EVIDENCE SUGGESTING THAT THE NADPH/NADP RATIO MODULATES THE SPLITTING OF THE ISOCITRATE FLUX BETWEEN THE GLYOXYLIC AND TRICARBOXYLIC ACID CYCLES, IN *ESCHERICHIA COLI*

Juan BAUTISTA, Jorgina SATRÚSTEGUI and Alberto MACHADO

Departamento de Bioquímica y Biología Molecular, Centro de Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid-34, Spain

Received 20 July 1979

1. Introduction

The growth of *Escherichia coli* on two carbon sources is brought about by the utilization of the glyoxylic acid cycle that provides the necessary biosynthetic intermediates. Isocitrate lyase (EC 4.1.3.1.) and malate synthase (EC 4.1.3.2.) are the enzymes of the cycle induced under these conditions [1]. During growth on two carbon sources, isocitrate is the common substrate of two metabolic pathways; it can be used by either NADP-isocitrate dehydrogenase (IDH) or by isocitrate lyase (IL). IDH has a higher activity and lower K_m for isocitrate than IL [2]. Since, in *E. coli*, both enzymes are located in the same cellular compartment, the operation of the glyoxylate cycle would therefore imply the inhibition of IDH in order to favour the utilization of this substrate by IL. In a number of sources [3–5] it has been proposed that a reduced flux through IDH is brought about by the concerted inhibition of the enzyme by oxaloacetate and glyoxylate. We have studied the characteristics of this concerted inhibition in *E. coli* IDH in order to ascertain whether it could account for the operation of the glyoxylate cycle. The results presented suggest that the concerted inhibition by oxaloacetate and glyoxylate has a doubtful regulatory role and we propose NADPH as the most likely physiological inhibitor of IDH.

2. Materials and methods

2.1. *Chemicals*

NADH, NADPH, glucose-6-phosphate and malate dehydrogenases were obtained from Boehringer, and DL-isocitrate, oxoglutarate (monosodium salt), NAD, NADP, lactate and glutamate dehydrogenases were obtained from Sigma. Other reagents were of the highest purity available from commercial sources.

2.2. E. coli *growth and harvest conditions*

The organism used was K2 strain of *E. coli* from the H.L. Kornberg collection. Cultures were grown aerobically at 37°C in synthetic media [6] containing 0.05 M glucose or 0.1 M acetate as carbon sources. The cells were harvested in the logarithmic growth phase. Crude extracts were obtained by cell disruption in a MSE-60 ultrasonic disintegrator and centrifuged at 3000 × *g* for 10 min. Supernatants were used for enzyme assays.

2.3. *Assay methods*

The activities of glucose-6-phosphate, 6-phosphogluconate, NAD- and NADP-glutamate dehydrogenases were determined as in [7]. NAD- and NADP-isocitrate dehydrogenases were determined by the method in [8], except that semicarbazide and cysteine were omitted. Isocitrate lyase was assayed as in [9].

2.4. *Determination of metabolites*

Samples (100 or 200 ml) of *E. coli* cultures were rapidly gathered in pre-cooled recipients and immediately immersed in liquid nitrogen. The frozen cultures were lyophilized, weighed, resuspended in acid or alkaline solutions [10] homogenized in a MSK (B. Braun) cell homogenizer at 2000 rev./min for 2 min, and used as alkaline and acid extracts for the

determination of metabolites by the methods in [10,11]. Measures were made with a Unicam SP 1700 Ultraviolet Spectrophotometer and a Gilson oxygraph K-IC. Metabolite concentrations are referred to the dry weights of the corresponding cultures obtained by filtration of 25 ml samples.

3. Results and discussion

3.1. *Concerted inhibition of* E. coli *NADP-isocitrate dehydrogenase*

Table 1 shows the inhibition produced by oxaloacetate and glyoxylate on *E. coli* IDH. When assayed separately, each of these compounds, at 3.3 mM, produced a very small inhibition of the enzyme. The mixture of the two components (0.06 mM each) produced a 39% inhibition of *E. coli* IDH that, compared to the inhibition reported on the enzyme from other sources such as *Tetrahymena* [5,12], *Rhodospirillum rubrum* [13] and rat liver [12], is particularly small. Besides, the physiological role of this inhibition is also controversial:

(1) The inhibition is apparently produced by the non-enzymatic condensation product of oxaloacetate and glyoxylate, namely oxalomalate [14].
(2) The inhibition has been shown to be non-reversible in vitro by the addition of lactate and/or malate dehydrogenases that would consume glyoxylate and oxaloacetate, respectively [15].

In the light of these facts, the physiological role of the oxaloacetate and glyoxylate concerted inhibition may be regarded as an open question.

However, the operation of the glyoxylate cycle requires a pronounced inhibition of *E. coli* IDH. This arises from the fact that IDH has lower K_m for isocitrate and higher activity than IL both with cells grown on glucose and acetate substrates, since not only IL but also IDH are induced in the second source (table 2). The induction of IDH in acetate grown cells could be the result of an increased importance of the function of the enzyme in those cells. Acetate-grown organisms have reduced levels of the pentose phosphate pathway enzymes that have a well-established NADPH producing function (glucose-6-phosphate and 6-phospho-gluconate dehydrogenases changing from 140 and 50 to 60 and 30 nmol .mg protein^{-1}.min^{-1} in glucose and acetate grown cells, respectively). This is not surprising since gluconeogenesis is primed with respect to glucose catabolism that is probably strongly blocked in acetate-grown organisms. Accordingly, the cellular NADPH demands must be met by an alternative pathway. We suggest that IDH becomes the major enzyme catalyzing NADPH production in acetate-grown cells.

Table 1
Effect of glyoxylate and oxaloacetate on the isocitrate dehydrogenase of *E. coli*

Compounds added	M × 10^{-3}	% Activity
None		100
Oxaloacetate	3.3	61.1
Glyoxylate	3.3	98.0
Oxaloacetate + glyoxylate	0.06 + 0.06	61.0

Table 2
Activities of isocitrate dehydrogenase and isocitrate lyase from *E. coli* grown in 0.10 M acetate and 0.05 M glucose, and K_m for isocitrate of both enzymes

	Glucose 0.05 M	Acetate 0.10 M	K_m for isocitrate
Isocitrate dehydrogenase	24	108	3.7×10^{-5} M
Isocitrate lyase	10	65	3.0×10^{-3} M

Enzyme activities are expressed in nmol .min^{-1} .mg protein^{-1}. The results are the means of 3 expt

3.2. *NADPH inhibition of* E. coli *NADP-isocitrate dehydrogenase*

In *Tetrahymena pyriformis* NADP-isocitrate dehydrogenase catalyzes the principal NADPH producing reaction of that organism. This enzyme is subject to a regulation similar to that described for the enzymes of the pentose phosphate pathway [12]. *Tetrahymena* IDH is strongly inhibited by NADPH with a K_i very similar to the K_m for NADP [12]. The inhibition of NADP-IDH by NADPH has also been reported for enzymes from other sources [3]. We have measured the NADPH inhibition of *E. coli* IDH compared to that produced on glucose-6-phosphate and 6-phospho-gluconate dehydrogenases of the same organism. The results are summarized in table 3. NADPH produces a similar inhibition on the three enzymes. The cellular NADPH/NADP ratio is widely accepted as the major regulatory parameter acting on

Table 3
Effect of NADPH on *E. coli* glucose-6-phosphate, 6-phosphogluconate and isocitrate dehydrogenases

Initial NADP (μM)	Initial NADPH (μM)	NADPH / NADP	% Inhibition by NADPH		
			Glu-6-P. DH.	6-P-Glu. DH	NADP-IDH
50	50	1	45	16	30
50	100	2	75	38	67
50	150	3	83	62	82

the pentose phosphate pathway. We propose that *E. coli* IDH is subject to the same control. Moreover, according to our proposal, the NADPH/NADP ratio would regulate the glyoxylate cycle by allowing the utilization of isocitrate by isocitrate lyase when this enzyme is fully induced. In addition, the inhibition of IDH by NADPH would eliminate the two following objections that faced concerted inhibition:

(1) It is not clear how glyoxylate and oxaloacetate could be produced in amounts needed for the inhibition of IDH when the enzymes of the glyoxylate cycle are not yet fully induced.
(2) Once the glyoxylate cycle enzymes are fully induced, and the production of glyoxylate and oxaloacetate is increased, the difficulty in overcoming the concerted inhibition by these compounds becomes obvious: (a) because of their increased levels whose decline is not evident; (b) because the inhibition is apparently not reversible [15].

The physiological importance of the inhibition by NADPH or by the NADPH/NADP ratio has none of the above objections and is supported by the following facts:

1. The cellular NADPH/NADP ratio from most sources where it has been determined is always > 1 and generally > 10 probably always lying within inhibitory values [16,17].
2. The production of a new metabolite is not needed in order to bring about the operation of the glyoxylate cycle.
3. Growth in acetate is a well known gluconeogenic condition under which lipid biosynthesis is likely to be repressed with a corresponding increase in the NADPH/NADP ratio, as has been reported in mammalian tissues [18]. The increase in NADPH/NADP could immediately produce an increase in IDH inhibition, with a resulting accumulation of isocitrate or citrate. These metabolites have been proposed as inducers of IL [1].

We have measured the cellular concentrations of several metabolites whose variations in glucose and acetate-grown cells could give some insight into the actual occurrence of NADPH inhibition of *E. coli* IDH. The results are shown in table 4. The 5-fold increase in citrate and the 2-fold decrease in oxoglutarate found in acetate-grown cells is consistent with an actual inhibition of IDH. According to our expectations, the NADPH/NADP ratio rises from 6.2–14 in glucose and acetate-grown cells, respectively, thus supporting the regulatory role of this parameter for the operation of the glyoxylate cycle. The regula-

Table 4
Effect of the carbon source on the concentrations of metabolites

Carbon sources	NADPH	NADP	NADPH / NADP	NADH	NAD	NADH / NAD	Citrate	Oxoglutarate
Glucose	0.24	0.04	6.2	0.41	2.10	0.19	3.56	1.05
Acetate	0.47	0.03	14	0.89	1.94	0.46	16.30	0.35

Concentrations are expressed in μmol/g dry wt. The values are the means of 3 expt

tion of the isocitrate flux through IDH to produce oxoglutarate and glutamate, and through IL to produce glyoxylate cycle intermediates, might be produced by oscillations of the NADPH concentration such as those found for NADH [19]. Oscillations in metabolite levels have now been found in a number of cases.

4. Conclusions

We have proposed and tried to demonstrate that in *E. coli* the NADPH/NADP ratio instead of the glyoxylate + oxaloacetate produced concerted inhibition, is the most important modulator of the glyoxylic acid cycle through the modification of the IDH activity. We also propose that NADPH/NADP ratio probably modulates the IDH activity from mammalian liver as well, and brings about its inhibition under gluconeogenic conditions [20].

Acknowledgements

This work was supported by Grants from the Comisión Administradora del Descuento Complementario del INP and from the Comisión Asesora de Investigación Científica y Técnica. We thank Miss María Victoria Mora Gil for her technical assistance.

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