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THE EFFECT OF MONOFLUOROACETIC ACID UPON THE CARBOHYDRATE METABOLISM OF OF *SACCHAROMYCES CEREVISIAE*

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Abstract—The effect of monofluoroacetic acid (HFA) upon the respiration rate of ethanol, acetaldehyde and lactate in resting cell suspensions of yeast cells has been investigated. Comparison with similar investigations already reported with glucose and with acetate shows that whilst the respiration rate with acetaldehyde is similarly effected by monofluoroacetate as is acetate, the respiration rate with ethanol and lactate behaves more akin to the respiration rate with glucose. Thus respiration with acetaldehyde as exogenous substrate is greatly inhibited at both pH 2·2 and pH 6·8 (~ 85 per cent) whilst the respiration rate with ethanol or lactate is greatly inhibited (66 and 91 per cent respectively) at pH 2·2 but much less inhibited at pH 6·8 (29 and 64 per cent respectively). Measurements of the extent of respiration achieved by glucose and ethanol show that, despite the differences in rate of respiration at pH 2·2 and pH 6·8, the exogenous substrate is oxidized to a similar extent at both pH values. It is argued that internal citrate oxidation is inhibited to the same extent at both pH values and that the differential effects upon respiration, glucose uptake and ethanol production previously reported are consequent upon the primary block in metabolism. Measurement of citrate levels in monofluoroacetate treated cells oxidizing glucose and acetate reaffirm the conclusion and mechanisms to explain the differential effect are discussed.

IN AN earlier paper[1] it was reported that treatment of resting cell suspensions of *Saccharomyces cerevisiae* with monofluoroacetic acid had similar effects upon glucose uptake, ethanol production and polysaccharide synthesis to those reported by Aldous and Rozee[2,3] upon the respiration of such cells when glucose was the exogenous substrate. Thus a much greater degree of inhibition was found in these parameters when cells were suspended at pH 2·2 after a preincubation with monofluoroacetic acid than when similar cells were resuspended at pH 6·8. It was argued that the prime effect of the monofluoroacetic acid in these cells was a consequence of processes leading to inhibition of glucose uptake at pH 2·2 and that an alternative metabolic route operative at pH 6·8, but not at pH 2·2, allowed glucose uptake to continue at the more neutral pH. This report is concerned with a comparison of the behaviour of various substrates subsequent to treatment of the cells by monofluorozcetic acid, in an attempt to localise and define the nature of this alternative metabolic route.

METHODS

The methods employed in this investigation have been described previously.[1] In brief, washed cells of bakers yeast (D.C.L.) were suspended in 0·1 M HCl/KCl

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buffer at pH 2·2[4] and to aliquots of these was added sufficient sodium monofluoroacetate to achieve a final concentration of 5 mM. After 30 min incubation at 25° the cells were centrifuged at 18,000 *g* for 15 min, the supernatant discarded and the cells were washed twice with the HCl/KCl buffer at pH 2·2.

For respiration studies these cells, in parallel with untreated control cells, were made up to 25 mg wet weight/ml in either HCl/KCl buffer at pH 2·2 or in double phosphate buffer[5] at pH 6·8. The oxygen uptake of the cells was then measured with the substrates indicated at a final concentration of 60 mM for a minimum of 2 hr.

Citrate determinations were carried out on such cells at the end of these respiration studies by stopping the reaction with 0·5 M trichloroacetic acid, extracting overnight, centrifuging off the deproteinised material and estimating the citrate level in the supernatant by the method of Taylor.[6]

In experiments to determine the extent of respiration substrate additions were limited to 7·5 μmole of glucose or 15 μmole of ethanol to allow completion of oxidation in a reasonable time. This lower substrate concentration showed the same degree of inhibition of respiratory rate as that shown in experiments using substrates at 60 mM.

RESULTS

(a) *The effect of monofluoroacetic acid (HFA) upon ethanol, acetaldehyde and lactate respiration*

In an earlier report[1] it was argued that the clearly established allosteric inhibition of yeast phosphofructokinase (E.C.2.7.1.11. ATP:D fructose-6-phosphate 1-phosphotransferase) by citrate[7] might be expected to cause an accumulation of hexose monophosphates in HFA treated cells when they were subsequently exposed to glucose. If such an accumulation occurred then inhibition of glucose uptake by product inhibition of hexokinase (EC. 2.7.1.1. ATP:D hexose-6-phosphate 6-phosphotransferase)[8] could be expected. This situation it was argued could arise at pH 2·2, but at pH 6·8, because glucose uptake was not so markedly inhibited, an alternative oxidative mechanism leading to the removal of hexose phosphates must exist. The hexose monophosphate shunt system was considered to be such a possible mechanism since the enzymes of this sequence are known to be present in yeast.[9–11]

If this hypothesis is correct, substrates such as ethanol, acetaldehyde and lactate, which are not oxidized via phosphofructokinase, would not be expected to show a differential inhibition but rather should behave like acetate[3] in that their respiration would be highly inhibited at both pH 2·2 and pH 6·8. The results of experiments using these substrates, together with the results obtained with glucose and acetate for comparison, are shown in Table 1.

It can be seen from this that acetaldehyde behaves like acetate in that the inhibition of respiration is high at both pH values and that there is no significant difference in the degree of inhibition at pH 2·2 and pH 6·8 ($P > 0{\cdot}1$). However ethanol and lactate show lower degrees of inhibition at pH 6·8 than pH 2·2 ($P < 0{\cdot}001$).

Thus the behaviour of acetaldehyde is consistent with the possible operation of the hexose monophosphate shunt at pH 6·8 as the alternative oxidative metabolic route but the results with ethanol and lactate certainly are not. Ethanol is reported to be oxidized via acetaldehyde and acetate in yeast[12,15] and lactate via pyruvate and acetyl CoA.[18] It seems difficult in the light of these latter results to maintain that the cause

TABLE 1. THE EFFECT OF MONOFLUOROACETIC ACID UPON THE RESPIRATION OF SUBSTRATES

Substrate	C 2·2* Rate†	S.E.M.	H 2·2* Rate†	S.E.M.	% Inhibition	C 6·8* Rate†	S.E.M.	H 6·8* Rate†	S.E.M.	% Inhibition
Glucose n = 12	920	45·5	395	26·0	57	963	9·6	812	11·4	16
Ethanol n = 3	838	41·0	285	23·3	66	1182	40·2	839	27·5	29
Lactate n = 3	817	16·8	76	2·2	91	815	29·4	289	16·6	64
Acetate n = 3	846	108·6	35	2·4	96	1112	6·1	60	1·8	95
Acetaldehyde n = 3	732	47·7	96	5·0	86	875	31·6	172	10·0	82

* C 2·2 = control at pH 2·2
H 2·2 = HFA treated cells at pH 2·2 etc.
† Rate = average values in μl/min/g dry wt. cells.
Comparison of respiratory inhibition at pH 2·2 v. pH 6·8:
glucose—$P < 0{\cdot}001$; ethanol—$P < 0{\cdot}001$; lactate—$P < 0{\cdot}001$; acetate—$P > 0{\cdot}1$; acetaldehyde—$P > 0{\cdot}1$.

of the lower respiratory inhibition of glucose at pH 6·8 is due to the operation of the hexose monophosphate shunt at this pH.

(b) *The effect of monofluoroacetic acid on the extent of oxidation of glucose and of ethanol.*

In a recent report Williamson[13] has argued for the reversibility of the inhibition of aconitase by monofluorocitrate, as first reported by Morrison and Peters,[14] The extent of respiratory inhibition, induced in perfused rat heart by monofluoroacetate treatment, could be reduced by feeding a citrate precursor (pyruvate). This reversal of inhibition was correlated with a large increase in citrate level.

Examination of citrate levels in these yeast cells showed that monofluoroacetic acid, whilst causing an elevation of citrate levels in the cells on subsequent exposure to glucose at both pH values, caused a considerably greater elevation at pH 6·8 (Table 2).

TABLE 2. THE EFFECT OF MONOFLUOROACETIC ACID TREATMENT ON CITRATE LEVELS IN CELLS RESPIRING GLUCOSE OR ACETATE

Substrate	C 2·2 μmole/g dry wt.	S.E.M.	H 2.2 μmole/g dry wt.	S.E.M.	% Increase	C 6·8 μmole/g dry wt.	S.E.M.	H 6·8 μmole/g dry wt.	S.E.M.	% Increase
Glucose (n = 12)	14·9	4·9	42·0	3·1	182	12·0	1·2	82·7	7·4	506
Acetate (n = 3)	13·3	2·6	42·6	0·9	221	18·2	2·3	76·2	5·6	465

It could be argued from these results with glucose that the greater synthesis of citrate that occurs at pH 6·8 is sufficient to allow reversal of the inhibition of citrate oxidation and that this alone is sufficient to explain the lower degree of inhibition of glucose respiration at this pH. However the results given for citrate accumulation with acetate as the exogenous substrate (Table 2) are difficult to equate with this interpretation. In this case where the differential effect on respiratory rate is absent, the increase in citrate level is very similar at both pH values to that shown with glucose. If there exists in the yeast cell a single mechanism for the oxidation of citrate

then it is difficult to see how a level of citrate produced from glucose could reverse a n inhibition in its oxidative degradation and yet a similar level caused by acetate metabolism be unable to do so. On similar grounds to this, Aldous[19] proposed two diverging mechanisms for citrate oxidation, one of which was blocked by monofluoroacetic acid and was the only one available in an acid environment, the other being unaffected by the inhibitor and was available only in an environment above pH 4·5.

A consequence of such an hypothesis would be that not only should the respiratory rate with glucose and ethanol be relatively unaffected at pH 6·8, but also the extent to which the substrates are oxidized in monofluoroacetic acid treated cells should be greater at this pH than at pH 2·2. The results of experiments to examine this are shown for glucose in Fig. 1 and ethanol in Fig. 2. It can be seen from these that although the respiration rate with both substrates is more greatly inhibited at pH 2·2 than pH 6·8, the monofluoroacetic acid treated cells eventually oxidise the substrates to exactly the same level at both pH values. It seems unlikely then that the presence of an alternative oxidative mechanism for internally produced citrate, available only at pH 6·8, is an explanation of the differential effects on the respiratory rates shown by these substrates.

Closer examination of the results shown in Figs. 1 and 2 indicates that at both pH values, glucose and ethanol are being oxidized to a similar extent in treated cells and that this extent is equivalent to their oxidation to the level of acetate. Thus in the case of glucose 7·5 μmole of substrate consumed 339 μl of oxygen at pH 2·2 and 320 μl at

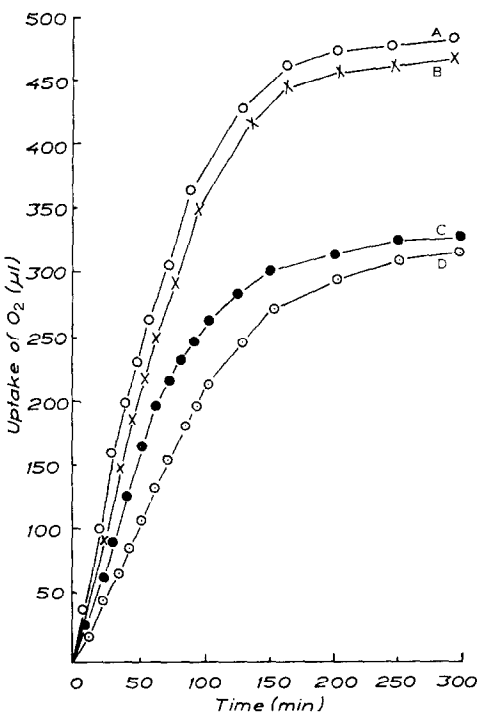

FIG. 1. The of 5 mM Fluoroacetate on the extent of oxidation of 7·5 μmole. Glucose.
A = Control cells at pH 2·2
B = Control cells at pH 6·8.
C = Fluoroacetate treated cells at pH 6·8.
D = Fluoroacetate treated cells at pH 2·2.

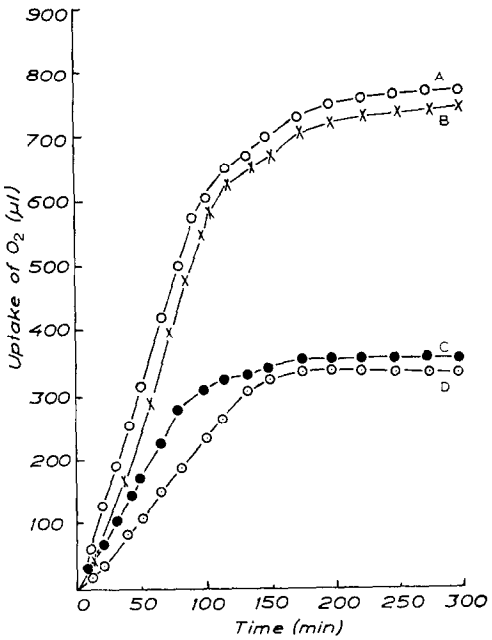

FIG. 2. The effect of 5 mM Fluoroacetate on the extent of oxidation of 15 μmole. Ethanol.
A = Control cells at pH 2·2
B = Control cells at pH 6·8
C = Fluoroacetate treated cells at pH 6·8
D = Fluoroacetate treated cells at pH 2·2

pH 6·8. This is equivalent to 45 μl oxygen/uM glucose and 43 μl oxygen/μm glucose respectively and represents 4·0 and 3·8 μg atom of oxygen/μM glucose. Oxidation to the level of acetate would require 4·0 μg atom of oxygen/μM glucose. In the case of ethanol the oxygen uptake is equivalent to 2·0 μg atom of oxygen/μM ethanol at both pH values and this is the uptake expected in the oxidation of ethanol to acetate. These results confirm earlier observations, at least for the case at neutral pH, reported for glucose metabolism after monofluoroacetate treatment in yeast by Eaton and Klein[15] and for ethanol by Kalnitsky and Barron.[16]

DISCUSSION

The evidence presented of the effect of monofluoroacetic acid on the extent of oxidation of externally supplied ethanol and glucose makes it difficult to argue that the lower level of inhibition of respiration, of glucose uptake and of ethanol production seen at pH 6·8.[1,2] is a consequence of a reversal of a competitive inhibition of citrate oxidation. Peters[21] in a re-examination of aconitase inhibition by monofluorocitrate has established that its reversal is considerably more difficult than first reported. Certainly the results reported here would confirm this. Equally the operation at pH 6·8 of an alternative citrate oxidation mechanism is unlikely. The extent of oxidation of these substrates in the monofluoroacetic acid treated cells would suggest a metabolic block at the oxidation level of acetate at both pH values. It is unwise to assume, in an organism like *Saccharomyces cerevisiae*, that these measurements of extent of oxidation are a direct indication of the site of a metabolic block, particularly when it has been reported that non-oxidative assimilations do occur under these conditions.[17,20]

However, if the reason for the inhibited cells giving a lower extent of oxidation than the controls was to be attributed to a greater proportion of the incorporated substrate being biosynthesised to either polysaccharide or to lipid, the change in substrate flux would have to be an increase of approximately 40 per cent in the case of glucose and approximately 120% in the case of ethanol. Such assimilations require energy and it is unlikely that in cells showing marked respiratory and substrate uptake inhibitions, energy is more readily available than in the control cells where such inhibitions are not present.

Equally the differential inhibition of respiration seen with ethanol and lactate makes it unlikely that the differential effect can be due to the operation of the hexose monophosphate shunt only at pH 6·8.

It seems reasonable then to argue from these results that citrate oxidation is inhibited to the same extent at both pH values and that the differential effects on glucose, ethanol and lactate respiration are due to a less effective inhibition at pH 6·8 of oxidative enzymes concerned with these substrates' metabolism. The substance or substances could be either normal metabolites accumulating as a consequence of the inhibition of citrate oxidation or could be fluorinated metabolites of monofluoroacetate. It is not possible to distinguish between these alternatives on the basis of these results.

It is possible, however, to localise the enzyme stages likely to be differentially affected. In the case of glucose oxidation apart from possible oxidation through the hexose monophosphate shunt, only two oxidative stages remain available to the cell in the inhibited condition. These are the oxidation of glyceraldehyde-3-phosphate and the oxidation of pyruvate to acetyl CoA. An effector molecule or molecules inhibiting at either of the stages would lead to strong respiratory inhibition and also to extensive inhibition of glucose uptake. In the case of ethanol oxidation, since acetaldehyde shows an equally high inhibition of respiration at both pH values, it must be concluded that the site of differential inhibition is alcohol dehydrogenase (EC. 1.1.1.1. Alcohol: NAD oxidoreductase). The results with lactate do not enable a distinction to be made between the affected site being lactic dehydrogenase (EC. 1.1.1.27, L-lactate: NAD oxidoreductase) or pyruvic dehydrogenase (EC. 1.2.4.1 pyruvate: lipoate oxidoreductase).

Experiments to establish the nature of the inhibitor arising as a consequence of the blockage of citrate oxidation and the site of its action are currently being undertaken

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