On the Regulatory Properties of a Halophilic Malic Enzyme from *Halobacterium cutirubrum*

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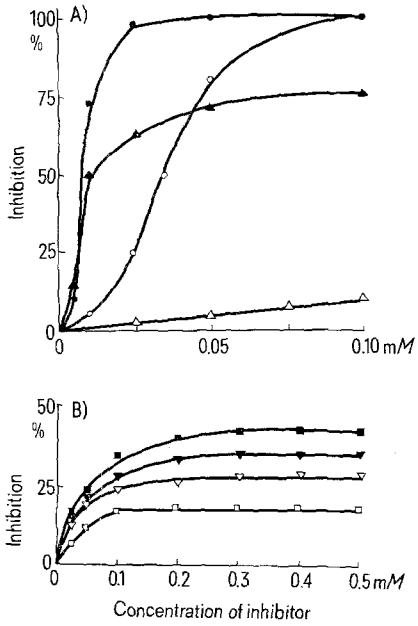
Summary. The NADP-linked malic enzyme from *Halobacterium cutirubrum* is strongly inhibited by acetyl-CoA and NADH, and rather weakly inhibited by oxaloacetate and glyoxylate, in the presence of very high KCl concentrations (3 *M*), considered physiological for the extremely halophilic bacteria.

NADP-linked malic enzyme (EC 1.1.1.40) is inhibited by several metabolites in microorganisms like *Escherichia coli*[2,3] and a marine Pseudomonad[4,5]. The extreme halophile, *Halobacterium cutirubrum*, contains a NADP-linked malic enzyme[6], which is strongly inhibited by acetyl-CoA[7]. We show in this communication that the enzyme is also inhibited by NADH, oxaloacetate and glyoxylate; these inhibitors, as well as acetyl-CoA, were effective in the presence of a high concentration of KCl (3 *M*), supposed to be physiological for the extreme halophile[8].

Malic enzyme was purified about 50-fold, with a yield of nearly 10%, by a method involving obtention of crude extract by sonic disintegration followed by treatment with deoxyribonuclease I[7], ultracentrifugation at 150,000 × *g* for 150 min, fractionation with saturated ammonium sulphate solution[9], the enzyme being precipitated between 3 and 3.7 *M* ammonium sulphate, and hydroxylapatite chromatography[10]. The latter was performed with buffer solutions containing 3 *M* KCl, 0.1 m*M* EDTA and increasing concentrations of potassium phosphate, pH 7.0; the bulk of the enzyme activity was eluted by the 250 m*M* potassium phosphate buffer. The partially purified enzyme (specific activity 0.3 μmoles of NADPH formed/min/mg of protein, when assayed as described in the legend to the Figure, in the presence of 5 m*M* L-malate) was dialyzed against 0.05 *M* *Tris*-HCl buffer (pH 7.6), 5 *M* NaCl, 1 m*M* EDTA and used for the experiments described. The enzyme preparation was free of acetyl-CoA-deacylating activity, and was unable to oxidize NADPH, either in the absence or in the presence of oxaloacetate or glyoxylate, or NADH. Further purification was prevented by the enzyme instability and its lack of reactivation from the salt-free state.



Inhibition of the malic enzyme from Halobacterium cutirubrum by acetyl-CoA, NADH, oxaloacetate and glyoxylate. The enzyme activity was assayed spectrophotometrically at 340 nm, at 30 °C, as previously described[7], in the presence of 1 m*M* L-malate, 0.1 m*M* NADP, 2 m*M* $MgCl_2$ and 3 *M* KCl (closed symbols) or 1 *M* NH_4Cl (open symbols). The inhibitors were added at the concentrations stated on the abcissa. The enzyme activity in the absence of inhibitors was 0.07 or 0.13 μmoles of NADPH formed/min/ml of enzyme preparation, in the presence of 3 *M* KCl or 1 *M* NH_4Cl, respectively. A) Inhibition by acetyl-CoA (○, ●) or NADH (△, ▲). B) Inhibition by oxaloacetate (□, ■) or glyoxylate (▽, ▼).

As previously described for crude enzyme preparations[6], the partially purified malic enzyme was optimally activated by 1 *M* NH_4Cl; KCl was also an effective activator, at concentrations of 3 *M* or higher. The divalent cation requirement characteristic of malic enzyme[11] was satisfied by Mn^{2+} or Mg^{2+}; the apparent K_a values (in the presence of 5 m*M* L-malate and 0.1 m*M* NADP) were about 5 μ*M* and 0.2 m*M*, respectively. These values were nearly the same in the presence of 1 *M* NH_4Cl or 3 *M* KCl. The apparent K_m value for L-malate (NADP fixed at 0.1 m*M*) was about 2.9 m*M* or 6.6 m*M*, in the presence of 2 m*M* $MnCl_2$ or $MgCl_2$, respectively. Since substrate inhibition was evident at L-malate concentrations higher than 5 m*M*, the apparent K_m values were obtained by extrapolation of the asymptotes to the curved double reciprocal plots, according to Cleland[12]. The apparent K_m value for NADP (L-malate fixed at 5 m*M*) was about 17 μ*M* or 25 μ*M*, in the presence of 2 m*M* $MnCl_2$ or $MgCl_2$, respectively. Although the nature of the monovalent cation activator did not significantly change the apparent K_m values for both substrates, the greater effectiveness of 1 *M* NH_4Cl was evidenced by apparent V_{max} values twice as high as those obtained in the presence of 3 *M* KCl. The dependence of the apparent K_m value for L-malate on the nature of the divalent cation activator is similar to that previously reported for the malic enzyme from a marine Pseudomonad[11].

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The halophilic malic enzyme was strongly inhibited by acetyl-CoA and NADH (Figure A) and rather weakly inhibited by oxaloacetate and glyoxylate (Figure B). The former inhibitions presented sigmoidal kinetics, suggesting that they are probably allosteric. The effects of oxaloacetate and glyoxylate, on the other hand, apparently followed hyperbolic kinetics. All the inhibitors, which were competitive towards the substrate L-malate (ref.[7] and unpublished results), were more effective in the presence of 3 *M* KCl than in the presence of 1 *M* NH_4Cl; this was particularly significant for NADH, which was very little effective in the presence of the latter monovalent cation activator (Figure A). Since the apparent K_m values for both substrates were the same in the presence of either salt, it seems likely that the inhibitory effects themselves required a high salt concentration, similar to that reported as present in living halophile cells[8]. A similar finding was reported by KUSHNER et al.[13,14] for the inhibition of the aspartate transcarbamylase from *H. cutirubrum* by CTP. The results shown in the Figure were obtained with Mg^{2+} as divalent cation activator; similar results were obtained in the presence of Mn^{2+}.

The experimental data presented here suggest that the malic enzyme from *H. cutirubrum*, in spite of its truly halophilic character, shown by its absolute requirement of high salt concentrations for both activity and stability[6], is similar to the malic enzymes from other microorganisms in both its kinetic and regulatory properties. The low concentrations of acetyl-CoA and NADH (about 10 μM) effective for 50% inhibition in the presence of 3 *M* KCl (Figure A) suggest that these metabolites may play an important role in the regulation of the enzyme activity in vivo.

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Food-Induced Changes in UV-Absorption Spectra of Isolated Chromatin from Liver of Rats under Controlled Feeding Schedules

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Summary. We have measured the UV-spectra of liver chromatin extracted at 2 different times of day, corresponding to low or high rate of RNA synthesis from rats maintained under controlled feeding schedules. Results show that food intake does modify the UV-spectra of liver chromatin.

The control of the feeding schedules on which the laboratory rat is maintained has been proved to be a very useful experimental method to study the physiological mechanisms which regulate nuclear RNA synthesis.

Liver cells respond to food intake with an increased rate of RNA synthesis[1] in rats maintained under the controlled feeding schedules developed by POTTER et al.[2]. Such increased rates of RNA synthesis can be ascribed both to an activation of nuclear genome by increasing the availability of chromatin DNA template[3,4] and to an increased activity of pre-existing RNA polymerase molecules[4].

Recently it has been shown that isolated nuclei and chromatin can be studied by optical methods[5-7] and that the changes of the UV-spectrum correspond to different functional states of chromatin and rates of RNA synthesis[8].

In the present communication, we report food-induced changes in UV-absorption spectra of purified chromatin obtained from the liver of starved or fed rats in relation

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UV-absorption of liver chromatin at 2 different times of day and in different feeding conditions from rats under controlled feeding schedules

Time of day	Feeding condition	Chromatin absorbance at 258 nm (O.D. units/mg DNA)	Nucleoplasmic RNA synthesis in purified nuclei[1] (pmoles AMP/mg DNA)	Actinomycin-D binding to chromatin[3] (μg/mg DNA)	Chromatin apparent K_M for form-B RNA polymerase[4] (μM deoxynucleotides)
09.00 h	Starved since 17.00 h of previous day	8.672 ± 0.09 (9)	740 ± 59	30.6 ± 2.3 (6)	53.4
15.00 h	Fed from 09.00 h	9.690 ± 0.12 (10)	1289 ± 83	56.9 ± 2.9 (6)	32.2
15.00 h	Starved on the day of experiment (since 17.00 h of previous day)	8.763 ± 0.08 (6)	720 ± 62	31.2 ± 2.7 (6)	51.8

Measurements were performed in 1 cm path length cuvettes on chromatin solutions containing 50–100 μg DNA/ml. Data are expressed as O.D. units at 258 nm/mg DNA ± SEM; in parentheses is the number of experiments performed in different occasions.