In vivo Evidence for a Functional Glycolytic Compartment in Synchronous Yeast Cells

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Continuous \(^{14}\)C\(_2\)O\(_2\) Evolution, Glycolytic Compartment, Pyruvate Metabolism, Synchronous, Yeast

The different metabolic behaviour of endogenously produced \([1-{\text{\(^{14}\)}\text{C}}]\)pyruvate derived from \([3,4-{\text{\(^{14}\)}\text{C}}]\)glucose and of exogenously added \([1-{\text{\(^{14}\)}\text{C}}]\)pyruvate were studied with synchronous yeast cell populations, under conditions which differentially influenced the activities of pyruvate decarboxylase in the cytoplasm and pyruvate dehydrogenase in the mitochondria. Endogenously produced \([1-{\text{\(^{14}\)}\text{C}}]\)pyruvate is decarboxylated almost exclusively by PDC under anaerobic conditions, in contrast to added \([1-{\text{\(^{14}\)}\text{C}}]\)pyruvate which is decarboxylated under aerobic conditions by the action of PDH mainly.

Whereas \(^{14}\)CO\(_2\) evolution from exogenous \([1-{\text{\(^{14}\)}\text{C}}]\)pyruvate can be diluted proportionally by addition of pyruvate, this is not the case for \(^{14}\)CO\(_2\) evolution from endogenous \([1-{\text{\(^{14}\)}\text{C}}]\)pyruvate. It is suggested that glycolysis or at least some constituents of it might be arranged in such a manner that the resulting complex behaves as a functional compartment, meaning that it is almost inaccessible to exogenously added pyruvate.

Materials and Methods

Lyophilized cells of S. cerevisiae (strain from Gist-Brocades, Delft) were soaked in a salt medium [11] at 28 °C under aerobic conditions. After a starvation period of at least 12 h before harvesting, the cells were assayed for respiration, glucose consumption and ethanol production under aerobic and anaerobic conditions.

Pyruvate uptake studies were performed with \([2-{\text{\(^{14}\)}\text{C}}]\)pyruvate according to Titheradge and Coore [12], however, instead of \(\alpha\)-cyano-3-hydroxycinnamate, \(\alpha\)-cyanocinnamate was used.

In order to obtain synchronous cell populations, the cell suspension was washed once with fresh salt medium and centrifuged for 3 min at 2000 \(\times\) g. The sediment was suspended in 15% mannitol and centrifuged for 5 min at 30 \(\times\) g; then the supernatant was centrifuged for 5 min at 300 \(\times\) g. This procedure was repeated two times. The resulting 300 \(\times\) g sediment was washed once with salt medium and the cell density was adjusted to about 4 \(\times\) 10\(^{6}\) cells/ml. The synchrony of cell suspensions obtained in this way were determined in a PYG-medium [11] by measurement of the bud index, increase in cell number, glucose consumption and ethanol production [13], by

Abbreviations: MES, 1 (\(n\)-morpholino) ethane sulfonic acid; PDC, Pyruvate decarboxylase E.C. 4.1.1.1; PDH, Pyruvate dehydrogenase E.C. 1.2.4.1; S. cerevisiae, Saccharomyces cerevisiae.

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measurement of oxygen consumption, of cell size distribution (modified Celloscope 202, Ljungberg, Stockholm) and of [2-3H]adenine incorporation (20 µCi/4 x 10^7 cells, 10 min, 28 °C) into DNA; labelled RNA was eliminated by RNase treatment (120 Kunitz units/4 x 10^7 cells, 60 min, 28 °C).

Glucose and ethanol were assayed with kits from Boehringer, Mannheim and Merck, Darmstadt, respectively. Respiration was monitored with an electrode of the Clark type. Continuous 14C CO2 evolution was measured as previously described [10]. Investigated cell suspension was kept in rotation in a circular incubation chamber by a gas lift system. Same gas bubbles expel metabolic 14CO2 and the gas is mixed in a chamber with NaOH-Triton X 100. Total CO2 including 14CO2 is bound completely and resulting foam is collected in 1 min fractions. After addition of scintillation liquid they were counted in a Tricarb scintillation counter. Sodium [1,14C]pyruvate, spec. act. 19.7 mCi/mmol, sodium [2,14C]pyruvate, spec. act. 18.3 mCi/mmol and [2-3H]adenine, spec. act. 23.8 mCi/mmol were purchased from Amersham Buchler, Braunschweig and [3,4-14C]glucose, spec. act. 13.2 mCi/mmol from NEN, Dreieichenhain. DNase free RNase was obtained from Serva, Heidelberg. All other chemicals and biochem icals were of the purest grade available.

Results and Discussion

Under the conditions used yeast cells do not produce ethanol from exogenous glucose during aerobiciosis, whereas they do after introduction of anaerobic conditions (Fig. 1). Their glucose consumption increases approximately 30% during anaerobiosis as compared to aerobiciosis. Similar values were published by Lagunas [14]. This clearly indicates that pyruvate decarboxylase predominates under anaerobic conditions. Evolution of 14CO2 following the addition of [3,4-14C]glucose, which is metabolized to 2 molecules of endogenous [1-14C]pyruvate by a suspension of synchronous yeast cells under aerobic conditions, thus has to be considered to be mainly the result of the action of pyruvate dehydrogenase (Fig. 2a and c).

On the basis of these observations it was suggested that 14CO2 evolution following the addition of [1-14C]pyruvate to a suspension of yeast cells was the result of the action of pyruvate decarboxylase or pyruvate dehydrogenase, depending on the incubation conditions.

The possibility of a limitation of pyruvate transport from the extracellular to the intracellular space was ruled out in parallel experiments under conditions as in Fig. 2a, b, d. Neither anaerobiosis nor cyanide were able to reduce the pyruvate uptake rate of 190 nmol/min x 10^8 cells. Anaerobiosis did not result in a stimulated 14CO2 evolution from added [1-14C]pyruvate although pyruvate decarboxylase is stimulated under these conditions as measured by ethanol production (Fig. 1). In contrast, the unexpected decrease in 14CO2 evolution could be reversed by aerobiciosis (Fig. 2b and c). Finally addition of cyanide inhibited pyruvate dehydrogenase (Fig. 2d) and enhanced glycolysis (Fig. 2d) as shown by a drastic decrease of 14CO2 evolution from exogenous [1-14C]pyruvate and an enhanced 14CO2 evolution from [3,4-14C]glucose (Fig. 2d).
The results found by addition of [1-14C]pyruvate suggest that exogenous pyruvate does not reach pyruvate decarboxylase and pyruvate dehydrogenase equally. Whereas it seems to have almost no access to the pyruvate decarboxylase reaction centre, it apparently has free access to the intramitochondrial compartment.

A difference between the metabolism of endogenous and of added pyruvate should be proven by addition of [3,4-14C]glucose. Indeed, Fig. 2 demonstrates a striking difference in 14CO2 evolution pattern after the addition of [3,4-14C]glucose as compared to the [14C]CO2 evolution pattern following addition of [1-14C]pyruvate to the cell suspension.

Under aerobic conditions a steady state is reached initially (Fig. 2a), which is then elevated by anaerobiosis (Fig. 2b). This indicates that endogenously derived [1-14C]pyruvate is metabolized by pyruvate decarboxylase as was shown in Fig. 1 by the production of ethanol. Again, increase of 14CO2 evolution from [3,4-14C]glucose during anaerobiosis was readily reversed by aerobicosis (Fig. 2c).

The presented data support the hypothesis of a complex of glycolytic enzymes which seems to be inaccessible to exogenous pyruvate. Concerning such enzyme interactions, only recently was a large body of evidence presented under the aspect of metabolic compartmentation for aldolase and glyceraldehyde-3-phosphate dehydrogenase of rabbit muscle [15, 16] and human erythrocytes [17]. However, the presented data did not rule out the possibility of metabolic dilution of exogenous [1-14C]pyruvate by enhanced pyruvate production under anaerobiosis or after the addition of cyanide. If the effects described in Fig. 2 were a result of metabolic dilution only, it should be possible to reach a higher dilution of evolved 14CO2 and thus a decreased 14CO2 evolution from [1-14C]pyruvate and from [3,4-14C]glucose by addition of increasing amounts of unlabelled pyruvate. Fig. 3 shows that such a dilution was only observed when exogenous [1-14C]pyruvate was the source of evolved 14CO2. In this experimental design, specific activity of [1-14C]pyruvate was chosen 40 fold that of [3,4-14C]glucose in order to be able to measure the penetration of pyruvate into the supposed functional compartment at least between pyruvate kinase and pyruvate decarboxylase.

Expectedly, the extracellular decrease of specific activity of [1-14C]pyruvate led to a decrease in 14CO2 evolution of the same order of magnitude as the dilution considering a present pyruvate concentration "at loco" of about 2 mM.
This value can be estimated from the data of Fig. 3 (●) and was already reported for a different system [18].

In the case of [3,4-14C]glucose as substrate (Fig. 3 (○)) such a dilution, even considering a 40 fold lower specific activity, was not observed.

The presented results tentatively might be interpreted on the basis of the observation of Lagunas [14]. This author was able to demonstrate an increasing Pasteur effect with prolonged ammonia starvation time. This might implicate that starvation enhances the degree of the cellular structural complexity for the benefit of a higher regulatory efficiency of the energy metabolism. We consider the presented data as further experimental evidence in favour of a glycolytic compartment for the starved and synchronous population of the strain of S. cerevisiae used in this investigation. This restriction has to be pointed out since the described effects are strongly reduced using nonsynchronous and unstarved, proliferating yeast cell populations.

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