SODIUM REQUIRING AND AVIDIN SENSITIVE OXALACETATE DECARBOXYLASE IN GLUCOSE GROWN TREPONEMA (BORRELIA) STRAIN $B_2 5$

Dileep S. Sachan

Department of Pharmacology, Meharry Medical College
Nashville, Tennessee 37208

Received July 12, 1974

Summary: Glucose grown Treponema strain $B_2 5$ has been shown to contain a sodium activated and avidin sensitive oxalacetate decarboxylase, which is unique for the bacteria grown on glucose.

Enzymes catalyzing irreversible decarboxylation of oxalacetate (OAA) fall into three classes: i) the divalent cation dependent, EDTA sensitive, OAA decarboxylase exemplified by the prototype enzyme first discoverd in M. lysodecticus (1), ii) the OAA decarboxylase activity of L-malic enzyme in pigeon liver (2) and in L. arabinosus (3) which is evident only at acid pH and which requires a divalent cation, and iii) the membrane bound, biotin containing OAA decarboxylase of A. aerogenes which requires Na^+ for its activity (4). The latter Na^+ requiring OAA decarboxylase is induced in A. aerogenes (4) and in S. typhimurium (5) only when these organisms are grown on citrate. No organism growing on glucose has been reported to contain this enzyme.

This paper will demonstrate that a Na^+ requiring and avidin sensitive OAA decarboxylase is present in the glucose grown rumen spirochete, Treponema $B_2 5$. This enzyme appears to be similar to the OAA decarboxylase of A. aerogenes.

METHODS

Treponema $B_2 5$ also called Borrelia Sp. $B_2 5$ is an obligatory anaerobic rumen spirochete known to ferment glucose (6) and to hydrogenate linoleic acid (7, 8). The organism was obtained from Dr. M. P. Bryant and was grown on glucose by Dr. M. T. Yokoyama of Department of Dairy Science, University of Illinois, according to the procedures already described (8). Washed

TABLE 1

Effects of Protein Concentration on the Decarboxylation of OAA.

Enzyme (mg Protein)	$-\Delta$ OAA μmoles/20 min
2.8	3.6
8.3	10.2
13.8	16.8

The reaction was run at 30° in presence of 5 mM $MgCl_2$ and values have been corrected for spontaneous decarboxylation of OAA.

cell pellet was stored at -20° in an atmosphere of hydrogen gas until free extracts were prepared. The frozen cells were disrupted by sonication in 3 volumes of .05 M Tris-HCl buffer, pH 7.4 for 2 min. at 4°. The sonicated suspension was centrifuged for 20 min. at 27,000 x g in a refrigerated Sorval RC-2B centrifuge. The clear supernatant was used in all experiments. Measurement of OAA decarboxylase activity was performed by following the disappearance of OAA and formation of pyruvate and CO_2 under the conditions described by Stern (4).

RESULTS AND DISCUSSION

The rate of OAA decarboxylation by the cell free extracts of Treponema $B_2 5$ was linear with protein concentration, as shown in Table 1. In order to determine the nature of this OAA decarboxylase for metal requirement and compare it with the already known OAA decarboxylases (1, 3, 4), the effect of various metal ions was tested. The data in Table 2 show that 1) Mg^{++}, Mn^{++} and Ca^{++} inhibited endogenous activity (activity without any added metal ions) of OAA decarboxylase, 2) the enzyme was significantly activated by Na^{+} ions, and 3) the enzyme was sensitive to avidin which suggests that enzyme is a biotino-protein. The high endogenous activity was perhaps due to residual Na^{+} already associated with the enzyme. The optimal Na^{+}

TABLE 2

Effect of Metal Ions on OAA Decarboxylase from Treponema $B_2 5$.

Additions[a]	$-\Delta$ OAA[b]	$+\Delta$ Pvruvate[b]	Specific Activity[c]
None	3.99	3.90	72.0
$MgCl_2$	2.21	2.69	39.8
$MnCl_2$	2.95	2.75	53.3
$CaCl_2$	2.02	2.11	36.5
$MgCl_2$ + Avidin	1.62	1.65	29.0
NaCl	5.53	4.61	100.0

a. Mg^{++}, Mn^{++} and Ca^{++} concentrations were 5 mmolar, Na^+ was 20 mmolar and avidin added was 0.8 units.

b. Values are umoles of OAA disappearing from or umoles of pyruvate appearing in the incubation medium per 20 mins, per 2.77 mg protein at 30°. All values have been corrected for the spontaneous decarboxylation of OAA.

c. nmoles of OAA disappeared per min. per mg of supernatant protein.

concentration for maximal activity was approximately 20 mM and higher levels of Na^+ appeared to be inhibitory (Table 3). This requirement for Na^+ was quite specific since other monovalent cations did not effectively substitute for Na^+ (Table 4). A slight increase in the activity of OAA decarboxylase due to Li^+, Cs^+, Rb^+, and NH_4^+ does not appear to be of great significance compared to the activation caused by Na^+.

In order to magnify Na^+ effect by lowering the endogenous activity of the OAA decarboxylase, a portion of the 27,000 x g supernatant was dialyzed for 5 hours against 0.1 M Tris-HCl, pH 7.0 and the effects of Na^+ and Cs^+ (singly and in combination) were evaluated (Table 5). It can be seen that endogenous activity of the decarboxylase in the supernatant was about equal to that found in the undialyzed preparation (Table 3 & 4) suggesting that Na^+ was undissociable from the enzyme protein. Whereas the activating effect of Na^+ was completely retained in the dialyzed preparation, effect of Cs^+ was inhibitory. This inhibitory

TABLE 3

Effect of Na^+ concentration on the Activity of OAA Decarboxylase from Treponema $B_2 5$.

Na^+ Conc. (mM)	$-\Delta$ OAA[a]	$+\Delta$ Pyruvate[a]	Specific Activity[b]
None	2.07	1.78	49.9
5	3.04	3.36	73.4
10	3.64	3.30	87.7
20	3.72	3.22	89.5
50	3.54	3.13	85.3
100	2.97	2.78	71.5

a. Values in μmoles per 15 min per 2.77 mg supernatant protein at 30°. All values have been corrected for spontaneous decarboxylation of OAA.

b. Specific activity expressed in nmoles/min/mg protein.

TABLE 4

Effect of Various Monovalent Cations on OAA Decarboxylase Activity.

Additions[a]	$-\Delta$ OAA[b]	Specific Activity[c]
None	2.41	43.7
NaCl	3.92	70.7
LiCl	2.94	53.2
CsCl	3.10	56.0
RbCl	3.00	54.3
NH_4Cl	3.09	55.8

a. NH_4Cl 25 mM, all other 20 mM.

b. μmoles of OAA/20 min/2.77 mg protein at 30°. All values corrected for spontaneous decarboxylation of OAA.

c. Values are nmoles/min/mg protein.

TABLE 5

Effect of Dialysis on the Activity of OAA Decarboxylase.

Additions[a]	$-\Delta$OAA[b]	Spedific Activity[c]
None	2.20	46.7
NaCl	3.57	75.6
CsCl	1.55	32.9
NaCl + CsCl	3.60	76.3

a. Each at 20 mM concentration.

b. µmoles of OAA disappearing at 30° in 15 min. per 3.15 mg of supernatant protein.

c. Values are nmoles/min/mg protein.

effect of Cs^+ was abolished, however, when saturating Na^+ was present i.e. when Na^+ and Cs^+ were added together.

From these preliminary data it can be concluded that Treponema B_25 possesses a Na^+ activated, and avidin sensitive, OAA decarboxylase which appears to be similar to that described in A. aerogenes grown on citrate (4). The function of this enzyme in Treponema B_25, which grows on glucose and ferments it to acetate, formate, lactate, succinate, CO_2 and ethanol (6) is not clear. It is not known if this organism can grow on citrate. However, Allison and Robinson (9) reported that they were able to grow this bacterium on succinate. In their experiments succinate was not converted to glutamate via the reductive carboxylation pathway. It is possible that this organism converts succinate to oxalacetate (via fumarate and malate) which is then decarboxylated by OAA decarboxylase to pyruvate and CO_2. Consequently the pyruvate is phosphoroclastically cleaved to acetate and formate, thus generating energy for growth. This pathway would yield 1 mole of ATP per mole of succinate utilized. Further studies are in progress to explore this and other possibilities.

ACKNOWLEDGMENT

The author is thankful to Dr. M. T. Yokoyama for growing the cells. This work was supported by the grant from the National Science Foundation (GB-8078).

REFERENCES

1. Krampitz, L. O. and Werkman, C. H. (1941). Biochem. J. 35: 595-602.
2. Ochoa, S., Mehler, A. H. and Kornberg, A. (1948). J. Biol. Chem. 174: 979-1000.
3. Korkes, S., delCampillo, A. and Ochoa, S. (1950). J. Biol. Chem. 187: 891-905.
4. Stern, J. R. (1967). Biochemistry 6: 3545-3551.
5. O'Brien, R. W. and Stern, J. R. (1969). J. Bacteriol. 99: 395-400.
6. Bryant, M. P. (1952). J. Bacteriol. 64: 325-335.
7. Sachan, D. S. and Davis, C. L. (1969). J. Bacteriol. 98: 300-301.
8. Yokoyama, M. T. and Davis, C. L. (1971). J. Bacteriol. 107: 519-527.
9. Allison, M. J. and Robinson, I. M. (1970). J. Bacteriol. 104: 50-56.