THE INDUCTION OF A C_4-DICARBOXYLIC ACID ANION TRANSLOCATOR IN *AZOTOBACTER VINELANDII*

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1. Introduction

Recently we described the existence of several inducible translocators for di- and tricarboxylix acid anions in *Azotobacter vinelandii* [1]. We have studied now more closely the conditions under which the translocator for C_4-dicarboxylic acid anions is induced. Furthermore, we have determined the number of inducer molecules that can be bound to the repressor, making use of a formula derived by Yagil and Yagil [2], based on the model proposed by Jacob and Monod [3]. From our results it appears that two molecules of the non-metabolizable inducer mesotartrate can be bound to the repressor.

2. Methods

Growing, harvesting and adaptation of *A. vinelandii* cells was done as described elsewhere [1]. In order to follow adaptation, samples were taken, quickly chilled and after centrifugation and washing, resuspended in distilled water. The oxidation activity, determined as described earlier [1], was used as a measure of the translocator activity. L-malate and glucose were determined enzymatically according to Schoner [4] and Lloyd and Whelan [5], respectively.

3. Results

We have demonstrated earlier [1] that a translocator for C_4-dicarboxylic acid anions can be induced in *A. vinelandii* by each of the 4 Krebs-cycle intermediates: succinate, fumarate, L-malate or oxaloacetate. Table 1 shows the activity of a number of other dicarboxylic acid anions in inducing this translocator, as measured by the rate of succinate oxidation. Nearly identical values were found for the oxidation of L-malate or fumarate, in contrast to findings of Repaske et al. [6].

Fig. 1 shows the final oxidation activity reached after induction for 2 hr with different concentrations of externally added succinate. Fig. 2 shows the disappearance of L-malate and glucose and the increase of the succinate oxidation during adaptation to L-malate. A major difficulty with natural inducers like L-malate or succinate is their disappearance during the adaptation process due to metabolism. However, fig. 2 shows also that the inducer does not need to be present during the whole induction process. This is in contrast to the glucose requirement. In table 2 is shown the rate of succinate oxidation after a 2 hr induction period as a function of the added glucose concentration. Removal of the glucose during the adaptation process immediately stops the induction in contrast to the removal of the inducer. Table 2 shows furthermore that some catabolite repression occurs at high glucose concentrations.

Recently a paper by Yagil and Yagil [2] appeared, in which a relationship was derived which allows one to determine graphically the number of inducer molecules that have to be bound to the repressor in order to inactivate it. In table 3 the rate of translocator synthesis (as measured by the succinate oxidation) as a function of the external mesotartrate concentration is given. Separate experiments have shown that meso-

Table 1
Induction of a C_4-dicarboxylic acid anion translocator in *Azotobacter vinelandii* by dicarboxylic acid anions.

Inducer	Concentration (mM)	Succinate oxidation (μ atoms 0/min/mg protein)
-	-	0.15
Succinate	0.10	1.80
Mesotartrate	1.0	1.50
D-malate	2.0	1.24
L-(−)-tartrate	2.0	0.70
Dihydroxyfumarate	2.0	0.70
Maleate, D-(+)-tartrate	2.0	0.50
Methylsuccinate, thiomalate α, β-Dibromosuccinate, D, L-bromosuccinate	} 2.0	0.15–0.20
Dimethylsuccinate ester	2.0	0.15

Sucrose-grown cells were induced for 2 hr as described in [1] with the inducer concentrations as indicated. Succinate oxidation was measured polarographically as described in [1].

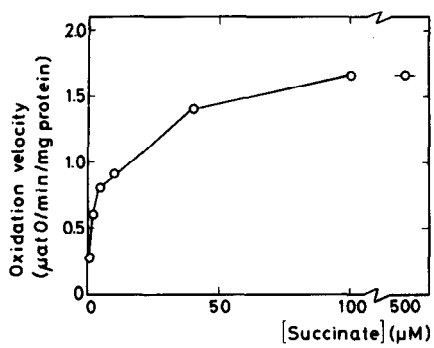

Fig. 1. Adaptation of sucrose-grown *A. vinelandii* to various external concentrations of succinate. Adaptation was carried out as described elsewhere [1]. Succinate oxidation of the induced cells was measured after 2 hr.

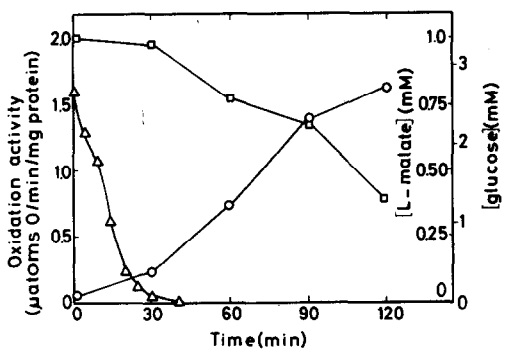

Fig. 2. Disappearance of L-malate and glucose and increase in succinate oxidation during adaptation of sucrose-grown cells to L-malate. Adaptation was carried out as described elsewhere [1]. Glucose and L-malate were determined in samples obtained by millipore filtration. Activity of the translocator was measured from the oxidation of succinate. (△–△–△): L-malate; (□–□–□): glucose; (○–○–○): succinate oxidation.

tartrate itself is metabolized at a negligible rate. Plotting the values of table 3 according to Yagil and Yagil [2] results in a straight line with a slope of 1.98, as is shown in fig. 3. In another experiment a value of 2.11 was found. This means that 2 molecules of mesotartrate are bound to the repressor.

4. Discussion

Although the process of enzyme induction has been studied for a long time, there exists uncertainty about the place (in a topological sense) where the inducer is acting. Recent reports by Winkler [7] and Dietz and Heppel [8] suggest that in certain cases the inducer acts only when present outside the cell. The experiments reported here point in the same direction. Low external concentrations of L-malate or succinate (50–100 μM) lead to maximal induction. However, during growth on sucrose or during sucrose or pyruvate oxidation by sucrose-grown cells, appreciable amounts of Krebs-cycle intermediates are found inside the cell (P. Postma, unpublished results). Apparently, generated intracellularly, the inducer does not lead to induction (cf. [8]).

Table 2
The effects of glucose during adaptation of *A. vinelandii* to succinate.

Glucose (mM)	Succinate oxidation (μ atoms O/min/mg protein)
–	0.28
0.06	1.0
0.36	1.24
0.82	1.60
3.0	1.40
55	1.35
110	1.25

Sucrose-grown cells were adapted in the presence of various concentrations of glucose and 1 mM succinate during 2 hr as described in [1]. Succinate oxidation was determined as described in [1].

Table 3
Rate of translocator synthesis during adaptation to different external mesotartrate concentrations.

Mesotartrate (mM)	Rate of translocator synthesis (μ atoms O/min/mg per hr)
1.5	1.66
0.8	1.49
0.4	1.20
0.2	0.58
0.1	0.16
0.05	0.085

Sucrose cells were adapted to mesotartrate at the concentrations indicated for 2 hr as described in [1]. At 30 min intervals samples were taken and the succinate oxidation activity was determined. The rate of translocator synthesis (as measured by the succinate oxidation) is expressed as the increase in oxidation activity per hr (μ atoms O/min/mg per hr).

By comparing the structure of several dicarboxylic acids anions that could or could not induce the translocator, we hoped to determine the part of the molecule essential for induction. However, from table 1 no clear picture emerges, in contrast to the findings with the lactose operon [9].

Several analogues of dicarboxylic acid anions were discovered that could induce the C_4-dicarboxylic acid anion translocator, whereas at the same time they were metabolized at a very low rate.

Because of its slow metabolism, mesotartrate was selected to determine the number of inducer molecules that can be bound by the repressor. As in the

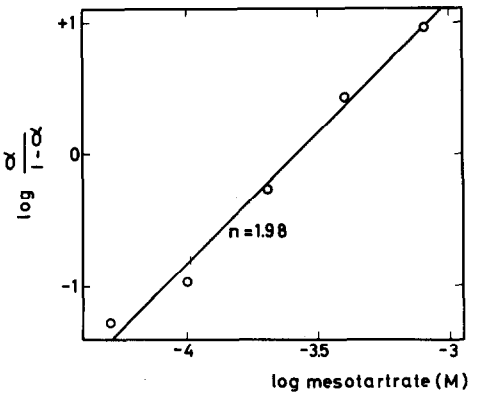

Fig. 3. Induction of the succinate oxidation activity by mesotartrate in *A. vinelandii*. The values of table 3 were plotted according to Yagil and Yagil [2]. α is the ratio between the rate of translocator synthesis at a given concentration of mesotartrate and this rate at a saturating concentration of mesotartrate.

case of the lactose repressor [2] it turned out to be two. Presently we attempt to determine this number also with the metabolizable Krebs-cycle intermediates using an experimental set-up in which the externally added inducer concentration is kept constant during the adaptation.

The reason for the requirement of glucose remains unclear. Repaske et al. [6] suggested that glucose merely provides energy. Glucose can be replaced by several sugars and also by pyruvate. However, the induction of the translocator for citrate needs almost no glucose. On the contrary, unpublished results (P. Postma) show that the induction of the citrate translocator in *A. vinelandii* is repressed by glucose, as was found also by Englesberg et al. in *Salmonella typhimurium* [10] and Villarreal-Moguel and Ruiz-Herrera in *Aerobacter aerogenes* [11].

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References

[1] P. W. Postma and K. van Dam, Biochim. Biophys. Acta 249 (1971) 515.
[2] G. Yagil and E. Yagil, Biophysical J. 11 (1971) 11.
[3] F. Jacob and J. Monod, J. Mol. Biol. 3 (1961) 318.
[4] W. Schoner, in: Methoden der Enzymatischen Analyse, 2nd ed., ed. H. U. Bergmeyer (Verlag Chemie, Weinheim/Bergstr., 1970) p. 1551.
[5] J. B. Lloyd and W. J. Whelan, Anal. Biochem. 30 (1969) 467.
[6] R. Repaske, J. Shroat and D. Allman, J. Bacteriol. 79 (1960) 394.
[7] H. H. Winkler, J. Bacteriol. 101 (1970) 470.
[8] G. W. Dietz and L. A. Heppel, J. Biol. Chem. 246 (1971) 2885.
[9] A. Kepes and G. N. Cohen, in: The Bacteria, Vol. 4, eds. Y. C. Gunsalus and R. Y. Stanier (Academic Press, New York, 1962) p. 179.
[10] E. Englesberg, J. A. Watson and P. A. Hoffee, Cold Spring Harbor Symp. 26 (1961) 261.
[11] E.I. Villarreal-Moguel and J. Ruiz-Herrera, J. Bacteriol. 98 (1969) 552.