The Effect of Diffusible Acids on Potassium Ion Uptake by Yeast

By H. RYAN, J. P. RYAN AND W. H. O'CONNOR

Department of Biochemistry, University College, Dublin, Irish Republic

(Received 23 July 1971)

1. When yeast oxidizes ethanol at different pH values the uptake of K^+ corresponds closely to the amount of acetate accumulated at each pH value. 2. The addition of semicarbazide to the suspension buffered at pH 4.75 inhibited both the K^+ uptake and the acetate accumulation by about 50%. 3. The addition of either acetate or propionate to the suspensions markedly increased the K^+ uptake. 4. The addition of acetate to the suspensions lowered the intracellular pH of the yeast from a resting value of pH 5.80 to 5.56. 5. The ratio of the initial rate of K^+ uptake to O_2 consumption was 0.77. This ratio was increased to 1.77 in the presence of 10 mmol of propionate/l.

When yeast ferments glucose in the presence of potassium chloride K^+ is taken up by the cells and an equivalent amount of H^+ is excreted (Conway & O'Malley, 1946). Conway, Duggan & Kernan, (1963) showed that the K^+ uptake was competitively inhibited by extracellular H^+ ions at pH 3.3 or lower. Armstrong & Rothstein (1964) studied the effect of extracellular H^+ ions on K^+ uptake over a wide range of pH values. They found that H^+ inhibited competitively below pH 3.5, but functioned predominantly as a non-competitive inhibitor between pH 3.5 and pH 5.0. At pH values between pH 5.0 and pH 8.0 H^+ had no apparent effect on K^+ uptake. Rothstein & Enns (1946) showed that K^+ uptake and acid excretion also occurred during the oxidation of ethanol by yeast at pH 4.6. In studies with ethanol as substrate, Ryan (1967) found that the K^+ uptake in an unbuffered medium amounted to 17.5 mequiv./kg in the first 10 min, but fell to 5.5 mequiv./kg when the medium was buffered at pH 7.4. These results indicated that extracellular H^+ ions did not affect K^+ uptake during ethanol oxidation in the same manner as when glucose was used as a substrate. In this paper the effect of extracellular pH on K^+ uptake is further investigated. Intracellular conditions affecting K^+ uptake are also considered, and a maximum ratio of K^+ uptake to O_2 consumption is determined.

MATERIALS AND METHODS

Yeast. Fresh baker's yeast (*Saccharomyces cerevisiae*, strain RN), as supplied by the Cork Yeast Co., Watercourse Road, Cork, Irish Republic, was used. Before use, washed yeast was 'starved' by oxygenation of a 1:10 (w/v) suspension for 24 h at 22°C.

Chemical determinations

Potassium. This was determined with a Beckman (model DU) flame spectrophotometer.

Acetic acid, propionic acid and bicarbonate. These were determined by the microdiffusion procedures described by Conway (1962).

Oxygen consumption. This was determined by the conventional manometric technique, with 3 ml samples of the yeast suspension/flask, and shaking at 120 cycles/min at 22°C. In a preliminary series of experiments it was found that the O_2 consumption for a 1:100 (w/v) suspension shaken in an atmosphere of air, was identical with that for a 1:20 (w/v) suspension shaken in pure O_2 . The former technique was used to obtain the initial O_2 uptake rate.

Experimental procedures

Aerobic suspensions. Samples of the 'starved' yeast were suspended (1:20, w/v) in the various media and bubbled with O_2 at 22°C. The yeast samples were harvested either by centrifugation for 10 min at 1000g, or on millipore filters (SM 5/ μ), the latter technique being preferred for K^+ determination. When the yeast was required for determination of acetic acid the samples were not washed, but allowance was made for acetic acid present in the interspace (Conway & Downey, 1950). For K^+ determination, 2 ml samples were removed, the yeast was harvested on millipore filters and washed twice with 4–5 ml of ice-cold glass-distilled water.

Intracellular pH. Samples of centrifuged yeast (2.5g) were frozen with liquid N_2 and thawed rapidly to 22°C. The pH of the resulting mixture was determined with a Beckman (model G) pH-meter. A small correction (<0.1 pH unit) was made for extracellular buffer in the interspace fluid.

The results of the various analyses are expressed throughout per kg (wet wt.) of centrifuged yeast.

Calculation of initial rate of K^+ uptake. Assuming a simple exponential reaction, where K_{∞}^+ is the total

amount of K^+ taken up by the yeast, and T is the time constant for the process (as calculated from semilogarithmic plots), the initial rate of K^+ uptake/h may be calculated as follows:

$$\text{Initial rate of } K^+ \text{ uptake} = 60 K_{\infty}^+ / T$$

The computed intercepts show an initial binding of some 6–7 mequiv. of K^+ /kg to the yeast in the first min. This does not represent a net flux of ions across the cell membrane, but probably represents a preliminary cation binding to the cell wall. Such a binding has been already postulated for K^+ uptake by *Neurospora crassa* (Slayman & Slayman, 1968).

RESULTS

Effect of pH on K^+ uptake. The effect of changing the pH of the suspending fluid on K^+ uptake was determined with either ethanol of glucose as a substrate. In these experiments the yeast was suspended (1:20, w/v) in solutions containing 0.01M-potassium chloride and 0.02M-citric acid, adjusted to various pH values with 1M-tris. The suspensions contained either 1.5% (v/v) ethanol or 5% (w/v) glucose, and were bubbled with O_2 at 22°C. The amount of K^+ taken up by the yeast in the first 10 min was determined in each suspension. The results in Fig. 1 show that, in the presence of either substrate, the amount of K^+ accumulated increases as the pH of the suspension is raised from pH 3.0 to 5.0. Further increases in pH, however, caused a considerable decrease in K^+ uptake in the presence of ethanol, but only a slight decrease in the presence of glucose. It is shown also in Fig. 1 that, when the suspensions containing ethanol were bubbled with $CO_2 + O_2$ (30:70), the effect of pH on K^+ uptake resembles closely that in the presence of glucose.

Acetate accumulation and K^+ uptake. Yeast oxidizes ethanol via acetaldehyde and acetate (Eaton & Klein, 1957). As undissociated acetic acid readily penetrates the cell (Conway & Downey, 1950), acetate may be expected to accumulate in the yeast as the pH of the suspending fluid is lowered. The accumulation of acetate, both in the yeast and the suspending fluid, was measured at different pH values. The experimental conditions were as described for Fig. 1, the substrate being ethanol (1.5%, v/v) and the suspensions were bubbled with O_2 at 22°C for a period of 10 min. The K^+ uptake in each suspension was also determined. In Table 1 it is shown that, although the total amount of acetate formed in the system is relatively constant between pH 4.0 and pH 7.0, the amount accumulated by the yeast decreases as the pH of the suspension is raised from pH 4.5 to pH 7.0.

Effect of semicarbazide on K^+ uptake and acetate accumulation. Acetaldehyde formed during ethanol oxidation may be 'trapped' by the addition of

Fig. 1. Effect of pH on K^+ uptake. The yeast was suspended (1:20, w/v) in fluids containing 0.02M-citric acid (adjusted to various pH values with 1M-tris) and 0.01M-KCl. The substrates were: glucose (5%, w/v) bubbled with O_2 at 22°C (●); ethanol (1.5%, v/v) bubbled with O_2 at 22°C (○); ethanol (1.5%, v/v) bubbled with $CO_2 + O_2$ (30:70) at 22°C (△).

semicarbazide to the suspending fluid (Silhankova, 1963). The effect of semicarbazide was investigated at pH 4.75, this pH being close to the optimum for both K^+ uptake (Fig. 1) and acetate accumulation (Table 1). Suspensions containing 0.2M-semicarbazide and no semicarbazide were prepared simultaneously. Duplicate samples were removed from each suspension at zero time (i.e. immediately after the addition of ethanol), 10 min and 20 min. The K^+ and acetate contents of the samples were determined. It is shown in Fig. 2 that the presence of semicarbazide decreases both K^+ uptake and acetate production by approx. 50%.

Effect of added acetate or propionate on K^+ uptake. Samples of yeast were suspended (1:20, w/v) in fluids containing 0.01M-potassium chloride, 1.5% (v/v) ethanol, 0.02M-citric acid and various amounts of acetic acid or propionic acid. The suspension fluids were adjusted to pH 4.75 with 1M-tris before mixing them with the yeast. The suspensions were bubbled with O_2 at 22°C and the K^+ uptake was determined after 10 min. It is shown in Table 2 that the uptake of K^+ increases to a maximum value as the concentration of acetate or propionate is raised to 5–10 mmol/l. Further increase in the concentration of acetate or propionate decreases the potassium uptake.

Intracellular pH changes and K^+ uptake during the oxidation of ethanol. Conway & Downey (1950) showed that when yeast fermented glucose in the presence of 0.1M-potassium chloride the mean intracellular pH rose from an initial value of pH 5.80 to pH 6.35 after 30 min. In studying the

Table 1. *Acetate accumulation and K⁺ uptake during the oxidation of ethanol*

The yeast was suspended (1:20, w/v) in fluids containing 0.01M-KCl and 1.5% (v/v) ethanol and 0.02M-citric acid adjusted to pH4.75 with 1M-tris. The suspensions were bubbled with O₂ at 22°C. The K⁺ uptake and acetate accumulation was determined after a period of 10 min. The yeast contained 105 mequiv. of K⁺/kg, and 4.7 mequiv. of volatile acid/kg, at zero time.

Extracellular pH	K ⁺ uptake (mequiv./kg)	Acetate formed (mequiv./kg)		
		Intracellular	Extracellular	Total
3.0	17.2	12.3	9.5	21.8
4.0	24.5	21.8	10.0	31.8
4.5	28.0	22.3	11.0	33.3
5.0	28.3	19.8	12.5	32.3
6.0	16.0	11.3	20.5	31.8
7.0	10.8	4.3	30.2	34.5

Fig. 2. Effect of semicarbazide on K⁺ uptake and acetate accumulation. The yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to pH4.75 with 1M-tris) and 0.01M-KCl and 1.5% (v/v) ethanol. Control for K⁺ uptake (○); K⁺ uptake with 0.2M-semicarbazide (●); control for acetate accumulation (△); acetate accumulation with 0.2M-semicarbazide (▲).

intracellular pH changes that occur during ethanol oxidation the general conditions were the same as described in Table 2. The suspensions contained either no added acetic acid or 0.01M-acetic acid adjusted to pH4.75 with 1M-tris. Samples of the yeast were removed at zero time and after 10min of oxidation. The intracellular pH was determined as described in the Materials and Methods section. Duplicate samples were analysed for both K⁺ and acetate content. It is shown (Table 3) that the initial intracellular pH was 5.80 in the absence of added acetate and 5.56 in the suspension containing 0.01M-tris-acetate buffer. The final intracellular pH values were approxi-

Table 2. *Effect of acetate or propionate on K⁺ uptake during the oxidation of ethanol*

The yeast was suspended (1:20, w/v) in fluids containing 0.01M-KCl and 1.5% (v/v) ethanol and 0.02M-citric acid with various concentrations of acetate or propionate. The fluids were adjusted to pH4.75 with 1M-tris before mixing them with the yeast. Suspensions were bubbled with O₂ at 22°C. The K⁺ uptake was measured after 10min. The yeast contained 112 ± 2 mequiv. of K⁺/kg at zero time.

Acetate or propionate (mmol/l)	Uptake of K ⁺ with added acetate (mequiv./kg)	Uptake of K ⁺ with added propionate (mequiv./kg)
0.0	27.4	26.0
1.0	29.0	26.3
2.0	—	28.5
3.0	33.6	—
5.0	36.2	34.8
10.0	37.4	34.2
20.0	32.4	24.5

mately the same in the two suspensions but both K⁺ and acetate accumulation were considerably increased in the presence of added acetate.

Ratio of K⁺ uptake to oxygen consumption. During the oxidation of ethanol the uptake of K⁺ in the first 10min reaches a maximum value when the suspension is buffered at pH4.75 (Fig. 1). This uptake at pH4.75 is considerably increased in the presence of 0.01M-propionate (Table 2). The initial rate of K⁺ uptake was determined at pH4.75, both with and without 0.01M-propionate. Samples of yeast were suspended in 0.02M-citric acid (adjusted to pH4.75 with 1M-tris) and 1.5% (v/v) ethanol. The suspensions were bubbled with O₂ for 5min at 22°C and 1M-potassium chloride was added (zero time) to give a final concentration of 0.01M-potassium chloride and a ratio of 1:20 (w/v) yeast to suspending fluid. In the initial stages samples were removed at 1min intervals by using the

Table 3. *Intracellular pH changes and K⁺ uptake during the oxidation of ethanol*

The yeast was suspended (1:20, w/v) in fluids containing 0.01M-KCl and 1.5% (v/v) ethanol and 0.02M-citric acid adjusted to pH4.75 with 1M-tris. The suspensions contained either 0.01M-acetate or no added acetate, and were bubbled with O₂ at 22°C.

Time	Without added acetate			With 0.01 M-acetate		
	Intracellular pH	K ⁺ content (mequiv./kg)	Acetate (mequiv./kg)	Intracellular pH	K ⁺ content (mequiv./kg)	Acetate (mequiv./kg)
0 min	5.80	111.2	4.9	5.56	114.0	24.5
10 min	6.13	141.7	21.0	6.12	161.4	53.8
Change	0.33	30.5	16.1	0.56	47.4	29.3

Table 4. *Effect of propionate on the initial uptake of K⁺ during the oxidation of ethanol*

The yeast was suspended in fluids containing 1.5% (v/v) ethanol and 0.02M-citric acid adjusted to pH4.75 with 1M-tris. The suspensions contained either 0.01M-propionate or no propionate and were bubbled with O₂ at 22°C for 5 min. The 1M-KCl was added (zero time) to give a final concentration of 0.01M-KCl and a ratio of 1:20 (w/v) yeast to suspending fluid. The values are the average from six experiments and represent mequiv. of K⁺/kg.

Time (min) ...	K ⁺ content (mequiv./kg)								
	0	1	2	3	4	5	10	20	40
Without propionate	101.1	110.7	114.6	117.6	119.9	121.9	126.7	133.3	134.2
With 0.01 M-propionate	102.5	116.5	122.5	126.6	129.2	131.2	139.4	152.0	155.9

Millipore filter technique. Samples were removed at intervals until the K⁺ uptake had ceased.

The yeast was analysed for K⁺ and the initial potassium content of the yeast was also determined. In a second experiment samples containing 0.01M-propionic acid were treated in a similar manner (Table 4). The amount of K⁺ that had entered after a period of 40 min was taken as K_∞⁺ (i.e. the end-point of K⁺ uptake). The differences, K_∞⁺ - K_t⁺, for each sample were plotted against time on semi-logarithmic paper. Straight lines were fitted to the results up to 5 min in the case of ethanol alone, and up to 3 min in the case of ethanol plus propionic acid. The intercepts and slopes of each line were used to determine the appropriate initial K⁺-bindings and time constants. These were 5.6 and 5.9 for ethanol alone and 7.6 and 6.6 for ethanol plus propionic acid. The initial rate of K⁺ uptake was calculated as described in the Materials and Methods section, the general procedure being that used by Slayman & Slayman (1968). The total K⁺ uptake with ethanol alone is only some 60% of that with ethanol plus propionic acid. Hence, although the time constants are similar for both systems, the initial rate of K⁺ uptake is significantly greater in the presence of propionic acid (Table 4).

The O₂ consumption was determined as described in the Materials and Methods section: the suspension fluids were identical with those used in the K⁺-uptake experiments. After 5 min of equilibration potassium chloride was added from the side

arm (zero time), and the O₂ consumption was measured for 20 min during which time it was linear. The O₂ consumption (mmol/h per kg wet wt. of cells) was calculated from the slope obtained, and this value was taken as the initial rate. In Table 5 the initial rates of K⁺ uptake and O₂ consumption are shown. The ratios of K⁺ uptake to O₂ consumption were calculated from these results, and are also shown in Table 5.

DISCUSSION

When yeast oxidizes either glucose or ethanol, the uptake of K⁺ is markedly influenced by the pH of the suspension fluid. In both systems the uptake of K⁺ increases as the pH is raised from pH 3.0 to pH 5.0. By further raising the pH from pH 5.0 to pH 7.0 the K⁺ uptake is considerably decreased when ethanol is the substrate, but only slightly decreased in the presence of glucose. As these differences are observed under similar experimental conditions, the factors controlling K⁺ uptake at pH 5.0 or higher, lie within the cell, rather than in the suspension medium.

Rothstein (1960) has suggested that the production of metabolic anions, such as bicarbonate and succinate, and the resulting change of cell pH to alkaline values may be predominant factors in limiting the amount of K⁺ that can be absorbed during glucose fermentation. An alternative interpretation is that the major role of the K⁺-H⁺

Table 5. *Initial rate of K⁺ uptake and oxygen consumption during the oxidation of ethanol*

The measurements were made both with and without 0.01 M-propionate at pH 4.75. The experimental conditions are described in the text. The results are the average values from six experiments.

	Without propionate (mmol/h per kg)	With 0.01 M-propionate (mmol/h per kg)
Initial rate of K ⁺ uptake	337	486
Initial rate of O ₂ consumption	436	274
$\Delta K^+/\Delta O_2$ ratio	0.77	1.77

ion-exchange system is to raise the intracellular pH from a resting value of pH 5.80 to a final value in the region of pH 6.2–6.4.

When acids formed metabolically cannot readily escape from the cell the K⁺ uptake is increased, this increase depending on the amount of anions accumulated. This is illustrated by comparison of the effect of extracellular pH on K⁺ uptake with glucose and with ethanol. Raising the pH of the suspension fluid to pH 7.0 has little effect on K⁺ uptake when glucose is the substrate, as the bicarbonate and succinate anions formed do not readily leave the cell under these conditions. On the other hand, the accumulation of acetate is a function of external pH, and thus when ethanol is the substrate both K⁺ uptake and acetate accumulation are decreased as the pH is raised. When the suspensions containing ethanol are bubbled with CO₂+O₂ (30:70), this decrease in the K⁺ uptake is not observed as bicarbonate now replaces any acetate that has been lost. In one such suspension, buffered at pH 5.7, the bicarbonate content of the cell rose by 26 mequiv./kg in the first 10 min, thus substituting for the acetate that diffuses out at this pH.

During the oxidation of ethanol the amount of K⁺ taken up in the first 10 min is maximum at approx. pH 4.75. At this pH both K⁺ uptake and acetate production are inhibited by about 50% when 0.2 M-semicarbazide is included in the medium. If, on the other hand, the amount of acid available to the cell is increased by the addition of either acetic acid or propionic acid to the suspension fluid, then the K⁺ uptake is also increased. In the absence of added acetic acid the intracellular pH was 5.80 at zero time and was 6.13 after 10 min. The buffering capacity of the cell fluid is 63 mequiv./l (Conway & Downey, 1950) or 34 mequiv./kg of wet yeast. From this it may be calculated that the pH change of 0.33 units would require a K⁺–H⁺ exchange of 11–12 mequiv./kg. Because there is a net accumulation of 16.1 mequiv. of acetate/kg, a total K⁺ uptake of 26–27 mequiv./kg is required to achieve a pH change of 0.33 units. A similar calculation of the pH change in the presence of added acetic acid further illustrates this point. Here the initial pH of the cell is 5.56 because of the increased acetate content.

Although the bulk of the K⁺ enters in the first 10 min, uptake continues for approx. 20 min. The final pH of the cell might, therefore approach pH 6.2–6.4, the value observed by Conway & Downey (1950).

Finally the ratio of K⁺ uptake to O₂ consumption was measured. These experiments were done at pH 4.75, which is the optimum for K⁺ uptake in the first 10 min. A $\Delta K^+/\Delta O_2$ ratio of 0.77 was found. In the presence of 0.1 M-propionic acid this ratio is increased to 1.77, even though the O₂ consumption is considerably decreased. This finding suggests that the $\Delta K^+/\Delta O_2$ ratio is dependent on the intracellular pH.

On the basis of the results it is suggested that intracellular pH is a major factor in the control of the K⁺–H⁺ exchange system. It is considered that this exchange operates only when the intracellular pH is below a value in the region of 6.2–6.4. When diffusible acids are added to the yeast suspension an increased uptake of K⁺ occurs, as such acids both lower intracellular pH and increase the buffering capacity of the cell fluid. Further, because the rate of K⁺ uptake is increased in the presence of added propionic acid, the intracellular pH may be the main factor in determining the efficiency at which the system operates.

REFERENCES

- Armstrong, W. McD. & Rothstein, A. (1964). *J. gen. Physiol.* **48**, 61.
 Conway, E. J. (1962). *Microdiffusion Analysis and Volumetric Error*, pp. 234, 201. London: Crosby, Lockwood and Sons Ltd.
 Conway, E. J. & Downey, M. (1950). *Biochem. J.* **47**, 355.
 Conway, E. J., Duggan, P. F. & Kernan, R. P. (1963). *Proc. R. Ir. Acad.* **63 B** 6, 93.
 Conway, E. J. & O'Malley, E. (1946). *Biochem. J.* **40**, 59.
 Eaton, N. R. & Klein, H. P. (1957). *Biochem. J.* **67**, 373.
 Rothstein, A. (1960). *Ciba Found. Study Group: Regulation of the Inorganic Ion Content of Cells*, p. 53. London: J. and A. Churchill Ltd.
 Rothstein, A. & Enns, L. H. (1946). *J. cell. comp. Physiol.* **28**, 231.
 Ryan, H. (1967). *Biochem. J.* **105**, 137.
 Silhankova, L. (1963). *Folia microbiol.* **8**, 32.
 Slayman, C. L. & Slayman, C. W. (1968). *J. gen. Physiol.* **52**, 424.