Patterns of nucleotide utilisation in bacterial succinate thiokinases

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1. INTRODUCTION

Our studies of the structural and functional diversity of citric acid cycle enzymes from different bacteria have revealed molecular size patterns with the 2 acyl-CoA-linked enzymes citrate synthase (EC 4.1.3.7) and succinate thiokinase (succinyl-CoA synthetase, EC 6.2.1.4 and 6.2.1.5) [1–3]. Both these enzymes exist in either a 'large' or 'small' form which differ in the number of subunits they contain. The striking feature is that the 'large' forms are restricted to Gram-negative bacteria, whereas the 'small' forms occur in the Gram-positive bacteria. Eukaryotic organisms appear also to contain only the 'small' forms of these enzymes. In the case of citrate synthase, the structural patterns are associated with regulatory differences [2–4] but, as yet, no comparable regulatory sensitivity has been detected for succinate thiokinase.

Succinate thiokinase catalyses the reversible reaction:

$$\text{succinyl-CoA} + \text{NDP} + P_i \rightleftharpoons \text{succinate} + \text{NTP} + \text{CoA-SH}$$

where NDP and NTP represent nucleoside diphosphate and triphosphate. The nucleotide specificity of the enzyme from a variety of sources has been studied. The mammalian enzyme was originally found to be specific for guanine and inosine nucleotides [5]. An adenine nucleotide-linked succinate thiokinase of animal origin was later reported [6] and this has recently been shown to be of wider occurrence in diverse animals [7,8]. On the other hand, the enzyme from plant sources appears to be specific for the adenine nucleotides [9–14] as was also reported to be the case with *Escherichia coli* [15,16]. Indeed, it has been suggested that bacterial succinate thiokinases might generally be specific for ADP/ATP [17]. However, the enzyme from *Rhodopseudomonas spheroides* has been shown to utilise ATP, GTP and ITP [18] and further investigation of the *E. coli* enzyme has revealed that GTP and ITP can also serve as substrates, albeit less effectively than ATP [19]. More recently, several other bacterial succinate thiokinases have been examined for their nucleotide utilisation and some variation in adenine *vs* guanine utilisation has been detected [20].

In view of our demonstration of molecular size differences among diverse bacterial succinate thiokinases [3] and our development of a convenient, sensitive and continuous polarographic assay for the enzyme, which is not restricted to low nucleotide concentrations [21,22], it seemed worthwhile to make a systematic study of succinate thiokinases from a range of bacteria drawn from diverse taxonomic groups. The results presented here indicate distinct patterns of nucleotide specificity which appear to show some correlation with bacterial classification.

2. EXPERIMENTAL

Bacillus stearothermophilus and *Rhodopseudomonas spheroides* were obtained as frozen cell pastes from the Centre for Applied Microbiology and Research (Porton). A culture of *Thermus aquaticus* was kindly provided by Dr P.H. Ray (University of Kentucky). The other bacteria used were from the culture collections of this laboratory or of the Department of Microbiology (University of Leicester).

Thermus aquaticus was grown at 70°C as in [23]; the other bacteria were grown in nutrient broth at

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30°C or 37°C. Cells were harvested and disrupted by ultrasonication, and the supernatant solutions obtained after centrifugation to remove cell debris were used without further treatment.

Succinate thiokinase was assayed polarographically by monitoring the rate of formation of CoA-SH at the dropping mercury electrode as in [3]. Assay mixtures contained 0.1 M Na/K phosphate (pH 8), 10 mM $MgCl_2$, 0.15 mM succinyl-CoA and varying concentrations of ADP or GDP; the reaction was initiated by the addition of enzyme to give a final total volume of 1.0 ml. 'Deacylase' rates were also measured by omitting the ADP or GDP, and these were subtracted from the rates measured with the complete system to give the true succinate thiokinase rates. For measurements of the reaction in the reverse direction, assay mixtures contained 0.1 M Tris-HCl (pH 8), 10 mM $MgCl_2$, 2 mM succinate, 0.05 mM CoA-SH and varying concentrations of ATP or GTP; the rate of CoA-SH consumption was followed polarographically.

3. RESULTS AND DISCUSSION

Succinate thiokinase activity was detected in extracts of all the organisms studied (see table 1) consistent with their possession of a complete citric acid cycle.

The polarographic assay for succinate thiokinase is particularly convenient and suitable for examining the nucleotide dependence of enzyme rate. Other assays employed for this enzyme are the hydroxamate method [24] which is discontinuous and less convenient for kinetic studies or the spectrophotometric method at 235 nm [25] which suffers from interference by absorbance of the nucleotide substrates. These disadvantages are eliminated by the polarographic procedure.

Studies in this laboratory have been concerned with some of the citric acid cycle enzymes of the bacterium *Acinetobacter calcoaceticus* (earlier designated *A. lwoffi*) [26,27]. When the succinate thiokinase of this organism was examined, a rather high K_m value ($\sim$ 1 mM) for ADP was found, but a much lower K_m ($\sim$ 0.02 mM) was measured when GDP was substituted as substrate. This contrasts with the reverse situation encountered with the *E. coli* enzyme where the K_m values were found to be in the region of 0.01 mM for ADP and 0.1 mM for GDP. When measurements were made on succinate thiokinase from *Pseudomonas aeruginosa* yet another type of behaviour was found; the K_m values for ADP and GDP were very similar at $\sim$0.02–0.04 mM. Fig.1 illustrates the nucleoside diphosphate substrate dependences of the succinate thiokinases from these 3 bacteria. When these enzymes were studied in the reverse direction, i.e., the formation of succinyl-CoA at the expense of ATP or GTP, a similar pattern of nucleotide substrate utilisation was observed; any 'preference' for ATP or GTP parallels that displayed towards ADP or GDP in the forward direction.

Estimation of the approximate K_m values for ADP and GDP of the succinate thiokinases from the other bacteria tested showed that they may all be classified into 1 of 4 groups (table 1):

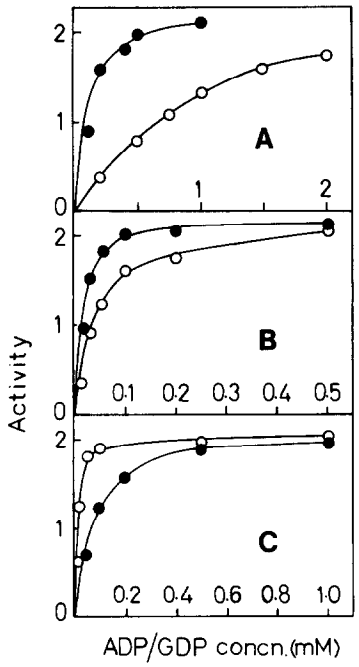

Fig.1. Dependence of activity on ADP and GDP for different succinate thiokinases. Assays were done as described in the text with ADP (○) or GDP (●), using the succinate thiokinase from *Acinetobacter calcoaceticus* (A), *Pseudomonas aeruginosa* (B) and *Escherichia coli* (C). Activity is in arbitrary units. In (A) the nucleotide concentration scales are different for ADP and GDP; for ADP, the numbers 1 and 2 refer to 1.0 and 2.0 mM, whereas for GDP the number 1 represents 0.1 mM.

Table 1

Nucleotide specificity patterns of succinate thiokinases

Group	Organism	K_m ADP	K_m GDP
I	*Acinetobacter anitratus* *Acinetobacter calcoaceticus* *Bordetella bronchiseptica* *Brevibacterium leucinophagum*[a] *Chromobacterium violaceum* *Mima polymorpha* *Xanthomonas hyacinthi*	Very high	Low
II	*Paracoccus dentrificans* *Pseudomonas aeruginosa* *Pseudomonas fluorescens* *Pseudomonas stutzeri* *Rhodopseudomonas spheroides* *Thermus aquaticus*	Low	Low
III	*Alcaligenes faecalis* *Arizona arizonae* *Escherichia coli* *Klebsiella (Aerobacter) aerogenes* *Serratia marcescens*	Low	High
IV	*Arthrobacter simplex* *Bacillus megaterium* *Bacillus stearothermophilus* *Brevibacterium linens* *Kurthia zopfii*	Low	No activity

[a] *B. leucinophagum* was previously classified with the Gram-positive *Brevibacterium* sp. but has now been shown to be Gram-negative [28]

(I) Those resembling *A. calcoaceticus* in having succinate thiokinases with a very high K_m for ADP ($\sim$ 1 mM) but a much lower K_m for GDP ($\sim$ 0.02 mM);

(II) Those with similar and low K_m ($<$ 0.05 mM) for both ADP and GDP, thus resembling *Ps. aeruginosa*;

(III) Those like *E. coli*, showing low K_m-values for ADP ($\sim$ 0.01 mM) mut significantly higher K_m-values for GDP ($\sim$ 0.1 mM);

(IV) Those having succinate thiokinases which functioned with ADP (K_m $<$ 0.1 mM) but which appeared to show no activity with GDP.

Our findings confirm the reports [18,19] that the *R. spheroides* and *E. coli* succinate thiokinases show activity with both adenine and guanine nucleotides, and emphasise that the idea that bacterial succinate thiokinases function specifically with adenine nucleotides must be abandoned. Kelly and Cha [20] have also reported nucleotide substrate preferences for a few bacterial succinate thiokinases. They restricted their studies to Gram-negative bacteria and did not identify distinct groups or patterns of behaviour, but their results are compatible with our scheme.

The data presented in table 1 are derived from a fairly small sample of diverse bacteria. Therefore, it may be premature to draw firm conclusions about the compositions of the 4 groups. There are, however, tentative indications of some grouping together of bacteria with established taxonomic relatedness. Groups I–III are all Gram-negative

bacteria, while group IV comprises Gram-positive bacteria. The enterobacteria come together in group III and the organisms in group II are either pseudomonads or bacteria considered to be related to them. The organisms in group I additionally share several highly distinctive features of 2 other citric acid cycle enzymes, isocitrate dehydrogenase and α-oxoglutarate dehydrogenase, as well as of pyruvate dehydrogenase, which are not exhibited by organisms in groups II–IV [29].

This scan of bacterial succinate thiokinases has exposed apparantly distinct patterns of enzyme diversity which may be correlated with bacterial classification, but further studies will be required to ascertain whether or not these patterns are more widely applicable. Such studies may also throw some light on the possibility that the patterns of nucleotide specificity may have physiological significance in terms of differences in other metabolic reactions in the diverse bacteria [20].

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