SUCCINATE THIOKINASE FROM CYANOBACTERIA

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1. Introduction

The citric acid cycle enzyme succinate thiokinase, also known as succinyl-CoA synthetase (EC 6.2.1.4 or 6.2.1.5), occurs throughout a range of natural organisms in either a 'large' or a 'small' form [1]. The 'large' form has mol. wt 140 000–150 000 and was found to be produced exclusively by Gram-negative bacteria. The 'small' form, on the other hand, has mol. wt 70 000–75 000 and occurs in Gram-positive bacteria and eukaryotic organisms. This distribution of 'large' and 'small' succinate thiokinases parallels strikingly the situation encountered with another citric acid cycle enzyme – citrate synthase. The latter also occurs in either a 'large' or 'small' form, the 'large' form being restricted to Gram-negative bacteria, whereas Gram-positive bacteria and eukaryotes produce the 'small' enzyme [2,3]. These patterns of enzyme size have been shown to be useful in bacterial classification [4,5] and, together with the distinctive catalytic and regulatory properties which are characteristics of the different enzyme forms [3,6–8], they may assist the exploration of natural relationships between organisms.

The cyanobacteria (blue–green bacteria) were previously considered as a class of algae known as blue–green algae or cyanophytes. This classification rested on their plant-like photosynthetic properties. However, these organisms have been shown to be typically prokaryotic and may be classified with other bacteria. Moreover, the structure and chemical composition of their cell walls indicate that the cyanobacteria are Gram-negative prokaryotes (see [9]). Consistent with this classification are our studies of cyanobacterial citrate synthases [8,10]; only the 'large' type of citrate synthase was encountered in several species examined.

Succinate thiokinase has been reported to be absent from cyanobacteria [11] but the method of detection [12] is rather insensitive and not very satisfactory with strongly coloured cell extracts. Using a more sensitive polarographic assay which is unaffected by light-absorbing components [13] we have re-examined cyanobacteria for the presence of succinate thiokinase. These show that the enzyme is present in all of several species of cyanobacteria tested and, on the basis of molecular sizes, confirm the relationship of cyanobacteria to other Gram-negative bacteria.

2. Experimental

The organisms used (see table 1) and details of their conditions of growth were as in [8]. Cells were harvested in late exponential phase and washed twice with 0.1 M Na/K phosphate buffer (pH 7). Cells (5 g wet wt) were then resuspended in 3 ml buffer at 4°C and sonicated for a total of 1 min (with intermittent cooling) in an MSE 100W sonicator operated at full power. Cell debris was removed by centrifugation at 10 000 × *g* and 4°C for 10 min and the supernatant solution used for gel filtration studies without further treatment.

Gel filtration was performed at 4°C on a column (2.5 × 35 cm) of Sephadex G-200 equilibrated with 0.1 M Na/K phosphate buffer (pH 7). Extract (2 ml), to which had been added 50 μl (0.25 mg) of lactate dehydrogenase (rabbit muscle; Boehringer) and 50 μl (20 μg) of citrate synthase (pig heart; Boehringer), was applied to the column and fractions (35 drops; ~2 ml) were collected with an LKB 'Ultrorac' fraction collector.

Succinate thiokinase was assayed polarographically [13] by following the formation of coenzyme A with

a dropping mercury electrode at a potential of −0.2 V relative to a saturated calomel anode; a Radiometer PO4 recording polarograph was used at a full-scale deflection of 0.2 μA [14]. Reaction mixtures contained 0.1 M Na/K phosphate (pH 8), 10 mM Mg^{2+}, 0.15 mM succinyl-CoA and 0.5 mM GDP. Assays were done at 25°C and were initiated by the addition of enzyme to give a total reaction mixture volume of 1.0 ml. Citrate synthase and lactate dehydrogenase were assayed spectrophotometrically as in [2].

3. Results and discussion

Following the procedure in [1] we employed the technique of gel filtration on Sephadex G-200 to determine the molecular sizes of succinate thiokinase in the cell extracts. Lactate dehydrogenase (mol. wt 140 000) and mammalian citrate synthase (mol. wt 100 000) serve as convenient 'markers'; 'large' succinate thiokinases are typically eluted just ahead of lactate dehydrogenase whereas the 'small' thiokinases are eluted after the 'small' citrate synthase.

We detected succinate thiokinase activity in extracts of each of the cyanobacterial species examined (see table 1). Assays were routinely conducted at 0.5 mM GDP; rather less activity was observed at the same concentration of ADP. The specific activity of succinate thiokinase in the various extracts was in the region of 10 nmol . min^{-1} . mg $protein^{-1}$ and we were able to assay the enzyme by incorporating 50 μl extract/ml reaction mixture. Attempts to measure the activity at the same enzyme concentration by the discontinuous colorimetric hydroxamate method [12] were unsuccessful. Furthermore, application of both assay methods to the determination of succinate thiokinase in extracts of *Escherichia coli*, where coloured components were not present to complicate measurements, clearly indicated that the polarographic assay is >20-times more sensitive than the colorimetric method. The failure of other investigators to detect succinate thiokinase in cyanobacteria [11] is thus likely to be due to the rather low level of activity present and to their use of a relatively insensitive assay procedure.

Table 1
Cyanobacteria examined for succinate thiokinase

Anabaenopsis circularis
Anacystis nidulans
Aphanocapsa 6714
Chlorogloea fritschii
Gloeocapsa alpicola
Nostoc sp.E
Plectonema boryanum

The specific activity of succinate thiokinase which we found in cyanobacteria is, nevertheless, of the same order as the specific activities of other cyanobacterial citric acid cycle enzymes [11]. These workers failed to detect succinate thiokinase activity in *Anabaena variabilis* or *Anacystis nidulans* but were able to measure 3-ketoacyl-CoA transferase activity and concluded that succinyl-CoA might therefore be formed from acetoacetyl-CoA and succinate. Our finding of succinate thiokinase in cyanobacteria, and at a level greater than that reported for the 3-ketoacyl-CoA transferase, suggests that formation of succinyl-CoA may proceed directly from succinate, CoASH and a nucleoside triphosphate.

The results of gel filtration experiments indicated that all the cyanobacterial succinate thiokinases are of the 'large' type, and fig.1 shows a typical elution profile. In some gel filtration runs, succinate thiokinase appeared to be eluted from the column further ahead of lactate dehydrogenase than is generally encountered with the enzyme from other Gram-negative bacteria. The possibility exists that some

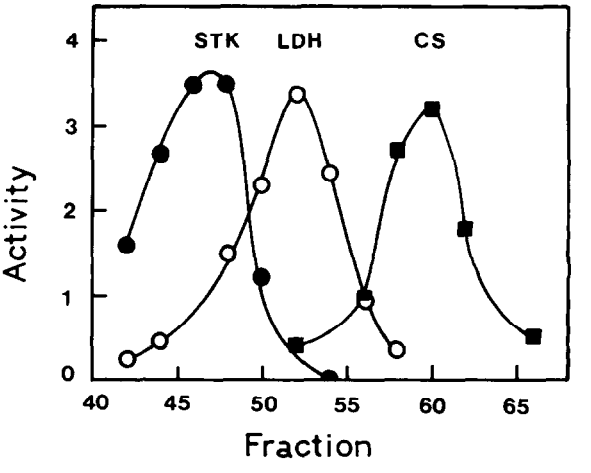

Fig.1. Gel filtration of cyanobacterial succinate thiokinase. A cell-free extract of *Aphanocapsa* 6714 was run on a column of Sephadex G-200 with rabbit muscle lactate dehydrogenase and pig heart citrate synthase as described in the text. Activity is in arbitrary units. (•) Succinate thiokinase (STK); (○) lactate dehydrogenase (LDH); (▪) citrate synthase (CS).

cyanobacterial succinate thiokinases may be >150 000 mol. wt. In no case was there any evidence of a 'small' succinate thiokinase.

These results augment and support the observations in [8,10] and show that both citrate synthase and succinate thiokinase occur in cyanobacteria in their 'large' forms, thereby emphasising the close relationship of cyanobacteria to other Gram-negative bacteria. In the case of citrate synthase we have found that although the cyanobacteria share with other Gram-negative bacteria the feature of possessing a 'large' enzyme, the cyanobacterial enzyme has quite distinct regulatory sensitivities [8,10]. It remains for further studies to ascertain whether cyanobacterial succinate thiokinase also exhibits any distinctive functional behaviour.

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