UPTAKE OF 2,4-DINITROPHENOL BY ANAEROBIC YEAST CELLS AND ITS RELATION TO THE ENERGY TRANSDUCTION IN THESE CELLS

G. W. F. H. BORST-PAUWELS

Laboratory of Chemical Cytology, Driehuizerweg 200, Nijmegen, The Netherlands

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The well known uncoupling agent of oxidative phosphorylation 2,4-dinitrophenol (DNP) also inhibits several energy requiring processes in anaerobic yeast without affecting glycolysis much [1-3]. Probably DNP and also azide interferes with the formation of an unknown high-energy compound formed under anaerobic conditions. The inhibition of phosphate uptake by DNP appeared to be accompanied by an increase of f which is the quotient of cellular and medium DNP concentration [3]. Jarett and Hendler [2] also showed that the accumulation of DNP increases more than proportional with the concentration of this inhibitor. Apparently an increase of the absorption capacity of the yeast for DNP occurs under the conditions that the energy transduction is blocked. Therefore, a study of the accumulation of DNP by yeast may be useful in elucidating the mechanism by which this inhibitor interferes with the energy transduction in anaerobic yeast.

Accumulation of ^{14}C labelled DNP (Philips Duphar, Pette, The Netherlands) by 1% w/v *Saccharomyces cerevisiae Delft 2*, in a 0.1 M sodium succinate buffer [3] at pH 4.5 and 25° for a 6 minutes period was investigated by measuring the decrease in radioactivity of the medium by means of liquid scintillation. The scintillation liquid consisted of 4 l toluene, 1 l nonidet P.80, 25 g PPO, and 1.5 g dimethyl-POPOP. To 10 ml scintillation liquid 0.5 ml samples were added together with two drops of 1 N $HClO_4$. The time of incubation suffices for complete entry of DNP [4]. The accumulation ratio of DNP increases strongly on increasing the DNP concentration from 10^{-6} M to about 4×10^{-5} M (fig. 1). In this range the inhibition of phosphate uptake is roughly proportional to the increase of f. Decreases of f observed at the higher concentrations of DNP are mainly due to a limited adsorption capacity of the yeast as will be shown below, and do not reflect the capacity of the yeast to perform chemical or osmotic work. Cells poi-

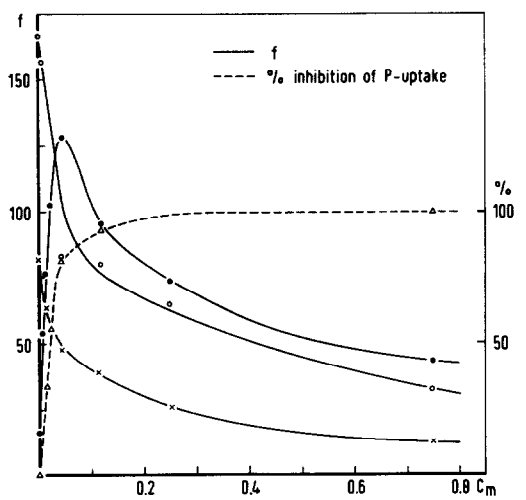

Fig. 1. The dependence of the ratio (f) of the concentration of 2,4-dinitrophenol in the yeast cell water and the final concentration of this compound in the medium upon the DNP concentration in the medium (C_m) in mM observed under different metabolic conditions. Comparison with the inhibition of phosphate uptake expressed in percents of total inhibition [3]. x resting cells; • yeast pre-incubated for 1 hr with 3% glucose; ○ yeast pre-incubated with 3% glucose for one hour and then with 3 mM monoiodoacetate for 6 min. The monoiodoacetate was not removed during the subsequent incubation with DNP for an additional period of 6 min.

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Table 1
Comparison of experimental and calculated values of the accumulation ratios of 2,4-dinitrophenol

	C_{DNP} (10^{-6}M)	cell pH	f	f	f_{calc}	f_{ads}	f'_{calc}
Resting cells	1	5.54 ± 0.07 (5)	94 ± 5 (9)		8	4.20	42
Metabolizing cells	1	5.89 ± 0.03 (14)	27 ± 4 (14)		19	3.75	92
Metabolizing cells	44	5.98 ± 0.07 (5)	124 ± 3 (3)		24	3.57	110
Cells poisoned with MIA	1	6.15 ± 0.05 (6)	179 ± 7 (2)		35	3.65	162

f = experimental DNP accumulation ratios; f_{calc} = accumulation ratios calculated according to ref. [5] from the differences in cell pH and medium pH; f_{ads} = quotient of bound and free cellular DNP calculated from the data of fig. 2; $f'_{calc} = f_{calc}(1+f_{ads})$ = correction of calculated accumulation ratios for binding of DNP to yeast cell macromolecules, The figures in parentheses denote the number of determinations.

soned with monoiodoacetate (MIA) or resting cells both of which hardly take up phosphate, show the highest accumulation ratio at very low DNP concentrations. On increasing DNP concentrations f gradually becomes smaller. Here again a parallel is shown between accumulation capacity of the yeast for DNP at relatively low concentrations of this compound and inhibition of phosphate uptake. The uptake isotherm for DNP is S-shaped in the case of metabolizing cells (fig. 2).

DNP only moves across the cell membrane in its undissociated form [5]. Therefore, accumulation of DNP will occur when the cell pH is higher than the medium pH, because of the greater dissociation of the acid in the cell. We have examined whether the differences in DNP accumulation ratios could be due to changes in cell pH. Cell pH's are determined by freezing and thawing [6]. The experimental accumulation ratios for cells poisoned with MIA or DNP or the ratios observed in the case of resting cells appeared to be much larger than those calculated (table 1). This points to adsorption of DNP to yeast cell compounds occurring under such conditions so that phosphate transport is blocked.

Direct proof that yeast can adsorb DNP came from the following experiment: Acetone powders of yeast washed free from small solutes are suspended in 0.5 molal NaCl provided with different amounts of DNP at pH 6.3. The Donnan potential between cells and medium did not contribute much to the uptake of DNP because of the high NaCl concentration applied. A large adsorption of DNP occurred (fig. 3). This adsorption appeared to increase when the pH of the medium is lowered. Presumably proteins are involved in the binding of DNP [7-9]. Correction of the calculated accumulation ratios for binding of DNP to cell proteins gives rise to values of the order of magnitude of those

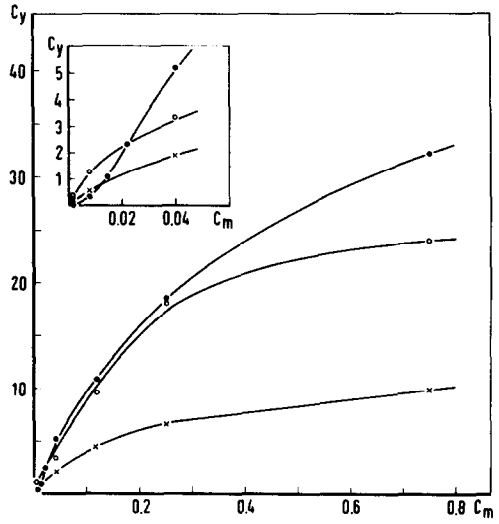

Fig. 2. Uptake of 2,4-dinitrophenol in the yeast as a function of the DNP concentration in the medium observed under different metabolic conditions. Same data as in fig. 1. Inset: enlarged part. Both the concentration of DNP in the yeast cell water C_y and the concentration of DNP in the medium C_m are expressed in mM.

observed experimentally in the case of inhibition of the energy transduction by either DNP or MIA. The uptake of DNP by resting cells, however, is about two times larger than expected. Probably, also small solutes are involved in the binding of DNP by resting cells.

Our results may support the hypothesis already proposed by Weinbach and Garbus for DNP binding to mitochondria, i.c. that DNP causes configurational changes in cell macromolecules [10]. Changes in pro-

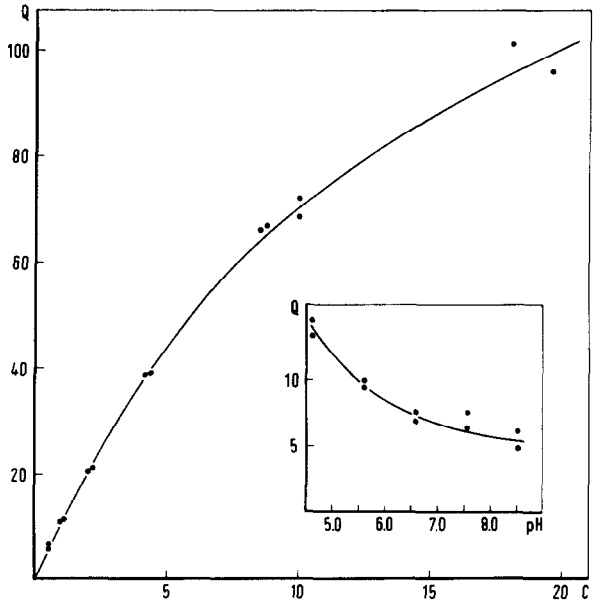

Fig. 3. Adsorption isotherm of 2,4-dinitrophenol observed with acetone powders of yeast at pH 6.3 in 0.5 molal NaCl. *Inset: the pH dependence of the adsorption of 0.5 mM dinitrophenol.* Adsorption of DNP (Q) is expressed in mmoles per kg dry weight of acetone powders. The line drawn is the calculated line for a Langmuir isotherm with a maximum adsorbed amount of 175 mmoles DNP per kg and a half value concentration of 15 mM. C is the concentration of DNP in the medium expressed in mM.

tein conformation induced by an effector generally give rise to an increased adsorption of the effector which will result in an S-shaped uptake isotherm as is found in fact by us. It is, however, difficult to visualize how such a large part of the cell proteins representing an adsorption capacity of 175 mmoles per kg of dry weight of acetone powders can be involved in conformation changes. Another possibility is that DNP is extruded actively from the cells after its permeation in the cell, and that this pump is inhibited under the same conditions as under which phosphate uptake is stopped. Until now we are not able to distinguish experimentally between these two possibilities.

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