DPNH OSCILLATIONS IN A CELL-FREE EXTRACT OF S. CARLSBERGENSIS[1]

Britton Chance, Benno Hess[2], and Augustin Betz[3]
Johnson Research Foundation
University of Pennsylvania
Philadelphia, Pennsylvania

Received May 1, 1964

In previous work we have examined cyclic and damped sinusoidal oscillatory responses of reduced pyridine nucleotide (RPN) in suspensions of yeast cells. The oscillation has been studied by metabolite assays which indicate a crossover point in the oscillation at phosphofructokinase (PFK)(Ghosh and Chance, 1964) and by analog computer studies (Higgins, 1964) which suggest that product activation of PFK is responsible for the sinusoidal waveform. This communication describes the observation of oscillations of DPNH level in a cell-free extract.

Preparations. S. carlsbergensis (ATCC 4228) is grown as described elsewhere (Chance, Estabrook, and Ghosh, 1964). Yeast cells are harvested and centrifuged to a hard pellet. The cells are mixed with a minimum of buffer and are ruptured in the Aminco pressure cell at a pressure of 8×10^3 lbs./sq. in. The cell contents are centrifuged from the debris and a translucent supernate of 93 mg protein/ml is obtained after centrifuging at 40,000 rpm in a Spinco Model L Centrifuge. The broken cell count is 10^4/ml, the intact cell count is 10^3/ml, as compared with almost 2×10^{10}/ml in the starting material. No supplements of enzymes or coenzymes were added to the cell extract; adequate activity remained for the rapid activation of DPN reduction on adding glucose-6-phosphate (G-6-P). Since the cells are washed in 0.1 M phosphate (KH_2PO_4; pH 6.5) no lack of phosphate is observed in these studies.

---

[1]This work was supported by PHS GM 12202

[2]Permanent address: Medical Clinic, Heidelberg University

[3]NATO Fellow

The supernate is studied without dilution in a 2 mm. optical path cuvette of a double beam spectrophotometer, employing interference filters (Chance and Legallais, in preparation). The wavelengths used are 345 mμ and 405 mμ. The recording was on an Esterline curvilinear chart, as indicated in Figure 1. The time scale is 4 min/large division, in contrast to 1 min/large division in the yeast cell experiments (Chance et al., 1964). Some non-linearity of the recording is encountered, since the absorbancy changes approach ± .20 per cent (corresponding to concentration changes of approximately 15 μM DPNH). The extract retained its oscillatory reaction for several hours.

EXPERIMENTAL RESULTS

Figure 1 indicates the oscillatory responses caused by the addition of 3 mM G-6-P to the yeast cell extract at 25°. Additions of ATP and DPN are not required. We have found it essential to evaluate these kinetics in terms of engineering parameters: the damping factor (the ratio of the amplitude in one direction to that in the opposite direction in a particular cycle of oscillation) and the "Q" (π times the number of oscillations required for the amplitude to fall by $^1/e$)(see Chance et al., 1964). A damped sinusoidal train of a frequency of 0.14 min^{-1}, a damping factor of 1.4, and a Q value of 4, is observed. For the intact cell oscillations, the frequency is 1.7 min^{-1}, the damping factor is 1.1, and Q is 8.

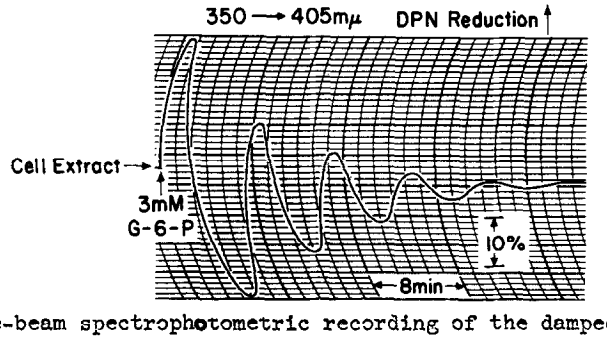

Fig. 1. Double-beam spectrophotometric recording of the damped sinusoidal oscillation of DPNH in a cell-free extract of S. carlsbergensis. pH, 6.5, temperature, 25°; protein, 93 mg/ml; optical path, 2 mm. (450-IV)

Effect of a temperature transient. With an extract aged less than several hours, no chemical supplement was needed to initiate the oscillations;

the temperature transient caused by warming the ice-cold extract to room temperature initiated an oscillation (Figure 2) with a frequency of 0.16 min^{-1} and a damping factor of 1.4. Apparently sufficient concentrations of endogenous intermediates were conserved at 0° that merely the transition to the higher temperature was needed to initiate the oscillations. This experiment suggests that the normal metabolic condition of the cell provides metabolite concentrations suitable for the oscillatory response.

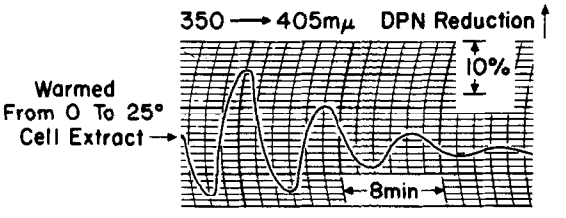

Fig. 2. Starting the oscillations by a temperature transient. The temperature of the extract, initially 0°, is allowed to rise at 25°. (Other conditions as in Fig. 1.)

Effect of fructose-diphosphate (FDP). After the oscillations due to warming to room temperature have subsided, the addition of 3 mM G-6-P (Fig. 1) or 3 mM FDP starts oscillations, with FDP an amplitude about 1/3 that observed with G-6-P (Fig. 3). The results clearly demonstrate that FDP itself may have an effect on PFK to initiate oscillations. As is characteristic of these extracts, the oscillations may be restarted by successive additions, and the latter part of Figure 3 indicates that a second addition of FDP starts the oscillations again with a readily measureable amplitude.

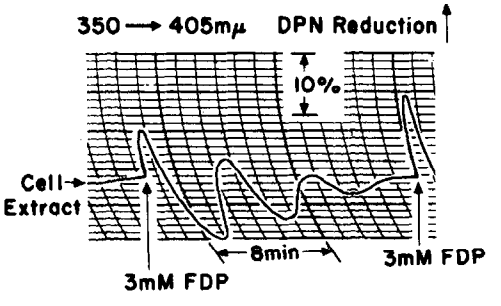

Fig. 3. Starting the oscillations by addition of 3 mM FDP. (Other conditions as in Fig. 1.)

Effect of iodoacetate. A cell-free system allows inhibitors such as iodoacetate to act rapidly at pH 6.5. In Figure 4, the system has been set into oscillation for some minutes by supplements of G-6-P and FDP. At the peak of the second oscillation cycle illustrated, 3 mM iodoacetate is added. In about a minute, the downward sweep of the trace indicates inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and consequent oxidation of DPNH by the accumulated acetaldehyde. Thus, the oscillations are rapidly terminated by inhibition of GAPDH. This result justifies our views concerning the role of this enzyme as the DPN-reducing enzyme in the oscillations (Chance et al., 1964). According to the mechanism for these oscillations, it is possible that fluctuations of the glyceraldehyde-3-phosphate (GAP) level would continue after the inhibition of GAPDH.

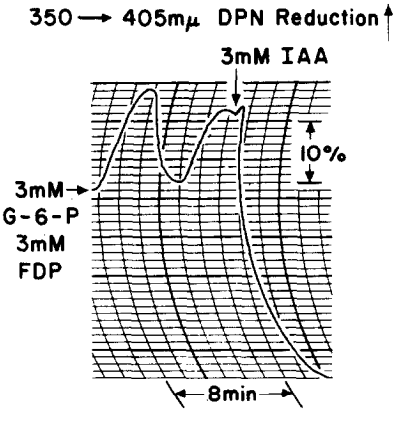

Fig. 4. Inhibition of the oscillations of DPNH by 3 mM iodoacetate. (Other conditions as in Fig. 1.)

Effects of ATP and AMP. The effect of ATP or AMP is generally to shift the steady state level of DPNH in the direction of oxidation or reduction, respectively. Their effects upon the oscillation depend upon the initial concentration already present and are in general small. Occasionally, AMP is observed to start the oscillations; G-6-P is far more effective.

DISCUSSION AND SUMMARY

The oscillations of DPNH can now be observed in a cell-free system. The period is 0.15 min^{-1}, approximately 10 times that observed in the intact cells (1.7 min^{-1}) at 25°. The damping factor is somewhat higher than observed in the intact cells (1.4 vs. 1.1) and the Q value has fallen to approximately 1/2 the maximum value observed (from 8 to 4). The oscillation will start spontaneously on warming the cell extracts from 0° to 25°, but optimal amplitude is obtained by adding FDP to the extract. The oscillations of DPNH are stopped rapidly by iodoacetate, leading to a large oxidation of DPNH. The experiments support in detail the previous data which indicated that GAPDH is the enzyme involved in the PN reduction observed in intact cells by absorption or fluorometry (Chance et al., 1964). The discovery of an essential role for G-6-P in the oscillation supports the proposed mechanism (Ghosh and Chance, 1964).

The remarkable characteristic of the oscillations is that they appear to have a period and a damping factor relatively independent of the three methods by which they may have been started.

The observation that the oscillation may be restarted by successive additions of G-6-P is of great interest, since it indicates the possibility of generating sustained oscillations by maintaining a constant level of G-6-P, either enzymatically or by the slow addition of this substrate.

This work has some significance to current views on metabolic control. While PFK plays a dominant role in the yeast cell oscillations, the phenomenon is relatively insensitive to AMP and ATP compared with G-6-P. Thus, the primary response in the Pasteur reaction may well be due to the effect of ADP on 1,3-diphosphoglycerate kinase, or of phosphate upon glyceraldehyde phosphate dehydrogenase (Estabrook, Maitra, and Chance, 1963).

It is obvious that a number of important experiments remain to be done with this extract, particularly the demonstration of sustained oscillations in the extract. However, these preliminary results lend strong support to the interpretation of our results in intact cells.

REFERENCES

Chance, B., Estabrook, R. W., and Ghosh, A., 1964. Proc. Natl. Acad. Sci., Vol. 51, in press.

Estabrook, R. W., Maitra, P. K., and Chance, B., 1963. In "Symposium on the Mechanism of Cellular Regulation in Bacteria", Marseilles, France. (J. Senez, ed.) CNRS, Paris, in press.

Ghosh, A., and Chance, B., 1964. Biochem. Biophys. Res. Comm. 16 174.

Higgins, J. J., 1964. Proc. Natl. Acad. Sci., Vol. 51, in press.