OSCILLATIONS OF GLYCOLYTIC INTERMEDIATES IN YEAST CELLS

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Received May 1, 1964

In previous papers we have described cyclic responses of reduced pyridine nucleotide (RPN) in yeast cell suspensions which have been observed by direct spectrophotometry at 340 mμ (Chance, 1952, 1954, 1955), and by fluorometric methods (Chance, 1964a). Considerable interest has been aroused by the observation of a damped sinusoidal train involving over 12 consecutive oscillations observed in Saccaromyces carlsbergensis (ATCC 4228)[2] (Chance et al., 1964a,b).

In this paper, samples are removed periodically from the yeast cell suspension in which the reduced pyridine nucleotide is observed by continuous fluorometric monitoring to oscillate sinusoidally, and the fluctuations of the concentrations of the intermediates are recorded in relation to the fluctuations of RPN. In order to identify the control sites of glycolysis involved in this oscillation, the crossover theorem derived for identifying control sites in the respiratory chain (Chance et al., 1958) has been applied to the glycolytic system. In addition, the phase relationships of the metabolites has been determined.

EXPERIMENTAL METHODS

Growth of the cells is described elsewhere (Chance et al., 1964b). Metabolite assays were made according to the method of Maitra and Estabrook (1964), based upon improvements on the methods of Bücher as described by

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* Information on these oscillations was communicated to Dr. F. Hommes of Nijmegen, who has confirmed the existence of the oscillations and the fluctuations of the metabolite as described here.

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Bergmeyer (1962). The phase and amplitude of the oscillations of RPN were monitored continuously by a fluorometer, the cuvette of which was connected to a larger reservoir by means of a pump. Samples were withdrawn periodically at intervals precisely timed with the peaks and the nodes of the fluorescence change for Fig. 1. For Figs. 2 and 3, samples were withdrawn every 10 sec. Although glucose could not be added simultaneously to the sampling reservoir and the fluorometer cuvette, a minute and a half elapsed between the addition of glucose and the initiation of oscillation due to the aerobic-anaerobic transition. Thus, the material in the fluorometer was well mixed with that in the main sampling chamber.

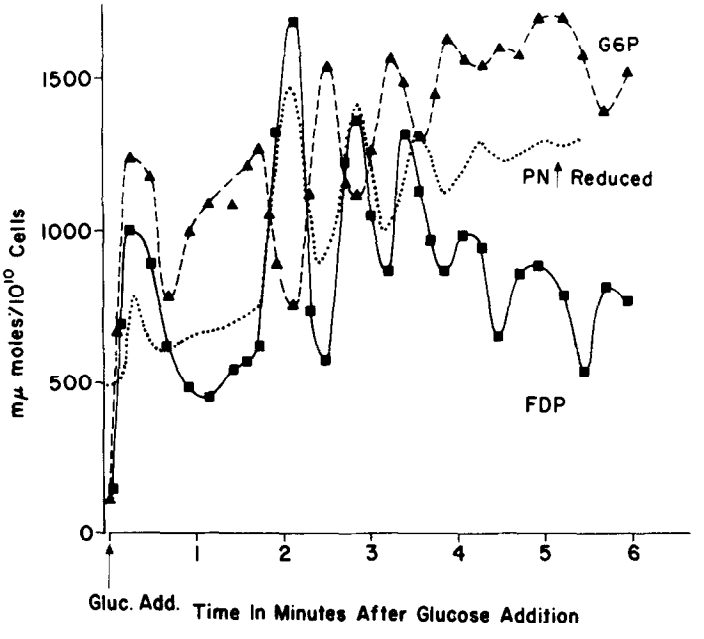

Figure 1. A damped sinusoidal oscillation of G-6-P and FDP measured simultaneously with fluorescence changes in a suspension of S. carlsbergensis (ATCC 4228). At the time of adding glucose (arrow) the cells are aerobic and spontaneously become anaerobic at approximately 100 sec after adding glucose. (Expt. 8-8-63).

EXPERIMENTAL RESULTS

Fructose diphosphate. A cyclic response of pyridine nucleotide to the addition of glucose or to the aerobic-anaerobic transition of Baker's yeast

cells is identified with the activation of DPN reduction by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Thus, the time course of changes of fructose diphosphate (FDP) concentration with respect to that of RPN is of greatest interest. Figure 1 shows damped sinusoidal oscillations of FDP that are in close synchrony with the oscillations of RPN, at least for one and one half full cycles of oscillation. It is apparent therefore, that the frequency and phase of the damped sinusoidal oscillations of FDP are closely similar to those of RPN. This is consistent with the idea that the oscillations of PN reduction observed are due to oscillations in the level of FDP.

Analyses were also carried out for glucose-6-phosphate (G-6-P). Here, the phase is shifted from that of FDP and RPN during the period for the two cycles in which RPN is oscillating sinusoidally. F-6-P was found to oscillate in phase with G-6-P.

Identification of the crossover point. While we are able to present assay data on many of the glycolytic intermediates, the value of such data is determined by their usefulness in identifying the control site of the oscillatory mechanism, i.e., the portion of the enzymatic system that is causing the oscillations. For this purpose, we employ the crossover theorem derived from the study of multisite controls in the multienzyme system comprising electron transport and oxidative phosphorylation (Chance, Higgins, Holmes and Connelly, 1958). A convenient graphical representation is to plot the name of the component in the linear sequence of enzymatic reactions against the percentage change of the component with respect to its mean value in a particular metabolic transition (a related plot has been used by Lowry et al. (1964a)). Referring to Figure 1 we identify a condition of maximum glycolytic flux with maximum pyridine nucleotide reduction and one of minimum glycolytic flux with minimum of pyridine nucleotide reduction. According to the crossover theorem, a control site will be identified under conditions of increasing flux by the point at which there is a crossover between a relative depletion of intermediates and a relative accumulation of intermediates, and vice versa with decreasing flux. Therefore we plot as

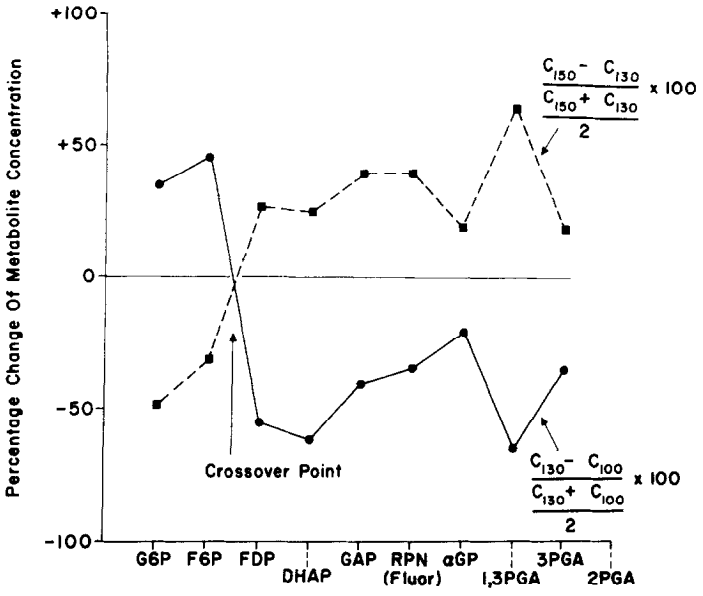

Figure 2. The crossover point for metabolite intermediates in damped sinusoidal oscillations of S. carlsbergensis (ATCC 4228). The abscissa represents the names of the intermediates and the ordinates, the percentage change of the intermediate, with respect to the mean value of its concentration. The concentrations are compared at 150 seconds with that at 130 seconds, and at 130 seconds with that at 100 seconds. A control site is identified by a depletion accumulation crossover point with increasing flux, and vice versa with decreasing flux. (Expt. 7-22-63)

values of the ordinate the change of concentration of the intermediates under conditions of increasing flux with reference to the average value of the concentration of the intermediates in the two states. In metabolite assays similar to those of Figure 1, a maximum of PN reduction was found at 150 sec after adding glucose and a minimum was found at 130 sec after adding glucose. We have therefore plotted percentage changes of the intermediates against the name of the intermediate in Figure 2 (dashed curve). It is apparent that intermediates G-6-P and F-6-P are depleted and intermediates from FDP to 3-phosphoglycerate (3-PGA) are accumulated, a clear example of a depletion-accumulation crossover point with increasing flux. We can also compare the minimum of PN reduction at 130 sec with the preceding maximum and thereby obtain the crossover point for conditions of diminishing

flux, as plotted as a solid line in Figure 2. Here, intermediates prior to the PFK step accumulate and those from FDP to 3-PGA are depleted. With decrease of flux, an accumulation-depletion crossover point marks the control site. Thus, we find that the same point is operative in increases and decreases of flux and conclude from this result that the metabolite control site in the oscillations of glycolytic intermediates is at PFK.

Phase plane plot. The kinetics of the glycolytic intermediates, in addition to indicating the control site according to the crossover theorem, may also provide incisive information on the type of oscillation mechanisms. The most appropriate graphical display of the oscillation characteristic is provided by the "phase plane plot", which in the terminology of biochemistry is represented by a graph of the concentration of one metabolite against that of the other one at various times throughout the oscillation cycles.

Three dominant characteristics of the phase plane plots can be recognized; first, a straight line at 45° slope indicates the two components to be oscillating in phase. Second, a straight line at 135° indicates that the phases of the two components are at 180°. An open figure indicates intermediate phase shifts between zero and 180°.

By taking samples every ten seconds throughout the interval from the addition of glucose until the completion of the oscillation, it has been possible to make incisive determinations of the phase relationships of the intermediates. Of particular interest are the relations of G-6-P and FDP, the two components on either side of the crossover point of Fig. 2. These data are plotted in Fig. 3. The values of the ordinate and the abscissa are in millimicromoles per 10^{10} cells. The graph starts at point 8, 80 sec after adding glucose, and then proceeds with points every 10 sec through to point 18, three min after adding glucose. Points 8 through 13 very nearly comprise a closed elliptical figure. A segment of a second elliptical begins with point 13 and continues through 15. There is apparently a distortion of the ellipticity at point 16, and beyond that the changes of G-6-P and FDP are proportional to each other. Also at this point the oscil-

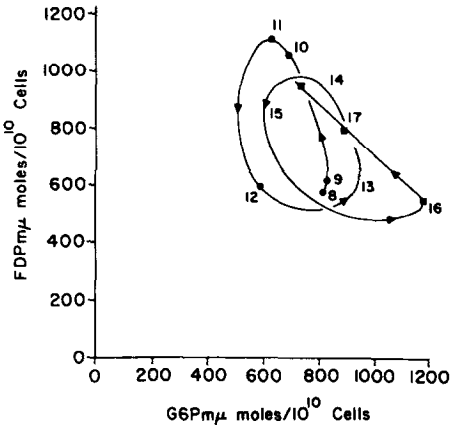

Figure 3. Phase plot diagrams for G-6-P and FDP. The concentration of G-6-P and FDP of Fig. 2 are plotted against one another at times after addition of glucose from 80 sec to 3 min. (Expt. 7-22-63)

lations observed fluorometrically have lost considerable amplitude. It is apparent, however, that the analytical data show an "open" phase plane plot for F-6-P and FDP with a center that moves toward higher G-6-P concentrations and lower FDP concentrations as the oscillations proceed. The "open" nature of the plot disappears as the oscillations subside.

DISCUSSION

The crossover theorem which requires only metabolite measurements corresponding to maxima and minima in the fluctuations has been applied to the identification of control sites in the respiratory chain and shows PFK to be the control site of oscillations in the glycolytic chain of S. carlsbergensis. A more incisive evaluation of the mechanism is provided by phase-plane diagrams which indicated a large phase shift between the substrate and the product at the PFK site. While it is possible that other factors contribute to the crossover phenomena involved, the identification of this site affords a basis for a chemical interpretation of the oscillation mechanism. This identification is supported by the characteristics of the oscillations in a cell free system (Chance, Hess and Betz, 1964). A minimum chemical mechanism for damped or continuous

sinusoidal oscillations involves a product activated step. PFK may be activated by FDP, albeit slowly (Lardy and Parks, 1956), or by ADP (Lowry and Passonneau, 1964b). This reaction combined with a first order step as a source and an enzymatic step as a sink will show damped or continuous oscillations (Higgins, 1964)

$$\text{GLU} \longrightarrow \text{F-6-P} \qquad (1)$$

$$\text{F-6-P} + E^{*} \longrightarrow E^{*} \cdot \text{F-6-P} \qquad (2)$$

$$E^{*} \cdot \text{F-6-P} \longrightarrow E^{*} + \text{FDP} + \text{ADP} \qquad (3)$$

At this point either ADP or FDP may convert an inactive form E^{+} of the enzyme to the active form (E^{*}). The activator may remain bound to the activated enzyme

$$E^{+} + \begin{matrix}\text{FDP} \\ \text{ADP}\end{matrix} \rightleftharpoons E^{*} \qquad (4)$$

Whichever is the activator, it must be expended, by aldolase in the case of FDP or by 1,3-diphosphoglycerate kinase in the case of ADP. In the case of FDP

$$\text{FDP} + E_2 \longrightarrow E_2 \cdot \text{FDP} \qquad (5)$$

$$E_2 \cdot \text{FDP} \longrightarrow E_2 + \text{GAP} \qquad (6)$$

SUMMARY

The damped sinusoidal oscillation of glycolytic intermediates of S. carlsbergensis (ATCC 4228) shows a close synchrony of FDP and RPN kinetics measured fluorometrically. A crossover point of the oscillatory metabolite pattern is found between G-6-P (F-6-P) and FDP identifying PFK as the control site. The phase relations of G-6-P and FDP are of especial interest; an open figure in the phase plane plot is obtained during maximal oscillations; the figure is more nearly closed as the oscillations subside. A reaction mechanism involving activation of PFK by one of its reaction products is proposed as the basic oscillator mechanism.

ACKNOWLEDGEMENT

The support of the National Institutes of Health and National Science Foundation for this research is gratefully acknowledged. The work was greatly assisted by Miss Sigrid Elsaesser.

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