VIII. EXPERIMENTS ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE OF *S. FAECALIS*

CHAPTER-VIII

EXPERIMENTS ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE OF S. FAECALIS

It was observed earlier that respiration in cell-suspension of S. faecalis was inhibited by SRC-820 (Chapter-V) at the initial stages and thereafter this inhibition was found to decrease. One of the explanations could be that an alternate pathway of utilization of glucose becomes operative under the influence of SRC-820. It was also reported that Glucose-6-Phosphate Dehydrogenase (G-6-PDH) is present in S. faecalis cells which can utilize glucose by H.M.P. pathway (Sokatch and Gunsalus, 1954; Cf. Gunsalus et al, 1955; Sokatch et al, 1956). It was therefore thought worthwhile to study the effect of this compound on the activity of G-6-PDH of the cell-extracts.

Glucose-6-Phosphate Dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, E.C.1.1.1.49) originally called 'Zwischenferment' catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconate in presence of NADP. Glucose-6-Phosphate occupies a pivotal position in carbohydrate metabolism as it forms a branch point between the EMP and H.M.P. pathways.

The enzyme G-6-PDH is widely distributed in animal tissues, plants and microorganisms and requires NADP for its activity. However, it has been reported that in some microorganisms, the enzyme lacks coenzyme specificity and can utilize both NAD^+ and $NADP^+$ (Wood and Schwerdt, 1954; DeMoss, 1955; Tabita and Lundgren, 1971; Benziman and Mazova, 1973; Vander Wyk and Lessie, 1974; Doelle, 1975). This lack of coenzyme specificity was explained as due to NAD(P) trans-dehydrogenase present in these cells (Colowick et al, 1952).

Results:

The activity of glucose-6-phosphate dehydrogenase was determined in extracts prepared from slow-vacuum dried cells of S.faecalis (see Methods and Materials). The assay of G-6-PDH was carried out using both the 2:6-Dichlorophenolindophenol (DCIP) (Mahler, 1955) and the spectrophotometric methods (Kornberg and Horecker, 1955).

It should be mentioned that the method of Lipman and Tuttle (1945), using hydroxylamine was not found to be suitable in this case, as it inhibited the activity of the enzyme.

A. Assay of G-6-PDH by Spectrophotometric Method:

1. Effect of Mg^{2+}, NaCl and Other Ions:

When G-6-PDH activity was followed spectrophotometrically, it was found that measurable activity was obtained only when the slow-vacuum dried cell-extracts was pre-incubated in buffer alongwith Mg^{2+} and NADP for 5 min. In the absence of added Mg^{2+} ions, the activity of G-6-PDH was found to be very poor. It was found that activity increases with increase in the concentration of Mg^{2+}. A range of Mg^{2+} concentrations from 3.3×10^{-4} M to 3.3×10^{-3} M was studied in this experiment. The maximum activity was reached at a level of 3.3×10^{-3} M Mg^{2+}. It was also observed that a concentration of Mg^{2+} below 1.67×10^{-3} M was ineffective in stimulating the enzyme activity.

As reported by other workers, that G-6-PDH from various microorganisms like *P. aeruginosa* (Lessie and Neidhadt, 1967), *T. ferroxldans* (Tabita and Lundgren, 1971), *E. coli* (Sanwal, 1970), *Zymomonas mobilis* (Sly and Doelle, 1968), *A. niger* (Jagannathan *et al*, 1956) and yeast (Glaser and Brown, 1955) was stimulated by Mg^{2+} ions similar to what was observed in the present investigation with *S. faecalis*.

In contrast, however, G-6-PDH from another organism belonging to the same family, L. mesenteroids was reported not to be stimulated by Mg^{2+} (Olive and Levy, 1967; Cf. DeMoss, 1953).

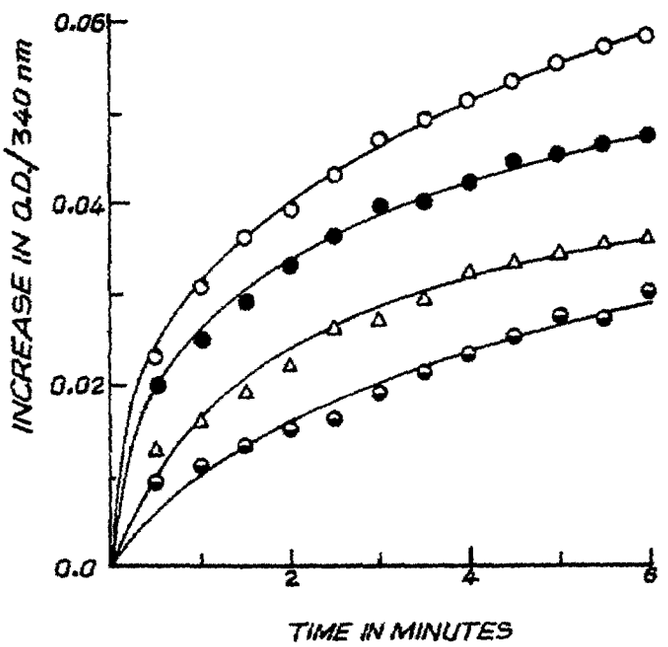
The activity of G-6-PDH was also assayed using several buffers like Tris, glycylglycine, phosphate and Krebs-Ringer phosphate buffer all at pH 7.5 (Fig.18). As can be seen from Fig.18 that among the buffers, highest activity could be seen in Krebs-Ringer phosphate buffer. The activity was found to be the least in Tris buffer.

High activity in Krebs-Ringer phosphate buffer can be due to the presence of Cl^- ions in this buffer which is known to have a stimulating effect on the enzyme (Mangiarotti and Garre', 1965; Mangiarotti et al, 1965; Cf. Pontremoli and Grazi, 1969). Addition of NaCl (1.23×10^{-1} M) to glycylglycine buffer, restored the activity to that obtained in Krebs-Ringer phosphate buffer, pH 7.5.

Effect of other ions on the activity of G-6-PDH such as Ca^{2+}, Zn^{2+}, K^+, SO_4^{2-} and PO_4^{3-} was also studied. Calcium ions (3.3×10^{-3} M) were also found to stimulate the enzyme activity. Zn^{2+}, on the other hand, at a level of 3.3×10^{-3} M exerted 50% inhibition. At a level of

Fig.18

Comparative activities of Glucose-6-phosphate dehydrogenase in different buffers.



Assay System:

Reaction mixture contained in a total volume of 3 ml (in µmoles): $MgCl_2$, 10 (not in Krebs Ringer phosphate); G-6-P, 5; NADP, 0.1; 3 hr. dialysed cell-extract (sup.), 0.5 ml equivalent to 8 mg of slow-vacuum dried cells and different buffers as following:

O—O Ca^{2+}-free Krebs Ringer phosphate, pH 7.5, 1 ml (3 times concentrated); ●—● glycylglycine buffer, pH 7.5 (20 µmoles); Δ—Δ phosphate buffer, pH 7.5, (50 µmoles) and ◒—◒ Tris-buffer, pH 7.5 (60 µmoles).

Cell-extract was pre-incubated with $MgCl_2$ at room temp. for 5 min. Where KR phosphate buffer was used, extract was pre-incubated with buffer as before.

Reaction started by addition of NADP.

2.3×10^{-5} M, very little inhibition was seen. K^+, SO_4^{2-} and PO_4^{3-} were found to be ineffective. In this connection, it should be pointed out that G-6-PDH from yeast was reported to be inhibited by K^+, Na^+ and NH_4^+ ions whereas Cl^- and SO_4^{2-} were reported to activate the enzyme (Pontremoli and Grazi, 1969).

2. Effect of SRC-820·HCl:

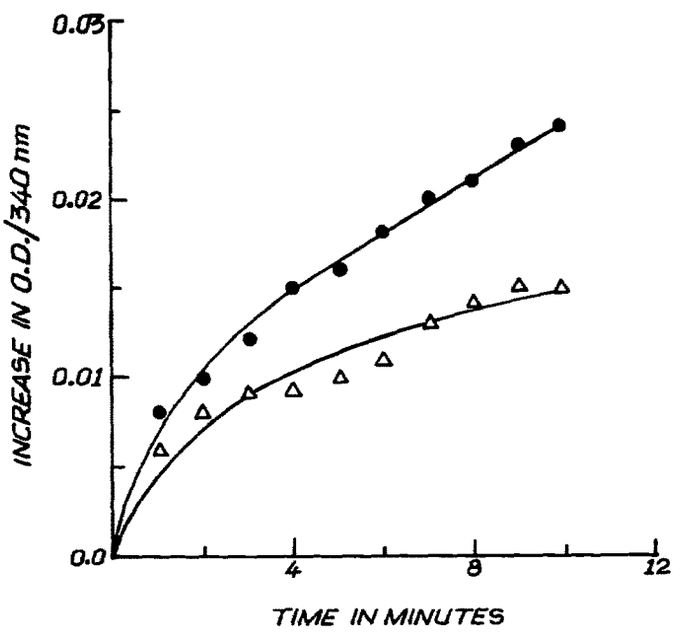
SRC-820·HCl (1×10^{-2} M) was found to exert an inhibitory effect on glucose-6-phosphate dehydrogenase activity. Inhibition to the extent of 50% was seen (Fig.19) when glycylglycine buffer (in the absence of NaCl) was used. Such an inhibitory effect observed in glycylglycine buffer was not seen when the experiment was carried out in Krebs-Ringer phosphate buffer probably due to the presence of a good amount of Cl^- ions in the buffer.

B. G-6-PDH by DCIP Method:

When the activity of G-6-PDH was followed by using the artificial electron acceptor, 2:6-dichlorophenolindophenol (DCIP), 50% more activity was seen in krebs-Ringer phosphate buffer than in glycylglycine buffer.

Fig. 19

Effect of SRC-820 on Glucose-6-phosphate dehydrogenase



Assay System:

Reaction mixture contained in a total volume of 3 ml (in μmoles): glycylglycine buffer, pH 7.5, 20; $MgCl_2$, 10; G-6-P, 5; NADP, 0.1 and 2 hr. dialysed cell-extract (sup.), 0.2 ml equivalent to 2 mg of slow-vacuum dried cells and SRC-820, 30 when present.

Cell-extract was pre-incubated with $MgCl_2$ and NADP at room temp. for 5 min.

Reaction started by addition of G-6-P.

●—● Control; △—△ With SRC-820 HCl.

It was also observed that NAD^+ at even 10 times more concentration cannot replace $NADP^+$ as coenzyme in this reaction.

Effect of SRC-820:

SRC-820·HCl (1×10^{-2} M) by this method was found to inhibit the activity by about 45% in glycyl-glycine buffer. However, in Krebs-Ringer phosphate buffer, the inhibitory effect of SRC-820 was found to be less (about 25%), similar to what was seen in the spectrophotometric assay.

SUMMARY

1. Activity of Glucose-6-phosphate dehydrogenase (G-6-PDH) was demonstrated in extracts from slow-vacuum dried cells of S.faecalis. The activity was followed spectrophotometrically as well as by 2,6-DCIP method using $NADP^+$ as the coenzyme.

2. NAD^+ cannot replace $NADP^+$ as coenzyme in this reaction.

3. G-6-P dehydrogenase from S.faecalis requires Mg^{2+} for its activity.

4. Activity of G-6-P dehydrogenase was highest in Krebs-Ringer phosphate buffer, pH 7.5 and lowest in Tris buffer of the same pH.

5. Chloride (as NaCl, 1.23×10^{-1} M) ions and Ca^{2+} (3.3×10^{-3} M) stimulate G-6-PDH activity of S.faecalis.

6. Zn^{2+} (3.3×10^{-3} M) inhibits G-6-PDH activity by about 50%. Other ions such as K^+, SO_4^{2-} and PO_4^{3-} do not affect the activity.

7. In the absence of added chloride ions, SRC-820·HCl (10^{-2} M) inhibits the activity of

G-6-PDH (spectrophotometric method) by about 50% (glycylglycine buffer, pH 7.5).

8. When G-6-PDH assay was carried out by DCIP method, SRC-820 (10^{-2} M) inhibited the activity in the absence of added chloride ions, by about 45%. Less inhibition (25%) was seen when Cl^- were present.