, and argon in a nutrient solution containing ^{32}P-labelled orthophosphate, and the soluble phosphates synthesized were studied by 2-dimensional paper chromatography and autoradiography as previously.[8] Before the experiment the yeast cells were grown for 2 h aerobically in a phosphorus-deficient medium [8] to which traces of biotin, pantotheine, and inositol were added, and agitated for 10 min in a completely phosphate-free medium at the temperature and in the atmosphere of the experiment, to "adapt" the cells to those conditions. Then 0.5 mC ^{32}P as orthophosphate and inactive carrier to make the solution 10^{-4} M were added and agitation continued for 20 min. Toluene was added to one of the cultures. The cells were then rapidly washed, extracted with cold 8 % TCA and, after removal of the TCA by ether extraction, chromatographed on paper as described earlier.[8] Other samples were taken after 1.5 h agitation.

Results of expt. No. 1.

In Table 1 is presented the distribution of radioactivity between the most radioactive compounds, which also represent the bulk of the soluble phosphate esters synthesized. As can be seen, trehalose phosphate was not synthesized at all at 30°C in normal aerobic or anaerobic conditions, but when the cells were agitated by pure oxygen, 3 % of the phosphate incorporated in the compounds isolated was found in trehalose phosphate. When the temperature was 38°C or higher, trehalose phosphate became strongly labelled in all conditions, "super" aerobic, normal aerobic and anaerobic. The metabolism of the cells, noticed as consumption of sodium hydroxide for maintaining pH 4.8, was very high when the yeast was agitated by O_2 at 30°C, and it is possible that trehalose phosphate mainly represents a storage of energy, phosphate, and glucose.

Experiment No. 2

The experiment was carried out with phosphorus-deficient cells of *Candida utilis*, using 9 ml samples of a 10 % suspension in a phosphate-free nutrient.

The cells were aerated at 45° and 48°C for 10 min, after which temperature was lowered to 30°C. Into both samples and a control that had been maintained all the time at 30°C, 0.1 mC ^{32}P as orthophosphate and enough carrier to make the solution 10^{-3} were added. The assimilation time was 10 min, after which the samples were analysed as in expt. 1.

Results of experiment No. 2

The results of this experiment are well illustrated by figures on the incorporation of radioactive phosphate into the soluble and non-soluble phosphates of the yeast, presented in Table 2. At 45°C uptake of orthophosphate was still

Table 1. Distribution of radioactivity between the acid-soluble compounds having the highest activity, as percentages of the total counted.

Sample	R5P	UMP	UDP	GMP	TreP	CDPCHO	UDPAG+	UDPG+ UDPGA	AMP— area *	ADP	ATP	IMP
20 min samples												
30°C air	12.8	3.2	—**	—	—	—	16.0	—	31.5	16.6	20.1	—
30°C oxygen	16.2	11.2	—	—	3.1	3.1	6.6	—	42.7	5.3	15.1	—
38°C air	4.6	1.6	—	—	2.3	—	35.3	—	24.8	10.6	20.9	—
38°C oxygen	9.6	1.3	—	—	3.7	—	9.2	—	34.5	17.2	24.6	—
45°C air	3.8	3.3	—	—	17.6	—	3.6		36.6	6.6	28.9	—
45°C oxygen	7.2	—	—	—	26.1	—	2.0	—	50.6	4.1	10.0	—
1 1/2 h samples												
38°C air	—	6.4	5.2	4.3	0.74	2.4	18.0	12.3	22.0	14.8	14.1	0.9
38°C argon	—	5.4	8.2	4.1	0.89	2.6	17.3	17.8	15.0	12.4	13.1	2.3

* = AMP+G1P+G6P

** = not found on radioautogram

Abbreviations: R5P = ribose-5-phosphate, UMP and UDP = uridine-5′-mono- and diphosphate, UDPAG = uridine diphosphate acetyl glucosamine, UDPG = uridine diphosphate glucose, UDPGA = uridine diphosphate glucuronic acid, GMP = guanosine monophosphate, TreP = trehalose phosphate, CDPCHO = cytidine diphosphate choline, AMP, ADP, and ATP = adenosine mono-, di- and triphosphate and IMP = inosine monophosphate.

Table 2. Non-reversibility of the thermo-inhibition of some phosphate-converting enzymes. The percentage of $^{32}PO_4$ assimilated and the distribution of activity between the TCA-soluble and non-soluble phosphates.

Sample No.	Temperature treatment	Temperature assimilation	Assimilated, per cent of given	TCA-soluble, per cent of assimilated	TCA-non-soluble, per cent of assimilated
1	30°C	30°C	99.5	73.2	26.8
2	45°C	45°C	99.6	84.0	16.0
3	45°C	30°C	99.8	80.3	19.7
4	48°C	48°C	73.0	92.8	7.2
5	48°C	30°C	61.1	91.0	9.0

good, but incorporation into insoluble phosphates was already diminished to about half the normal. A little more was incorporated at 30°C after aeration at 45°C, but much less than normally (sample 1). At 48° even uptake was inhibited and this inhibition was practically irreversible. The same thing was visibly evident from the paper chromatograms — after treatment at 45 to 48°C trehalose was accumulated even when the labelled phosphate was given at 30°C. The enzymatic block is irreversible.

Experiment No. 3

Two batches of phosphorus-deficient *Candida utilis* cells were aerated at 45°C, one in phosphate-free, the other in phosphate- and sucrose-free nutrient solution for 10 min. At the beginning 0.1 mC ^{32}P as orthophosphate and enough carrier to make the solution 10^{-3} M were added to 9 ml of 10 % cell suspension.

Radio-paperchromatograms of the TCA extracts showed that trehalose-6-phosphate became labelled only when there was sucrose in the nutrient medium. α-Glycerophosphate and fructose diphosphate became heavily labelled in both cases. This proves that glycolysis took place even in absence of exogenous sucrose, but not trehalose synthesis.

The distribution of radioactivity is presented in Table 3. As can be seen, in both cases assimilation was nearly quantitative, but in the absence of sucrose, incorporation of phosphate into the insoluble fraction was greater.

Table 3. Assimilation of $^{32}PO_4$ in the absence and presence of sucrose in the nutrient medium at 45°C.

Sample	Assimilated, per cent of given	TCA-soluble per cent of assimilated	TCA-non-soluble per cent of assimilated
without sucrose	99.5	80.9	19.1
with sucrose	99.8	91.5	8.5

A considerable part of this was evidently polymetaphosphate, which is well known to accumulate in phosphorus-deficient yeast, especially in the absence of carbohydrate. When sucrose was given, part of this phosphate was found as trehalose phosphate, instead.

DISCUSSION

We believe that the present isotope experiments have clarified many points in the synthesis of trehalose phosphate. The compound is formed in both yeasts studied at temperatures above 38°C independently of the presence or absence of oxygen. Its accumulation evidently represents a heat-resistant enzymatic side-track which comes into use when the main route is blocked. Agitation by pure oxygen causes some inhibition and accumulation even at the optimum temperature (30°C). Trehalose phosphate is evidently synthesized from exogenous phosphate and carbohydrate and represents a convenient auxiliary means of storing energy, phosphate, and glucose, all in one small-molecular compound. Otherwise its role remains obscure.

α-Glycerophosphate and fructose diphosphate also accumulate at temperatures above 38°C, especially in the *Candida utilis* yeast, but their accumulation does not depend on the presence of exogenous sucrose. As suggested earlier,[7] their accumulation evidently results from heat-inhibition of triose phosphate dehydrogenase and aldolase, respectively. Another possibility is the inhibition of the respiratory processes, which normally produce the acceptors. Accumulation of these glycolytic intermediates seems not to have any direct connection with that of trehalose phosphate, although the two processes take place simultaneously.

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REFERENCES

1. Robison, R. and Morgan, W. J. T. *Biochem. J.* **22** (1928) 1277.
2. Veibel, S. *Biochem. Z.* **239** (1931) 350.
3. Sato, T. and Tsumura, N. J. *Agr. Chem. Soc. Japan* **28** (1954) 624.
4. Leloir, L. F. and Cabib, E. *J. Am. Chem. Soc.* **75** (1953) 5445.
5. Panek, A. *Arch. Biochem. Biophys.* **98** (1962) 349.
6. Elander, M. *Arkiv Kemi* **21** (1963) 317.
7. Savioja, T. and Miettinen, J. K. *Acta Chem. Scand.* **20** (1966) 2444.
8. Savioja, T. and Miettinen, J. K. *Acta Chem. Scand.* **20** (1966) 2435.

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