Pyridine nucleotide reduction associated with glucose metabolism by *Escherichia coli*

Pyridine nucleotides occupy a central role in substrate oxidations. The interplay of coupled enzyme systems, either dehydrogenases and cytochrome systems aerobically or two or more dehydrogenase systems during anaerobic fermentation, is directly reflected by the alteration of the steady-state reduction of endogenous pyridine nucleotides. This is illustrated by CHANCE's[1,2] observations on glucose metabolism with yeast and ascites tumor cells. Such studies, however, are often complicated by the presence of compartmentation of nucleotides or metabolites into cytoplasmic and mitochondrial pools[3,4]. Since bacteria may serve as a source of cells which obviate the inclusion of a compartmentation hypothesis, studies have been initiated to determine whether comparable reactions of pyridine nucleotide could be measured with *Escherichia coli*. In addition bacteria, with their variety of metabolic pathways, offer an alternative method of investigating the parameters affecting the function of pyridine nucleotides in integrated enzyme systems.

The present report illustrates preliminary studies employing a sensitive fluorometric technique to monitor the changes in steady-state reduction of the endogenous pyridine nucleotides of *E. coli* during glucose utilization and to describe the results of some studies with chemicals known to alter oxidative and phosphorylative reactions. The fluorescence of reduced pyridine nucleotide was measured with a modified Eppendorf fluorometer in a manner analogous to that described by DUYSENS AND AMESZ[5] or CHANCE *et al.*[6]. In these studies, *E. coli* strain B was grown for 1.5 h in the synthetic medium M-52 as described by COHEN[7] employing glucose as carbon source. The cells were harvested by centrifugation, washed with 0.9% NaCl, and finally suspended in 0.9% NaCl to a concentration of about 10^{11} cells/ml. Under these conditions the contribution of endogenous substrates interfering with the metabolism of exogenous glucose is minimized.

The result of a typical experiment is shown in Fig. 1. When the resuspended cells of *E. coli* are diluted with 0.1 *M* phosphate buffer at pH 7.4 in the presence of NH_4^+, a slow oxidation of intracellular pyridine nucleotide occurs. This is associated

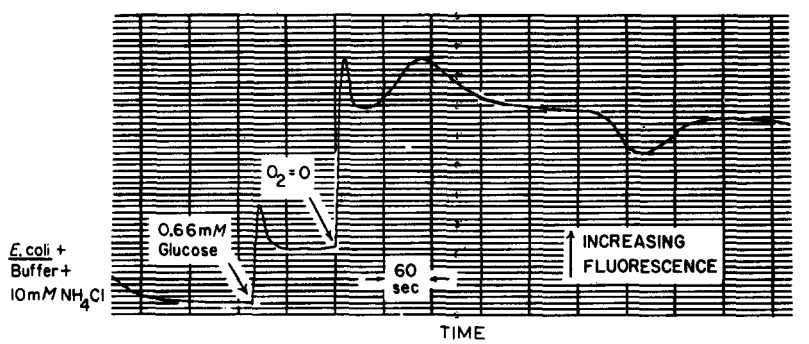

Fig. 1. 0.2 ml of a washed suspension of *E. coli* (containing approx. 10^{11} cells/ml) was diluted to 3.2 ml with 0.1 *M* phosphate buffer (pH 7.4) containing 9 m*M* NH_4Cl. At the time indicated 0.02 ml of a 0.1 *M* solution of glucose was added. An upward deflection on the tracing indicates an increase in fluorescence. The depletion of oxygen is indicated by the abrupt change in the extent of reduction of pyridine nucleotide. Temperature, 25°.

with the slow depletion of the residual endogenous substrate as determined in parallel polarographic measurements of the rate of oxygen utilization. The addition of glucose to the suspension of cells causes a rapid increase in fluorescence, indicative of the formation of reduced pyridine nucleotide. Concomitant with this pyridine nucleotide reduction is an increase of greater than 10-fold in the rate of oxygen utilization. In a parallel experiment samples were withdrawn every 15 sec and the reaction terminated by addition of the samples to a protein precipitant (trichloroacetic acid or KOH). Chemical analyses of these extracts were carried out to determine the nature of the pyridine nucleotide reduced during the aerobic cycle of reduction, as illustrated in Fig. 1. These analyses showed only DPN^+ or DPNH to be present in trichloroacetic acid extracts or alkaline extracts respectively (during the aerobic steady state of glucose oxidation the concentrations of DPNH and DPN^+ are $4 \cdot 10^{-10}$ and $16 \cdot 10^{-10}$ mole/mg dry wt., respectively). These values are in agreement with the determination of total pyridine nucleotide content of *E. coli* as recently reported by IMSANDE[8] as well as by KAPLAN[9]. Repeated analyses failed to demonstrate any measurable concentration of TPN^+ or TPNH under these conditions. This failure to observe TPN^+ or TPNH may be a consequence of the low levels of TPN in *E. coli*, *i.e.* less than 10% of the concentration of DPN (*cf.* IMSANDE[8]). The observation that DPN is reduced upon addition of glucose implies that the enzymes of glycolysis, notably triosephosphate dehydrogenase, are predominantly operative[10]. This interpretation that the initial reduction of pyridine nucleotide results mainly from oxidative reactions associated with the glycolytic pathway rather than the pentose phosphate shunt is also supported by other studies on the pattern of pyridine nucleotide reduction and the inhibition of oxygen uptake observed in the presence of 1.5 m*M* iodoacetic acid. In this case addition of glucose causes a rapid reduction of pyridine nucleotide and then a slower (approx. 30 sec) reoxidation to the level observed prior to glucose addition.

As shown in Fig. 1 the initial reduction of pyridine nucleotide observed on glucose addition is transient, followed by a slower decrease in the level of reduced pyridine nucleotide and the establishment of an aerobic steady state of oxidation. This aerobic oxidation of pyridine nucleotide is similar to that observed with yeast by CHANCE[1] and DUYSENS AND AMESZ[5] and could result from the interaction of DPNH either with the electron-transport system or with other coupled reactions at the substrate level. Experiments with *E. coli*, pretreated with lactate, did not alter this aerobic response of reduced pyridine nucleotide precluding the interpretation that the lactate – pyruvate ratio is effective in modifying the slower aerobic oxidative phase of the cycle. A similar interpretation is applicable to the initial anaerobic cycle of reduction. Preincubation of the cells with 2-deoxyglucose, however, completely abolishes the aerobic overshoot without altering the respiratory rate.

Fig. 1 also shows that about 2 min after the addition of glucose there is a rapid increase in the concentration of reduced pyridine nucleotide. Parallel polarographic measurements of oxygen utilization indicate that the oxygen dissolved in the reaction medium is exhausted at this time. This onset of a change in metabolism is reflected in a cyclic response of the pyridine nucleotide, in this case illustrative of the transition to anaerobic fermentation. Associated with anaerobic fermentation are three distinctive transitions of the steady state of pyridine nucleotide. These have been designated as the first, second, and third anaerobic cycles. Interference of glycolysis by the addition of iodoacetic acid or sodium arsenate markedly alters the response

of pyridine nucleotide (the first anaerobic cycle) observed during the transition to anaerobiosis. In the presence of these inhibitors, pyridine nucleotide becomes reduced on the transition from aerobiosis to anaerobiosis, but with no "overshoot" of reduction. Titration studies with the uncoupling agent, dibromophenol, indicates that a concentration of 0.6 m*M* is sufficient to suppress this first anaerobic cycle of pyridine nucleotide reduction. The second and third anaerobic cycles depend upon the presence of NH_4^+ in the reaction medium. The second cycle, however, is variable and not always as pronounced as shown in Fig. 1. In addition, the association of the third anaerobic cycle with glucose depletion is suggested from studies showing that the time of onset of this cycle is directly dependent on the concentration of bacteria and glucose employed, *i.e.* its time of appearance is more prolonged at low concentrations of bacteria or with higher concentrations of glucose. Analysis of glucose uptake during this reaction shows that this third anaerobic cycle appears when the level of glucose is decreased to about 0.1 m*M*.

The present demonstration of the ability to measure changes in pyridine nucleotide reduction during bacterial metabolism offers a method, previously unexamined, for extending our knowledge of bacterial metabolism and the factors controlling this metabolism. An examination of the effect of inhibitors or uncoupling agents on the transient steady-state changes of pyridine nucleotide reduction should afford an intimate view of the enzyme systems operative during the integrated metabolism of this organism as well as other bacteria.

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