DIAUXIE IN TARTRATE-UTILISING STRAINS OF PSEUDOMONAS AND ITS CONTROL BY OXALOACETATE

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The synthesis of inductive enzymes is frequently inhibited by substrates such as glucose (Monod, 1941; Cohn, 1956) and recently, this effect has been interpreted in terms of the intracellular accumulation of an inhibitor of inductive enzyme formation (Cohn & Horibata, 1959; Neidhardt, 1960). This inhibitor is considered to be identical with a repressor formed in lower concentrations under all conditions of growth and which is responsible for making the enzyme an inductive one. Not all results can, however, be accomodated in such a hypothesis (Mac Quillan, Winderman & Halvorson, 1960) and further observations on various microorganisms and on the effect of substrates other than the sugars generally used would appear to be desirable. To this end, inhibition of induction by tartaric acid isomers in Pseudomonas sp. has been investigated in the present work.

The strains employed (Shilo, 1957; Shilo & Stanier, 1957) metabolise the three optical isomers of tartaric acid inductively, each tartrate being dehydrated to oxaloacetate by an inducible dehydrase. Three dehydrases, each specific for one of the tartrates, are known and these are induced only by their particular substrates.

Cells were grown with one of the tartrate isomers as carbon and energy source and a second tartrate isomer was added during the logarithmic phase of growth as inducer. Two strains (md9 and lmdl) were used and of the six possible combinations of growth on one tartrate and induction by either of the two other isomers, all six were tested on strain md9 and growth on meso-tartrate and induction by d- or l-tartrate on strain lmdl (Table 1). In strain md9 meso-tartrate when given as the carbon and energy source prevented subsequent induction by the d- or l- isomers. In all other combinations, including

Table 1

Effect of growth on one tartrate isomer on subsequent induction by another tartrate isomer

Growth substrate (3g/l)	Inducer (6.6 mM)	O_2 uptake on addition of inducer 0-30min. (μl O_2/mg dry weight)	Differential* rate of meso- or d-tartrate dehydrase synthesis
Strain md9			
d-tartrate	d-tartrate	87.5	68 (d)**
meso-tartrate	"	10	< 1.7 "
l-tartrate	"	109	54.4 "
l-tartrate	l-tartrate	108	-
meso-tartrate	"	8.5	-
d-tartrate	"	115	-
meso-tartrate	meso-tartrate	121	169 (m)**
d-tartrate	"	122	73.2 "
l-tartrate	"	96	-
Strain lmdl			
d-tartrate	d-tartrate	-	105 (d)**
meso-tartrate	"	-	121 "
meso-tartrate	l-tartrate	132	-

The basal medium contained/l : $(NH_4)_2SO_4$, 1g; KH_2PO_4, 1.5g; Na_2HPO_4, 0.25g; $MgSO_4.7H_2O$, 50mg; $(NH_4)_2SO_4.FeSO_4.6H_2O$, 5mg; Yeast extract (Difco), 25mg; pH 6. Cultures were grown in shake flasks at 30°. For dehydrase assays cells were lysed by addition of versene and lysozyme (Repaske, 1958). For d-tartrate dehydrase assays, extracts were incubated for 10 min. at 30° with 4 mM versene, 12.5 mM d-tartrate, 50 mM tris buffer pH 8.7. For meso-tartrate dehydrase assays extracts were incubated for 10 min. at 30° with 4 mM versene, 12.5 mM meso-tartrate 22 mM phosphate buffer pH 7.2. The accumulation of keto acids from tartrates proceeds quantitatively in the presence of dehydrase and versene (Shilo, 1957); keto acids were measured by the method of Friedman and Haugen (1943). A dehydrase unit was defined as that amount which forms 0.1 μmole keto acid/h. Under the growth conditions used, increase in the amount of dehydrase gives a straight line when plotted against increase in bacterial dry weight.

*Differential rate of dehydrase synthesis is given as increase in dehydrase units/0.5mg increase in bacterial dry weight.

**(d) d-tartrate dehydrase, (m) meso-tartrate dehydrase.

strain lmdl growing on meso-tartrate, cultivation on one isomer did not inhibit induction by a second. That metabolism of meso-tartrate but not of other tartrate isomers can inhibit inductive enzyme synthesis is striking when it is considered

that the metabolic pathway of all the tartrate isomers is likely to be a common one after dehydration to oxaloacetate.

Indications that meso-tartrate does not inhibit induction by competing with the inducer for entry into the cell in strain md9 were obtained from experiments with cells fully induced to d-tartrate. Such cells continue metabolism of the d-isomer in the presence of meso-tartrate concentrations six times greater than those which stop induction.

Response to induction was next studied during growth on substrates other than tartrates. When the two strains were cultivated on oxaloacetate, succinate or l-malate, d- or l-tartrate failed to induce specific dehydrase formation (Table 2). With the above substrates meso-tartrate behaved differently in each of the two strains, inducing in strain md9 and failing to induce in strain lmdl. When α-ketoglutarate, citrate or glucose were given as carbon and energy sources, d-tartrate dehydrase was formed on addition of inducer, though at a reduced rate. Growth on pyruvate allowed rapid synthesis of the d-tartrate dehydrase (Table 2).

The inhibition of dehydrase synthesis by oxaloacetate appears of particular significance since this compound is the immediate product of dehydrase action. The data are thus consistent with the existence of a feed-back mechanism (Magasanik, 1957) for controlling tartrate dehydrase formation, this feed-back mechanism acting through the accumulation of oxaloacetate. The operation of such a mechanism could explain the observed inhibitions of induced enzyme synthesis. The single instance where growth on a tartrate isomer inhibits induction by a second tartrate is during growth of strain md9 on meso-tartrate. This is also the one case where the synthesis of the tartrate dehydrase functioning in the growing cells is not inhibited by oxaloacetate. A relatively high concentration of internal inhibitor may thus be accumulated by the action of the meso-tartrate dehydrase and this could then inhibit the induced synthesis of d- or l-tartrate dehydrase. During growth of md9 on other isomers, or of lmdl on meso-tartrate, the concentration of intracellular inhibitor should be relatively low due to operation of the feed-back mechanism. In these instances, all tartrate isomers are able to induce their respective dehydrases.

Table 2

Induction by tartrate isomers during growth on organic acids and glucose*

Growth substrate (3g/l)	Inducer (6.6 mM)	Differential rate of meso- or d-tartrate dehydrase synthesis Strain md9	Strain lmdl
Oxaloacetate	meso-tartrate	99 (m)	< 3.3 (m)
Succinate	"	40.5 "	< 3.6 "
l-malate	"	-	< 2.3 "
Oxaloacetate	d-tartrate	< 2.6 (d)	19 (d)
Succinate	"	< 1.5 "	< 2.2 "
l-malate	"	< 1.5 "	< 2.9 "
fumarate	"	< 4.4 "	-
citrate	"	10.5 "	-
α-ketoglutarate	"	18.5 "	-
glucose	"	25.9 "	-
pyruvate	"	89 "	-
oxaloacetate	l-tartrate	29**	31.7**

*Details of growth conditions, dehydrase assays and definition of differential rate of synthesis as described in Table 1.

**μl O_2/mg dry weight taken up after inducer addition 0-30 min. Since l-tartrate dehydrase cannot be assayed under the conditions used for d- or meso-tartrate dehydrase assay (Shilo, 1957) induction was followed manometrically.

The failure to respond to inducer observed during growth on C4 dicarboxylic acids and the partial inhibitions during growth on α-ketoglutarate, citrate or glucose may again be explained by intracellular accumulation of oxaloacetate.

In the above arguments, it is postulated that these strains may in accordance with enviromental conditions accumulate differing amounts of intracellular inhibitor. Support for this hypothesis has been obtained by comparing induction of cells of strain md9 growing exponentially on l-tartrate and cells metabolising this isomer in washed suspensions (Table 3). Cells growing with l-tartrate as substrate are readily induced by the d-isomer, while in resting cells l-tartrate inhibits induction by d.

The interpretation placed on diauxic inhibitions by Cohn and Horibata (1959) and Neidhardt (1960) would thus appear to apply to the tartrate system only if the inhibitor responsible

Table 3

Effect of metabolism of l-tartrate on subsequent induction by d-tartrate in growing and resting cells of strain md9*

Time	Tartrate added	d-tartrate dehydrase units/ml in cells previously grown on l-tartrate	
		Washed cells resuspended in buffer**	Washed cells resuspended in complete medium
0-30 min.	l-tartrate 20mM	<1.4	<0.8
30-105min.	l-tartrate 20mM d-tartrate 6.6mM	<1.3	21
105-190 min.***	d-tartrate 6.6.mM	10.6	-

*Details of medium and definition of dehydrase unit as given in Table 1.

** 22 mM phosphate buffer pH7.2

*** Cells centrifuged, washed in phosphate buffer 22mM pH7.2 and resuspended in buffer and d-tartrate.

for feed-back control (oxaloacetate) is identical with the inhibitor which makes the enzyme an inductive one. Since enzyme systems whose synthesis is controlled by an immediate product of enzyme action and which at the same time are inducible have not been widely studied, little can be said at present about the relation of the inhibitors responsible for the two effects.

The finding that induction in resting cells may be prevented by a substrate which does not inhibit growing cells is similar to results obtained by Mandelstam (1957) and McFall and Magasanik (1960) on induced synthesis of β-galactosidase. The explanation suggested for this effect in the β-galactosidase system has been the accumulation of intermediary metabolites which in growing cells would be used for synthetic reactions and which act as inhibitors of adaptive enzyme synthesis (Neidhardt, 1960). In the case

of tartrate induction, a more specific explanation than general metabolic changes caused by absence of essential nutrients could possible be advanced on the basis of the feed-back mechanism controlled by oxaloacetate accumulation. This mechanism would ensure a low intracellular concentration of repressor only in growing and not in resting cells.

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