BBA 45797

STUDIES ON THE MECHANISM OF THE STIMULATION OF GLYCOLYSIS AND RESPIRATION BY K^+ IN *SACCHAROMYCES CEREVISIAE*

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(Received November 18th, 1968)

SUMMARY

1. The effect of K^+ on the respiration and glycolysis of yeast was investigated. Measurement of the levels of ADP, ATP and P_i revealed that K^+ stimulates an enzymatic activity that diminishes ATP and increases ADP. This increase in the ADP level seems to be the responsible for the stimulation of both glycolysis and respiration.

2. When glucose was used as substrate, the increase in the levels of P_i upon the addition of K^+ was found to be greater than either the ATP decrease or the ADP increase. This might mean that the ion induces the breakdown of ATP by an indirect mechanism, with another phosphorylated compound being hydrolyzed directly. The amount of ATP would decrease at the rate at which it was used to replace this hypothetical compound.

3. The studies on the mechanism by which K^+ induces the stimulation of glycolysis and respiration suggest that it is the presence of the ion inside the cell or some consequence of this that induces the changes necessary to produce the stimulation of "ATPase" activity responsible for the activation of glycolysis and respiration. The possibility that the presence of the ion outside the cell or the energy required for its transport causes the changes appears to have been eliminated.

INTRODUCTION

In a previous study[1], the stimulatory effect of K^+ in the incubation medium on the anaerobic glycolysis of yeast was explored. The explanation offered for the stimulation produced by K^+ was based on a decrease of the concentrations of both ATP and ADP observed after the addition of K^+ to the incubation mixture. ROTHSTEIN AND DEMIS[2] found that externally added K^+ could also increase the respiratory rate of yeasts when glucose was used as substrate; this effect, however, was not studied further.

This communication attempts to give an explanation for the observed stimulation of glycolysis and respiration by K^+. The data presented here include a modification of the results previously published on the changes observed in the concentrations of adenosine phosphates and P_i when K^+ was added. The erroneous results previously reported were due to the low concentration of perchloric acid employed

in the extraction procedure of these chemicals, a fact already observed by BETZ AND CHANCE[3].

The effects of K^+ were also studied as a function of pH (at pH 4.0 and 7.5), since it has been reported that the stimulatory action of this ion on glycolysis can only be obtained at rather low pH values and that high pH alone affects glycolysis similarly to the presence of K^+ (ref. 2).

CONWAY AND BRADY[4] demonstrated that K^+ are transported into the yeast cell *via* an exchange system for H^+; this transport occurs against a concentration gradient, which must require the expenditure of energy. In view of this, the possibility was also investigated that the stimulation of glycolysis and respiration was a response to the extra energy required for transport. The possibility of the stimulation of glycolysis through the direct activation by external K^+, postulated by ROTHSTEIN AND DEMIS[2], was also studied.

MATERIALS AND METHODS

Most of the materials and procedures employed throughout the work were previously described[1]. In this work, the extraction of the adenosine phosphates was carried out with 0.5 vol. of either 30% trichloroacetic acid or 60% perchloric acid. The tubes were left to stand for 20 min in an ice-water bath before centrifuging. P_I was extracted with 15% final concentration of trichloroacetic acid. The extraction of the glycolytic intermediates was found to be adequately accomplished with the low concentration (5% final) of perchloric acid used in previous work. The extraction of the adenosine phosphates with trichloroacetic acid has the disadvantage that even if neutralized, it strongly inhibits the glucose-6-phosphate dehydrogenase employed in the enzymatic analysis[3]; this inhibition of the enzyme could not be completely avoided even after 6 washings of the extract with ether. Because of this, perchloric acid extraction was preferred in most experiments.

In some experiments in which ethanol or butanol was used as substrate, the incubation mixture was placed in a beaker maintained at constant temperature, and with vigorous magnetic stirring, in order to obtain an adequate oxygenation of the yeast cells.

O_2 consumption was measured in a Model 53 Biological Oxygen Monitor, Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.

RESULTS

Fig. 1 shows the results obtained when the respiration of yeast cell suspensions was measured using glucose or ethanol as substrates, at pH 4.0 and 7.5. When a rather constant rate of respiration was attained, K^+, in the form of KCl, was added. At the lower pH (4.0), the respiratory rate, without added K^+, was lower than at pH 7.5. At pH 4.0, K^+ produced an acceleration of respiration with both substrates; the stimulation observed was somewhat greater when ethanol was the substrate. At pH 7.5 K^+ did not stimulate respiration. This same lack of a K^+ effect at a high pH was reported concerning glycolysis[2], and has been confirmed in our laboratory with the strain of yeast used in our experiments.

It was observed previously[1] that the anaerobic reduction of NAD^+, when

glucose was the substrate, was higher in the presence of K^+. The possibility that these ions could influence the reduction of NAD^+ with ethanol as substrate was investigated. On Fig. 2, the tracings of the reduction of NAD^+ with this substrate are presented. The presence of K^+ in the medium produces only a negligible increase in NADH.

Butanol has been reported to be oxidized by yeast only to the aldehyde state[5,6]. This makes it a very good substrate for eliminating possible effects on further steps of its metabolism, as might happen with ethanol, for instance. Experiments similar to those carried out with ethanol were made with butanol as substrate. The behavior of respiration and NAD^+ reduction with this substrate, with or without K^+ added, was

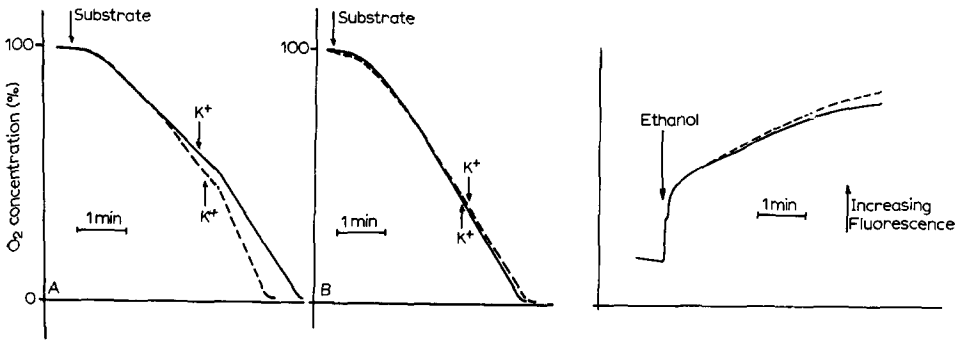

Fig. 1. The effect of K^+ on the respiration of yeast cells at pH 4.0 (A) or pH 7.5 (B) with ethanol (– – –) or glucose (———) as substrate. Incubation mixture: 0.05 M phthalate–Tris buffer (pH 4.0 or 7.5); 0.05 M glucose or ethanol; yeast, 50 mg wet wt. Final vol., 5 ml; temp., 25°. 100 μmoles of KCl were added at the arrows.

Fig. 2. Fluorometric tracing of the reduction of NAD^+ (365–460 mμ) with ethanol as substrate in the presence (– – –) or absence (———) of K^+. Incubation conditions were as described for Fig. 1, except that KCl concentration was 13 mM, 200 mg of yeast (wet wt.) were used, and the final vol. was 3.0 ml.

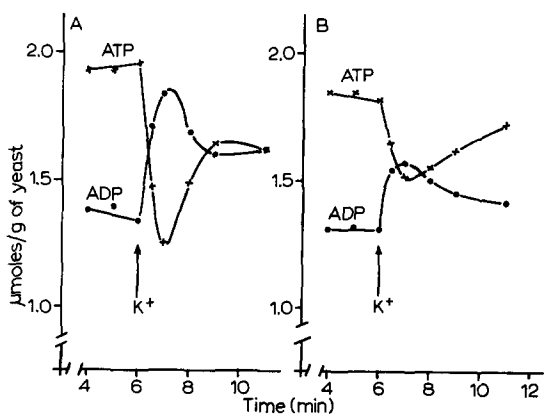

Fig. 3. The effect of K^+ on the levels of ADP and ATP with ethanol (A) or butanol (B) as substrates. Incubation conditions: 0.1 M phthalate–Tris buffer (pH 4.0); 0.05 M ethanol or butanol; yeast cells (10 g wet wt.); final vol., 50 ml; temp., 25°. The mixture was incubated in a beaker stirring vigorously, and 5-ml aliquots were obtained at the indicated times. The samples were extracted with 0.5 vol. of 60% perchloric acid as described under MATERIALS AND METHODS and then treated as described before[1]. 2 M KCl was added at the arrows to give a 40 mM final concentration.

identical to that with ethanol, except for the fact that maximal respiration or NAD^+ reduction with butanol required higher concentrations of substrate (about 0.05 M).

In view of the fact that tracings of NAD^+ reduction did not show any important differences in the presence or absence of K^+ using ethanol or butanol as substrate, the analysis of the adenosine phosphates was carried out in experiments in which, after a few minutes of incubation, KCl was added to the incubation mixture to observe the changes produced in the concentrations of these nucleotides. As Fig. 3 shows, after the addition of K^+, when ethanol was the substrate (Fig. 3A), a fast and very marked increase in the concentration of ADP, and a corresponding decrease in ATP were observed immediately after the addition of K^+. After about 1 min, these changes showed a tendency to return to previous levels without quite reaching them, *i.e.*, ATP remained lower, and ADP higher, than the levels present before the K^+ addition. Fig. 3B shows the results obtained in a similar experiment in which butanol was used as substrate. The changes observed in the levels of ATP and ADP upon the addition of K^+ are very similar to those observed when ethanol was the substrate.

When glucose was the substrate, the results shown in Fig. 4 were obtained. The changes observed in the concentrations of ATP and ADP are of a similar qualitative nature, but much slighter. These changes were different from those reported before[1].

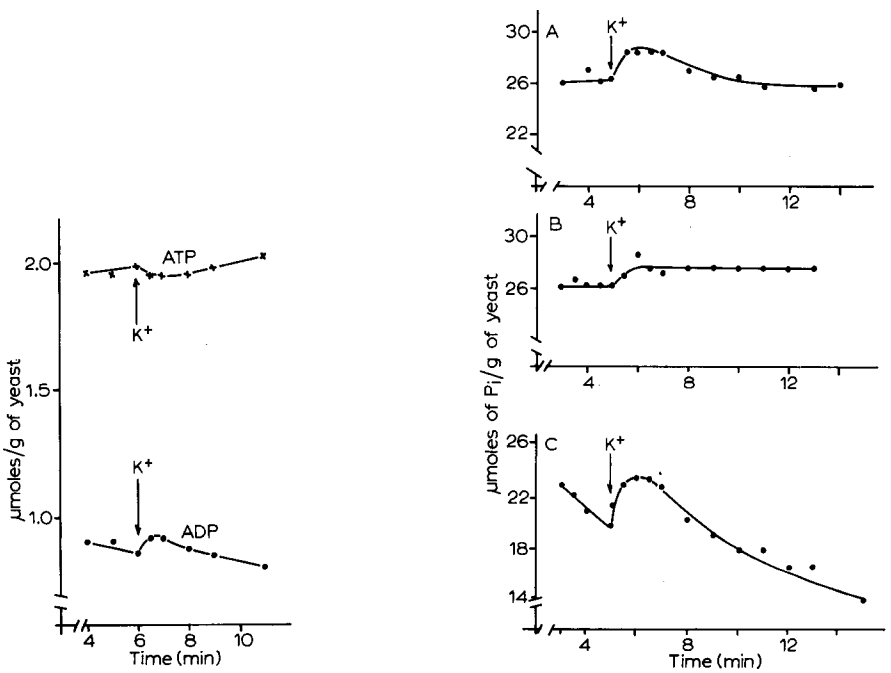

Fig. 4. The effect of K^+ on the levels of ADP and ATP with glucose as substrate. Experimental conditions as for Fig. 3, except that 0.1 M glucose was the substrate.

Fig. 5. Effect of K^+ on the levels of P_i with ethanol (A), butanol (B) or glucose (C) as substrate. Experimental conditions as for Fig. 3. The substrate was 0.05 M ethanol or butanol or 0.1 M glucose. P_i was extracted with 0.5 vol. of 30% trichloroacetic acid.

and could be observed only by changing the extraction procedure. The low concentrations of perchloric acid employed before were not enough to extract the adenosine phosphates completely, and a false decrease in both ADP and ATP had been reported.

The analysis of the changes observed in the P_i concentrations in similar experiments showed an increase of this chemical immediately after the addition of K^+ (Fig. 5). Similar results were obtained employing ethanol, butanol or glucose as substrates. It should be pointed out that an important increase in the level of P_i was observed with glucose as substrate, in spite of the fact that very slight changes were observed in the concentrations of ADP and ATP under the same conditions.

Comparative results of the effect of the addition of K^+ on the levels of ADP and ATP with ethanol as substrate at pH 4.0 and 7.5 with the same batch of yeast are presented in Fig. 6. At the higher pH, lower basal levels of ATP and higher levels of ADP were observed. Moreover, the immediate decrease of ATP and the increase of ADP when K^+ was added, were smaller than those observed at pH 4.0. After the initial decrease in concentration, ATP showed an increase, followed by a further decrease, in an oscillatory way, with ADP showing opposite changes. When glucose was used as substrate at pH 7.5, no changes were observed in the levels of adenosine phosphates.

The changes observed in the levels of ATP, ADP and P_i upon the addition of K^+ to the incubation medium suggested the stimulation of an ATPase activity by the ion. ROTHSTEIN AND DEMIS[2] postulated that it was the external concentration of K^+ which was responsible for the acceleration of glycolysis. This aspect of the problem was investigated further. Fig. 7 shows the ethanol production observed when different amounts of K^+ were transported, employing two different yeast concentrations at various concentrations of K^+. At both yeast concentrations, the curves of ethanol production and K^+ transport were closely parallel, which might indicate that it is the

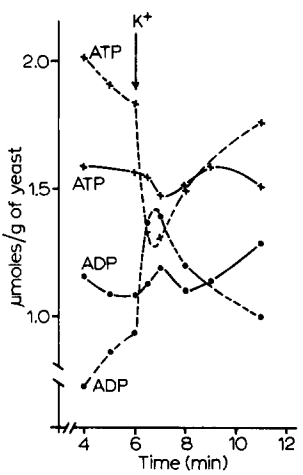

Fig. 6. The effect of K^+ on the levels of ATP and ADP with ethanol as substrate at pH 4.0 (– – –) or 7.5 (———). Experimental conditions as for Fig. 3, except that 0.1 M Tris–phthalate buffer was used in the pH 7.5 experiment. The extraction was carried out by the addition of 0.5 vol. of trichloroacetic acid. After centrifuging, the acid was washed 6 times with ether from the supernatant, and the enzymatic determination of the adenosine phosphates was carried out in the washed material after neutralization.

amount of K^+ transported which produces the activation of glycolysis, and not its external concentration.

In the experiment presented in Fig. 8, ethanol production and K^+ disappearance from the medium were measured at two different external concentrations of K^+ at several time intervals. It was found, in the first place, that a similar activation of ethanol production took place at both initial K^+ concentrations, and secondly, that the activation of glycolysis persisted long after the K^+ uptake had ceased. The activation of glycolysis at the 4 mM concentration did not decrease in spite of the fact that after about 13 min, the final level of external K^+ became less than 1 mM.

DISCUSSION

The stimulatory effect of K^+ on the respiration of yeast cells observed at low pH values, similar to that found by ROTHSTEIN AND DEMIS[2] on glycolysis, pointed to some connection between the two effects. The results of the NAD^+ reduction with ethanol, *i.e.*, the same levels of NADH in the presence and absence of K^+, suggested that the effect was not located at the dehydrogenating steps involved in its metabolism. Moreover, the use of butanol, which is not metabolized further after its first dehydrogenation as substrate, indicated that the effect could be on some step regulating the rate of electron flow through the respiratory chain. This common effect on respiration and glycolysis, with similar characteristics, pointed to a common cause in both cases.

The increase in the ADP concentration found upon the K^+ addition can adequately explain the stimulation of both glycolysis and respiration. The phosphate increase, on the other hand, possibly does not influence any of the metabolic processes; although the increases in its concentration are important, the relative increases, taking into account the total amount of phosphate, are very small, which would make it difficult to consider this chemical to be the limiting factor in either respiration or glycolysis.

The changes in the concentrations of the adenosine phosphates using ethanol or butanol as substrates clearly suggest the stimulation of an ATPase activity, which is reinforced by the findings in the changes of P_i. The question remains of whether the ATPase activity represents a direct hydrolysis of ATP, or whether this takes place through some intermediary step. A datum which might indicate that ATP hydrolysis is not the result of a single reaction is the very small change observed in the concentrations of ATP and ADP, accompanied by a significant increase in the P_i level upon the addition of K^+ when glucose was used as substrate. This could mean that the phosphorylated compound first hydrolyzed is not ATP, and that the decrease in ATP only reflects its expenditure to replace some phosphorylated compound whose hydrolysis is induced by the presence of K^+.

The absence of the stimulatory effect of K^+ at the higher pH value appears to be due to the absence of the stimulation of this ATPase activity which was observed at the lower pH; further studies are required to clarify this point. The data in Fig. 6, however, show that there is a higher ADP level at pH 7.5, than that observed at pH 4.0, the reverse being true for ATP before the addition of K^+. It looks as if at the higher pH, a higher expenditure at ATP is already present, which would maintain both respiration and glycolysis at an accelerated rate. Under these conditions K^+ could be unable to induce a further expense of ATP. Preliminary data from this

laboratory on the effect of pH on yeast glycolysis and respiration seem to indicate that the changes observed when the pH of the incubation mixture is suddenly increased are very similar to those observed when K^+ is added, which might mean that both effects are of a similar nature.

As to the possible mechanism by which K^+ induces this expenditure of ATP, three main possibilities exist: (a) energy is expended to fulfill the requirements for ion transport; (b) the external concentration could influence the activity of an enzyme or enzyme system localized on the cell surface[2], and (c) the presence of the ion inside the cell might produce some effect on a given enzyme(s). The third possibility could have an alternative, *i.e.*, it was not the presence of K^+ *per se*, but some consequence of its presence which produced the effect. It is known, for example, that K^+ transport occurs as an exchange for H^+, and that the transport of K^+ produces an increase in the pH of the intracellular contents[4].

The experiments on Fig. 7 showed a close parallelism between the amount of K^+ transported and the extra amount of ethanol produced, this being observed at two different concentrations of yeast. The maximal stimulation of glycolysis was observed at different concentrations of K^+ with different amounts of yeast, which could be an indication that the possible influence of the extracellular concentration of K^+ on the activation of glycolysis could be disgarded. This experiment, however, does not permit a choice to be made between the first and the last possibilities.

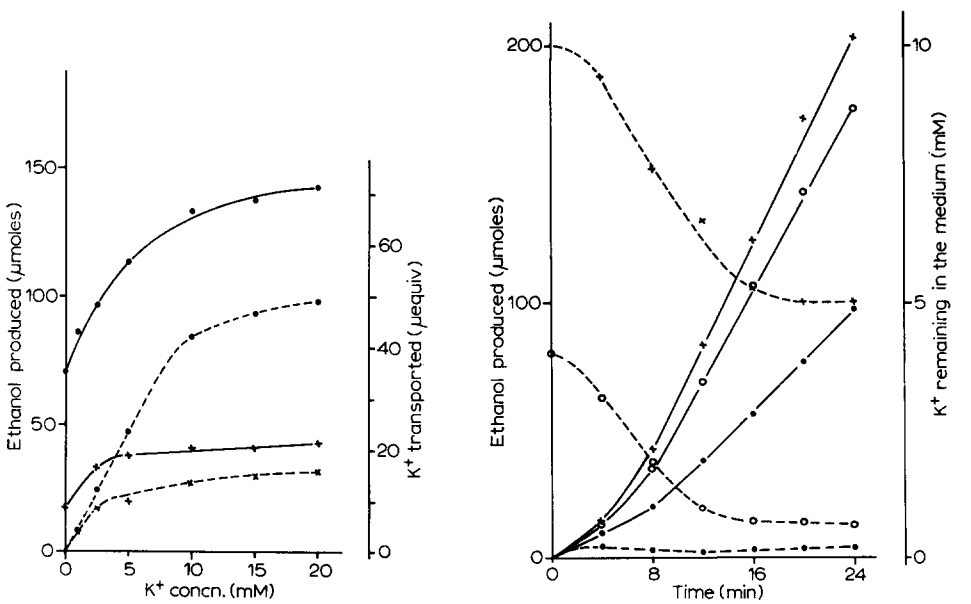

Fig. 7. Ethanol production (———) and K^+ uptake (– – –) at different K^+ concentrations and two yeast concentrations (1 g, ●, and 0.25 g, ×). Experimental conditions as described for Fig. 4, except that the incubation was carried out in centrifuge tubes and KCl was added at the indicated concentrations. Final vol. was 5 ml. The incubation was stopped after 10 min by cooling in an ice-water bath. The tubes were centrifuged in the cold, and K^+ and ethanol were measured in the supernatant.

Fig. 8. The effect of two different concentrations of K^+ on ethanol production (———) and K^+ transport (– – –). Experimental conditions as for Fig. 7, except that 0.5 g of yeast (wet wt.) was used per tube. Control, ●; 4 mM KCl, ○; 10 mM KCl, ×.

The data of Fig. 8 allow the elimination of possibilities (a) and (b). It was observed that a similar activation of glycolysis occurred at both K^+ concentrations, in spite of the fact that at the low concentration, the K^+ level in the medium became less than 1 mM after about 13 min. On the other hand, the activation of glycolysis in the tubes containing K^+, did not disappear when the transport of these ions had stopped. These facts demonstrate that the expenditure of ATP is not a consequence of the energy requirements of the K^+ transport system. The activation of the ATP-utilizing system seems, therefore, to be a consequence of the presence of K^+ in the yeast cell or of some other condition created concomitantly with its transport. The most obvious condition of this sort would be the pH increase created inside the cell as H^+ is exchanged for K^+.

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