ACCUMULATION OF INOSINE IN YEAST TREATED WITH 2-DEOXY GLUCOSE

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2-Deoxy-D-glucose (2-dGl) is a potent inhibitor of cell growth and fermentation in yeast (Cramer and Woodward, 1952; Woodward et al., 1953; Kiesow, 1960, 1961). Serving as a substrate to hexokinase (Woodward and Hudson, 1955; Sols et al., 1958), the 2-dGl-6-P produced is an inhibitor of phosphoglucoisomerase (Wick et al., 1957) and is also a substrate for the G-6-P dehydrogenase (Barban and Schulze, 1961; Eichhorn , 1964).

In a recent study, Heredia et al. (1964) have concluded that the severe effect of 2-dGl on yeast must be much more complicated than just an inhibition of glycolysis by the formation of 2-dGl-6-P. Interference with polysaccharide biosynthesis may occur in view of the formation of UDP-2-deoxyhexoses in yeast incubated with 2-d-D-galactose (de Robichon-Szulmajster, 1961; Fischer and Weidemann, 1964).

Experiments reported in this communication show that the most striking change in the free nucleotide pool of yeast cells incubated with 2-dGl is the appearance of relatively large amounts of inosine.

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* Experiments described in this work are taken from a Ph. D. Thesis to be submitted to the Hebrew University.

EXPERIMENTAL AND RESULTS

Saccharomyces cerevisiae strain RASE XII was grown on a circulatory shaker for 22 hours at 34°, in a medium consisting of KH_2PO_4, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.05%; $(NH_4)_2SO_4$, 0.2%; yeast extract (Difco), 0.1%; glucose, 1.5% and biotin 0.1 μg/ml. Cells were washed thrice in cold sterile water and then suspended in a nitrogen free sterile solution of KH_2PO_4, 2 mM; $MgSO_4$, 7 mM, Na_2SO_4, 14 mM and biotin 0.1 μg/ml with or without glucose and 2-dGl. The suspensions containing about 2 mg dry cells/ml were incubated for 6 hours with shaking at 34°. Cells were collected by centrifugation, washed with water, extracted twice by 73% ethanol (v/v) at 80° for 10 minutes and then recentrifuged. The ethanolic supernatants were evaporated to dryness in vacuum. The dry residue was dissolved in 20% ethanol (v/v) and extracted with ether to remove fatty materials. 2 volumes of petroleum ether were added to the etheral supernatant and the small amount of aqueous phase which separated was combined with the first solution and reduced in volume by lyophilization.

Constituents of the extracts obtained were separated quantitatively by chromatography on Whatman No. 1 filter paper using ethanol-1 M ammonium acetate (6:4 v/v) pH 7.5 as the solvent. The UV absorbing spots were cut out, washed with absolute ethanol then extracted with water. The isolated components were characterized for their spectra and analyzed by standard chemical and enzymatic procedures (Table 1). Samples were rechecked in comparison with authentic samples by thin layer chromatography on cellulose-D (Camag AG, Muttenz, Switzerland) using ethanol-1 M ammonium acetate, (7:3 v/v) pH 7.5 as the solvent.

Table 1

Identification of nucleoside and nucleotide components in extracts of yeast

Component	R_f [a]	Spectral type [b]	Other reactions [c]	Identification
1	0.18	Adenosine	Hexokinase system	ATP
2	0.26	Adenosine	Pyruvate kinase and hexokinase system	ADP
3	0.32	Adenosine	Adenylic acid deaminase	AMP
4	0.40	Uridine		UMP
5	0.46	Uridine	Glucose by chromatography and by glucose oxidase after hydrolysis at pH 2.0	UDPGl
6	0.55	Uridine		UDP-X [d]
7	0.72	Inosine	Hydrolysis and xanthine oxidase	Inosine [e]
8	0.71	Adenosine		Adenosine
9	0.85	Uridine		Uridine

a) In ethanol: 1 M ammonium acetate (6:4 by vol.) pH 7.5 on Whatman No. 1 filter paper.
b) Spectra at pH 2, 7 and 11 were taken in a recording spectrophotometer and compared with standard curves (Pabst Circular OR 10).
c) All components were analyzed in comparison with authentic samples, colorimetrically for total phosphate, for ribose in the orcinol reaction, for total sugar in the phenol-H_2SO_4 reaction and by chromatographic migration as described in the text.
d) Preliminary identification showed that at least part of this sugar nucleotide fraction is UDP-N-acetylglucosamine.
e) After prolonged incubation with 2-dGl, the inosine spot was found to be accompanied by hypoxanthine. The two were separated by paper chromatography with n-propanol:ethylacetate:water (7:1:2 by vol.) and the hypoxanthine directly measured by xanthine oxidase.

In the experiments reported, batches of about 1 g dry cells were analyzed in each case. 9-10 nucleotides could be detected in the extracts (Table 1), 0.1 μmole nucleotide/g dry cells was the limit of

Table 2

Nucleosides and nucleotides in cell extracts

Numbers indicate μmole/g dry weight

Nucleotide	Washed cells	After 6 hours incubation with					
		Buffer	33 mM glucose	33 mM glucose + 0.1 M 2-dGl	5 mM glucose + 33 mM 2-dGl	33 mM 2-dGl[a]	0.1 M 2-dGl[a]
ATP	1.35	0.75	1.70	1.20	0.25	0.22	0.20
ADP	0.15	0.30	0.20	1.00	0.35	0.31	0.42
AMP	1.65	1.25	0.70	0.40	0.77	0.68	0.57
UMP	0.75		0.96	0.47	0.32	0.40	0.52
UDPG	0.53	1.45	0.54	0.48	0.40	0.20	0.18
UDPX	0.54	1.15	0	0.25	0.46	0.54	0.51
Adenosine	0.37[b]	0	0.20	0	0	0	0
Inosine	0	0.41	0	3.00	5.70	1.80[c]	1.64[c]
Uridine + uracil	0.42	0.60	0.60	0.60	0.35	0.40	0.35
Total	5.76	5.91	4.90	7.40	8.60	4.55	4.39
Total purines	3.52	2.71	2.80	5.60	7.07	3.01	2.83

a) Many commercial preparations of 2-dGl were found to be highly contaminated by glucose. In this case a pure sample was obtained from Calbiochem, Los Angeles.

b) Freshly washed cells sometimes contained inosine (0.3-0.5 μmoles/g) but no adenosine. This conversion presumably depends on washing time, age of cells and other undetermined physiological factors.

c) In several comparable experiments, 50-60% of the inosine spot isolated was identified as hypoxanthine. This was not observed in systems incubated with glucose.

detection by the methods employed. It is evident that 2-dGl by itself caused a marked increase (to about 60% of the total purines) in the inosine content with a concomitant drop in the level of adenine nucleotides (Table 2). In the presence of both 2-dGl and glucose, the amount of inosine accumulated as well as total purine content was even larger.

To test for leakage of nucleotides, the external media obtained after incubation with 2-dGl were reduced in volume by lyophilization, treated with Norit A-Celite 535 (1:1 parts by weight) at pH 2, washed with large volumes of 1 mM HCl and then eluted with 1% NH_4OH in 50% ethanol. Examination of the concentrates by spectrophotometry, paper and thin layer chromatography failed to reveal any significant amounts of nucleotides, indicative to the negligibility or absence of their leakage during the experimental period.

DISCUSSION

Wu and Racker (1959) have shown that the inhibition of glycolysis in ascites cells by iodoacetate brings about a decrease in the adenine nucleotide pool in the cells. These authors also noticed that inosine, probably the product of adenine nucleotides catabolism, leaked from such treated cells. Recently it was observed that 2-dGl also causes a marked reduction in the level of ribonucleotides in various mammalian cells (Ibsen et al., 1962; Klenow, 1963; Letnansky, 1964; Urbahn et al., 1964).

In contrast to these observations, the ribonucleotide level in yeast treated by 2-dGl does not decline and inosine is accumulated within the cells. The supply of external glucose in addition to 2-dGl

usually augmented inosine accumulation. This effect requires further study since it might have been expected that an internal supply of glucose from the reserve carbohydrates (trehalose and glycogen) could have been provided. Whether the increase in total purines under these conditions is due to a de novo synthesis, is to be determined.

It can be assumed that inosine is formed by the deamination of adenosine, as no IMP or IDP were detected in the extracts studied. The inhibition of ATP regeneration in the cell due to the 2-dGl-6-P trap, presumably brings about an extensive degradation of ADP to adenosine which is then deaminated to inosine. Preliminary evidence indicates that hypoxanthine also accumulates in the yeast when incubation time with 2-dGl is prolonged. Hypoxanthine probably arises from a further degradation of inosine.

The accumulation of inosine, in addition to that of 2-dGl-6-P may be one of the causes to the 'lethal effect' of 2-dGl in yeast (Heredia et al., 1964). The kinetics and the relationship of this phenomenon to endogenous carbohydrate metabolism and to cell viability, is currently being studied in our laboratory.

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