OCCURRENCE OF 'LARGE' OR 'SMALL' FORMS OF SUCCINATE THIOKINASE IN DIVERSE ORGANISMS

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1. Introduction

Succinate thiokinase, also known as succinyl-CoA synthetase (succinate:CoA ligase (GDP), EC 6.2.1.4 and succinate:CoA ligase (ADP), EC 6.2.1.5), is an enzyme which has attracted considerable attention, largely because of its unique substrate phosphorylation role in the citric acid cycle and the complexity of the reaction catalysed. The enzymes from *Escherichia coli* and pig heart have been investigated in some detail [1] and one striking difference to emerge is in their molecular weights. The *E. coli* enzyme has mol. wt 140 000–150 000, whereas the pig enzyme is approx. 1/2 this size with mol. wt 70 000–75 000. There is experimental evidence that the *E. coli* enzyme is a tetramer and the pig enzyme a dimer. The molecular weights of 3 other bacterial succinate thiokinases were reported to be very similar to that of the *E. coli* enzyme [2] but, apart from this, little information is available concerning the molecular sizes of succinate thiokinases from other sources.

We have shown that there is a marked division of another citric acid cycle enzyme, citrate synthase, into 'large' and 'small' types, the former (a tetramer) corresponding to mol. wt ~250 000 and the latter (a dimer) to about 100 000 [3,4]. The 'large' citrate synthases occur exclusively in Gram-negative bacteria whereas Gram-positive bacteria and all eukaryotic organisms produce 'small' citrate synthases.

We therefore thought it possible that succinate thiokinases might also divide into 'large' and 'small' types along a similar taxonomic boundary and undertook a survey, by gel filtration, of the molecular sizes of a range of succinate thiokinases. The results reported here indicate that this enzyme does indeed show a clear-cut division into two molecular types according to the status of the source organism.

2. Experimental

Bacillus stearothermophilus and *Rhodopseudomonas spheroides* were purchased as frozen cell pastes from the Microbiological Research Establishment, Porton Down, Wilts. The other bacterial strains used were from the culture collection of this laboratory and each was grown aerobically in 1 litre nutrient broth for 24 h at 30°C. The cells were collected by centrifugation at 25 000 × *g* for 10 min, suspended in 5 ml 0.1 M phosphate, pH 7, and disrupted by treatment in an MSE 100 W sonicator for 2 min at full power with cooling. After recentrifugation, the supernatant solutions were used without further purification. Extracts of the purchased cells were prepared in a similar way.

Extracts of baker's yeast (Distillers Co. Ltd.) and wheat germ (Bemax) were obtained by passing chilled suspensions of these materials in 0.1 M phosphate, pH 7, through a French press. After centrifugation, the supernatant solutions were treated with protamine sulphate (1 mg/10 mg protein) to remove nucleic and then recentrifuged. The clear supernatant solutions were fractionated with ammonium sulphate; material precipitating at 50–60% saturated ammonium sulphate was redissolved in small vol. 0.1 M phosphate, pH 7, and used for the studies described. Fresh pig liver (120 g) was homogenised in 100 ml 0.1 M phosphate, pH 7. The mixture was centrifuged, treated

with protamine sulphate (as above), recentrifuged, and the clear supernatant used for the enzyme studies.

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

Succinate thiokinase was assayed polarographically [5]. The formation of coenzyme A was continuously monitored with a dropping mercury electrode at a potential of −0.2 V relative to a saturated calomel anode using a Radiometer PO4 recording polarograph at a full-scale deflection of 0.2–0.5 μA [6]. The reaction mixtures contained 0.1 M phosphate, pH 8, 10 mM Mg^{2+}, 0.15 mM succinyl-CoA and 0.5 mM ADP. In the case of the enzyme from pig liver, GDP was substituted for ADP [7]. Reactions were done at 25°C and were initiated by the addition of enzyme. Citrate synthase and lactate dehydrogenase were assayed spectrophotometrically as in [3].

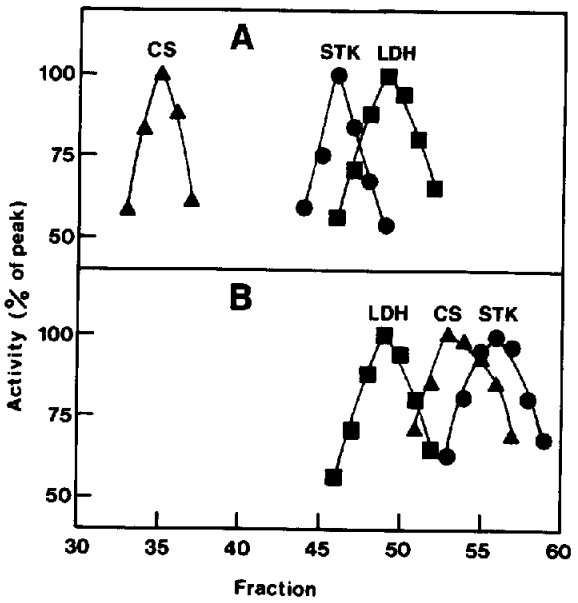

Fig.1. Gel filtration of succinate thiokinases. Cell-free extracts were run on a column of Sephadex G-200 with lactate dehydrogenase as in the text. (A) Extract of *E. coli*; (B) extract of baker's yeast. (•) Succinate thiokinase (STK); (▪) lactate dehydrogenase (LDH); (▲) citrate synthase (CS).

3. Results and discussion

The polarographic assay allowed succinate thiokinase to be measured readily in all the crude extracts examined and is better suited to this type of study than the spectrophotometric assay methods generally used for this enzyme.

Gel filtration was used to obtain an approximate measure of the molecular sizes of the various succinate thiokinases. Lactate dehydrogenase (mol. wt 140 000) served as a 'marker' protein and the citrate synthase of each particular organism served as an additional marker. It was noted above that the molecular weights of *E. coli* and mammalian succinate thiokinases are 140 000–150 000 and 70 000–75 000, respectively. Consistent with these values we observed that the *E. coli* enzyme was eluted from the G-200 column slightly ahead of lactate dehydrogenase whereas the pig liver enzyme was eluted considerably later than lactate dehydrogenase. All the organisms examined had succinate thiokinases conforming to one of these two types, which we have referred to as 'large' or 'small'. Figure 1 shows typical elution profiles for the two types of enzyme, and table 1 lists the organisms examined according to enzyme type. It is immediately apparent that only Gram-negative bacteria produce 'large' succinate thiokinases and that Gram-positive bacteria and diverse eukaryotic organisms produce the 'small' type.

The molecular weight values for the enzyme from three other Gram-negative bacteria (*Aerobacter aerogenes, Pseudomonas citronellolis,* and *Herellea vaginicola*) were recently found by gel filtration to be approx. 155 000 [2]. These results conform to the pattern of enzyme types and support our conclusion that the 'large' succinate thiokinase is a particular feature of Gram-negative bacteria. Hitherto, there have been no data on the molecular weights of succinate thiokinase from Gram-positive bacteria and it has been implicitly assumed that all bacterial succinate thiokinases resemble that of *E. coli*. Our results clearly show that this is not the case; rather, the Gram-positive bacterial enzymes resemble those of eukaryotic organisms, at least in molecular size.

There is thus a striking correlation between the incidence of 'large' and 'small' succinate thiokinases

Table 1
Molecular size of succinate thiokinases

'Large' enzyme	'Small' enzyme
Acinetobacter calcoaceticus	*Bacillus megaterium*
Brevibacterium leucinophagum[a]	*Bacillus stearothermophilus*
Escherichia coli	*Brevibacterium linens*
Pseudomonas aeruginosa	*Corynebacterium rubrum*
Rhodopseudomonas spheroides	Baker's yeast (*Saccharomyces cerevisiae*)
Xanthomonas hyacinthi	Wheat germ
	Pig liver

[a] *B. leucinophagum* was previously mistakenly classified with the Gram-positive *Brevibacterium* spp. but has now been shown to be Gram-negative [9]

and that of 'large' and 'small' citrate synthases [3,4]. This is also illustrated in fig.1 which shows that citrate synthase is eluted from the gel column either well before, or well after, lactate dehydrogenase, corresponding to a 'large' form (mol. wt ~250 000) or a 'small' form (mol. wt ~100 000), respectively.

It would thus appear that both citrate synthase and succinate thiokinase can be produced in tetrameric or dimeric forms, and the similarity in their distribution in nature is intriguing. In the case of citrate synthase, only the 'large' enzymes are sensitive to allosteric inhibition by NADH [4]. By analogy, some regulatory sensitivity might be expected for the 'large' succinate thiokinases but, as yet, no such properties have been observed.

It is noteworthy that both citrate synthase and succinate thiokinase utilize an acyl-CoA as substrate. Moreover, Gram-negative bacterial citrate synthase has a distinct (regulatory) nucleotide binding site and succinate thiokinase has a catalytic nucleotide binding site. These superficial resemblances, together with the molecular size similarities, prompt the speculation that there may be some evolutionary link between the two enzymes.

An extensive survey of malate dehydrogenases [8] has shown that the enzymes from all animal and plant sources, as well as from a number of both Gram-negative and Gram-positive bacteria, have a mol. wt 60 000. However, some Gram-positive bacteria, particularly *Bacillus* spp., possess a malte dehydrogenase of approx. 2 times this size. Thus, although there are 'large' and 'small' forms of malate dehydrogenase, their occurrence does not follow the division between Gram-negative and Gram-positive bacteria exhibited by citrate synthase and succinate thiokinase. Nor does other published information indicate comparable molecular size patterns for any of the other citric acid cycle enzymes.

The correlation between succinate thiokinase molecular size and taxonomic grouping suggests a potential value of the examination of succinate thiokinase for bacterial classification. We have demonstrated the value of citrate synthase size estimation in such classification [9–11], and measurements on succinate thiokinase might be used in conjunction. This approach can contribute to the correct classification of bacteria which do not give an unequivocal Gram reaction and may even permit the correction of misclassification [9].

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