VII. ENOLASE IN ACETONE POWDER EXTRACTS OF S. FAECALIS

CHAPTER-VII

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Introduction:

The results obtained in the study with viable-cell-respiration (Chapter-V, "Experiments on S.faecalis Respiration") indicated that the metabolism of glucose in this organism takes place by the Embden-Meyerhof pathway and is inhibited by sodium fluoride. When sodium fluoride and SRC-820 were present together, respiration was almost completely abolished (see Fig.10 in Chapter-V), and this observation led us to study the effect of SRC-820 on the activity of enolase in cell-extracts.

Enolase (2-Phospho-D-glycerate hydro-lyase, EC.4.2.1.11) catalyzes the removal of a molecule of water from 2-phosphoglyceric acid (2-PGA) (Warburg and Christian, 1941; Holt and Wold, 1961). The enzyme from both yeast and animal as well as the plant cells was reported to require divalent metal ions such as Mg^{2+}, Mn^{2+} or Zn^{2+} for activity (Stumpf, 1950; Malmström, 1953, 1955).

Zn^{2+} and Mn^{2+} are more effective at lower concentrations than Mg^{2+} but they inhibit at higher concentrations (Malmström, 1953). It is well known that, in the presence of Mg^{2+} and phosphate, fluoride exerts a strong inhibitory effect on enolase. A magnesium-fluorophosphate complex is presumed to be formed thereby effectively removing Mg^{2+} from the reaction mixture (Warburg and Christian, 1941; Cf. Malmström, 1961 and Wold and Ballou, 1957).

Results:

The activity of enolase was followed in acetone powder extracts using 3-phosphoglyceric acid (3-PGA) as substrate (to generate 2-PGA). The formation of phosphoenolpyruvate (PEP) was determined spectrophotometrically at 240 nm according to the method of Warburg and Christian (1941) (Cf. Bücher, 1955). Also the effect of sodium fluoride and EDTA as well as the requirement for Mg ions for the activity were determined by this method.

The influence of SRC-820 could not be demonstrated by the spectrophotometric method as this compound has high absorption at 240 nm even at very low concentrations & for this, estimation of released inorganic phosphate (Simmonsen et al, 1947) after alkaline hypoiodide treatment (Lohman and Meyerhof, 1934) was determined.

The acetone powder extract was found to be active in catalyzing the formation of PEP from 3-phosphoglyceric acid at pH 7.0. The activity was found to increase with increase in the amount of extract used. Thus, with 0.2 ml of extracts equivalent to 2 mg of acetone powder, a rise of 0.16 in O.D. at 240 nm in 10 min was observed.

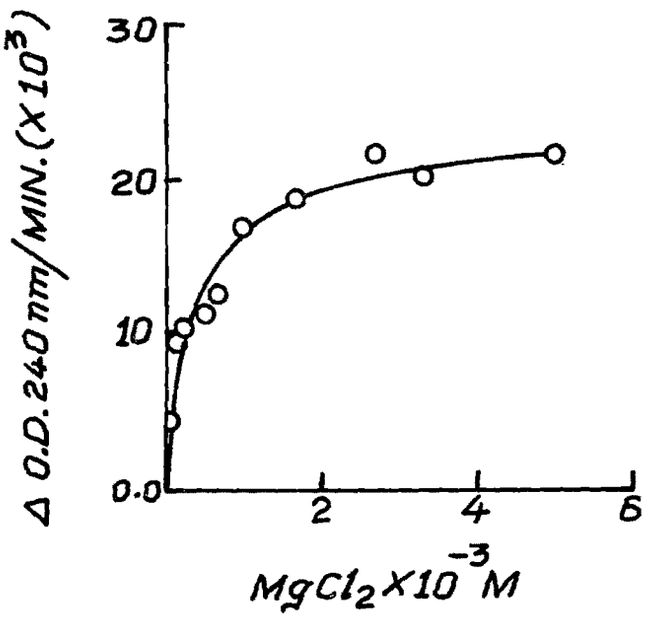
Different concentrations ofm 3-PGA ranging from 1.67×10^{-3} M to 16.7×10^{-2} M were studied and it was found that the activity increases upto a level of 3.3×10^{-3} M substrate concentration. Beyond this level, no further increase was seen. In subsequent experiments, the concentration of substrate was kept constant at 3.3×10^{-3} M.

1. Requirement of Mg^{2+} :

It was observed that in S. faecalis also, magnesium ions are required for PEP formation from 3-PGA. Increasing the amount of magnesium showed a proportional increase in PEP formation. In the absence of added Mg^{2+}, a slight increase in O.D. at 240 nm could be seen only after 2 min of incubation period. Fig.16 represents the rate of PEP formation against the concentration of Mg^{2+}. As can be seen from Fig.16, magnesium at a concentrations of 2.67×10^{-3} M gave the maximum activity.

Fig. 16

Effect of Mg^{2+} on Phosphoenolpyruvate formation.



Assay System:

Reaction mixture contained in a votal volume of 3 ml (in μmoles): Tris-buffer, pH 7.0, 55; 3-PGA, 10; 16 hr. dialysed cell-extract (sup.), 0.1 ml equivalent to 1 mg of acetone powder and $MgCl_2$ as shown in figure.

Reaction started by addition of cell-extract.

2. Effect of Sodium Fluoride:

Complete inhibition of activity was observed when 1.67 X 10^{-2}M concentration of NaF was present. As can be seen from Fig.17, inhibition of PEP formation increases with increase in concentration of NaF. About 16% inhibition was observed at a level of 1.33 X 10^{-3}M of NaF and about 60% inhibition at a concentration of 2.67 X10^{-3}M.

Bergmeyer (1965) had reported that 10^{-3}M fluoride causes a 60% inhibition in muscle enolase activity also in phosphate free medium.

3. Effect of EDTA:

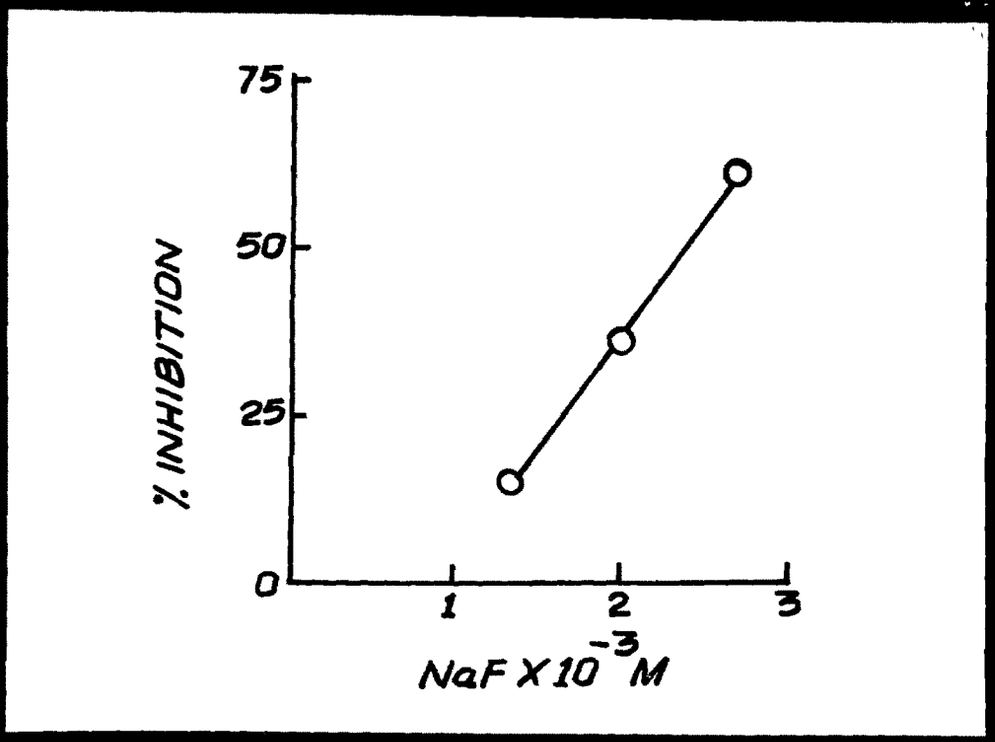
Disodium ethylenediaminetetraacetate (EDTA) at a level of 6.7 X 10^{-3}M completely inhibited PEP formation. EDTA at a concentration of 1.67 X 10^{-3}M exerted about 57% inhibition. The concentration of Mg^{2+} in this experiment was kept at a level of 10 uM/3 ml of reaction mixture.

4. Effect of EDTA, fluoride and SRC-820 on PEP formation:

In the following experiment, the influence of SRC-820·HCl was studied on PEP formation using less amount of Mg^{2+} (i.e. 5.5 µM/3 ml of reaction mixture) and the concentration of EDTA was also reduced to a level of 1.17 X 10^{-3}M.

Fig. 17

Effect of sodium fluoride on Enolase activity.



Assay System:

Reaction mixture contained in a total volume of 3 ml (in μmoles): Tris-buffer, pH 7.0, 100; $MgCl_2$, 10; 3-PGA, 10; 16 hr. dialysed cell-extract (sup.), 0.1 ml equivalent to 1 mg of acetone powder and the concentrations of NaF, as shown in figure.

Reaction started by addition of cell-extract.

Effect of SRC-820 either alone or with EDTA (System-A, Table-XII) or with fluoride (System-B, Table-XII) was studied. As can be seen from Table-XII, when Mg concentration was kept low (5.5 µM/3 ml of reaction mixture), SRC-820·HCl alone exerted an inhibitory effect of about 28% on PEP formation while EDTA inhibited the activity by about 24%. When both these inhibitors were present together, the inhibition was found to be not the sum but nearly the same as that exerted by either of these two substances.

Inhibition due to SRC-820·HCl was found to be reversed when magnesium concentration was increased to 10 µM/3 ml of reaction mixture. Under these conditions, fluoride exerted an inhibition of about 76% and when both SRC-820·HCl and fluoride were present together, no synergistic effect could be seen as in the case of respiration experiments (Chapter-V).

TABLE-XII

EFFECT OF EDTA, FLUORIDE AND SRC-820 ON PHOSPHOENOLPYRUVATE (PEP) FORMATION

Additions	PEP formed uM/3 ml Reaction Mixture	Inhibition (%)
System-A		
No inhibitor (3)	1.19 ± 0.04	-
+ Edetate (1.17 X 10^{-3}M) (4)	0.90 ± 0.07	24.0
+ SRC-820 (1 X 10^{-2}M) (4)	0.85 ± 0.08	28.1
+ Edetate (1.17 X 10^{-3}M) and SRC-820 (1 X 10^{-2}M) (4)	0.82 ± 0.02	31.1
System-B		
No inhibitor (5)	1.13 ± 0.01	-
+ Sodium fluoride (4) (2.5 X 10^{-3}M)	0.27 ± 0.03	76.1
+ SRC-820 (1 X 10^{-2}M) (4)	1.01 ± 0.03	10.6
+ Sodium fluoride (3) (2.5 X 10^{-3}M) and SRC-820 (1 X 10^{-2}M)	0.22 ± 0.02	80.6

Figures in parentheses represent number of experiments.

System-A: Contained in a total volume of 3 ml (in µmoles): Tris-HCl (pH 7.0), 60; $MgCl_2$, 5.5; 3-PGA, 10; cell-extract, 0.2 ml equivalent to 2 mg of acetone powder. Incubation for 2.5 min at 37°C.

System-B: Contained in a total volume of 3 ml (in µmoles): Tris-HCl (pH 7.0), 110; $MgCl_2$, 10; 3-PGA, 10; cell-extract 0.1 ml equivalent to 1 mg of acetone powder. Incubation for 5 min at 37°C.

SUMMARY

1. Acetone powder extracts of S.faecalis catalyze the conversion of 3-phosphoglyceric acid (3-PGA) to phosphoenolpyruvic acid (PEP). The activity was followed spectrophotometrically as well as colourimetrically by estimating the released inorganic phosphate from PEP.
2. S.faecalis enolase requires Mg^{2+} for its activity.
3. Sodium fluoride (2.67×10^{-3} M) inhibits enolase activity, by about 60%.
4. EDTA (1.67×10^{-3} M) also inhibits the activity of enolase by about 57%.
5. SRC-820 HCl (10^{-2} M) inhibits the activity by about 28%.
6. Inhibition due to SRC-820·HCl can be reversed by addition of more magnesium.
7. SRC-820 (10^{-2} M) either alone or with EDTA (1.17×10^{-3} M) exerts nearly the same amount of inhibition as that of either of these two inhibitors and not the sum.
8. Similarly, SRC-820 (10^{-2} M) either alone or with NaF (2.5×10^{-3} M) exerts nearly the same inhibition as that of sodium fluoride.