PROPERTIES AND REGULATORY EFFECT ON TETRAPYRROLE BIOSYNTHESIS OF SUCCINYL CoA SYNTHETASE ISOLATED FROM SOYBEAN CALLUS TISSUE SYSTEM

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1. Introduction

Succinyl-CoA is involved in the early steps of the biosynthesis of tetrapyrrole compounds such a haem and chlorophyll and its formation is catalysed by succnicyl-CoA synthetase (succinate: CoA ligase (GDP or ADP) EC 6.2.1.4 or 6.2.1.5). It has been purified from mammalian, bacterial and plant tissues such as spinach [1], wheat leaves [2], artichoke mitochondria [3] and tobacco [4].

This communication reports some properties of succinyl-CoA synthetase isolated and partially purified from soybean callus, a highly dividing tissue, useful for detecting active systems as was described by Miller [5]. The inhibitory effect of protoporphyrin and haemin on the enzyme is briefly discussed in relation to its probable regulatory action on the tetrapyrrole biosynthetic pathway.

2. Material and methods

Soybean callus was grown according to Miller [5]. The enzymic incubation medium contained tris buffer (pH, 8.2), GSH, ATP, sodium succinate, hydroxylamine and $MgCl_2$. The enzyme preparations were preincubated for 30 min at 37°. The reaction was started with CoA, incubated for 30 min at 37° and terminated with a mixture of 25% trichloroacetic acid-3 N HCl-5% $FeCl_3$ (1:1:1, v/v/v). The succinyl hydroxamate formed was determined as described by Kaufman and Alivisatos [1]; a synthetase unit is defined as the amount of enzyme that catalyses the formation of 1 μmole of succinyl hydroxamate in 30 min under standard conditions described. Free protoporphyrin IX was a generous gift of Dr. Batlle and it was prepared from blood by the method of Grinstein [6].

3. Results and discussion

3.1. *Enzyme isolation and purification*

Acetone powder of callus tissue was prepared as previously described [7]. The enzyme was extracted from acetone powder stocks with 0.1 M glycine-NaOH buffer (pH: 9.0; 1:30, w/v). This crude enzyme extract was purified 16-fold by ammonium sulphate fractionation (50–75% saturation), 2% protamine sulphate treatment (0.25 ml/ml of enzyme solution) and gel filtration on Sephadex G-100; protein was eluted with 0.05 M tris-HCl buffer, pH 9.0. Properties were studied in 16-fold purified preparations; purification was further improved to 140-fold by acetone fractionation (37.5–54.5% saturation) and gel filtration on Sepharose 6B, but the yield was very low.

3.2. *Glutathione requirement and thiol inhibitor effect*

It is an interesting finding, not reported for other synthetases, that the soybean enzyme was activated by preincubation with GSH (table 1); GSH cannot be replaced by cysteine, thyoglycollate and dithiothreitol. The GSH effect cannot be attributed to CoA protection because it increased the enzymic activity up to 30 min of preincubation in the absence of CoA. Thiol inhibitors such as iodoacetamide, GSSG and *n*-ethyl maleimide (1–5 mM, final concn.) produced partial inhibition of enzymic activity; without preincubation with inhibitors, inhibition was increased, probably due

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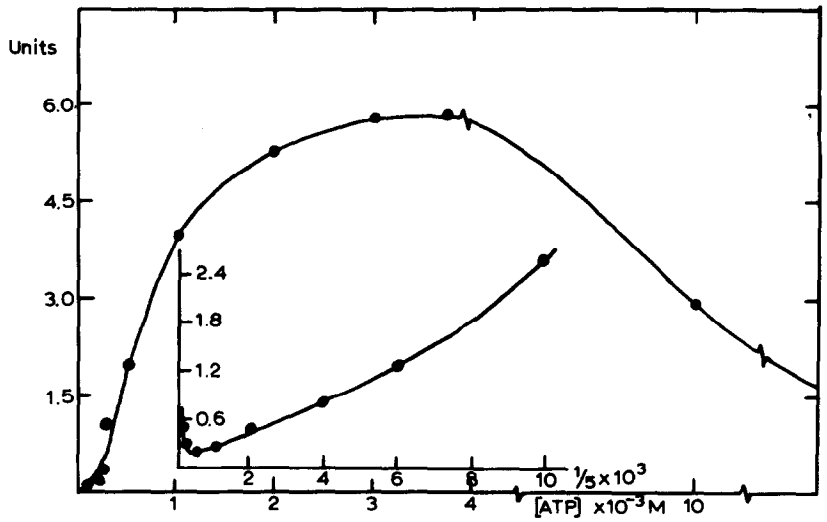

Fig. 1. Effect of ATP concentration on succinyl-CoA synthetase. The inset shows a double reciprocal plot of velocity and substrate. Incubation conditions as described in Methods.

to the rapid reaction of inhibitors with the CoA added to initiate the reaction; thus, it is possible that during preincubation, the enzyme reacted immediately with the inhibitor, leaving insufficient inhibitor to react with the CoA. Preincubation with GSH reversed the inhibitory effect. Despite the fact that one of the substrates is CoA, these findings suggest that the presence of –SH groups is related to catalytic activity.

Table 1
Effect of glutathion on succinyl-CoA synthetase.

Addition	Final concn. (mM)	% of activity at min				
		0	15	30	45	60
None	–	100	–	–	–	–
GSH	2.5	–	–	163	–	–
GSH	5.0	116	155	198	184	171
GSH	10.0	–	–	198	–	–

The enzyme was incubated as described in methods, except that GSH and preincubation were omitted at the control tube. Enzymic activity on this tube was considered 100%. The enzymic reaction was initiated with CoA.

3.3. *Metal requirement and nucleotide effects*

This enzyme has an absolute requirement for either Mg^{2+} or Mn^{2+} ions at an optimal final concentration of 5 mM. A kinetic study of the effect of ATP (fig. 1)

Table 2
Effect of protoporphyrin IX and haemin on the activity of succinyl-CoA synthetase.

Addition	Final concn. (mM)	Remaining activity (%)
Protoporphyrin IX	5.0	65
Protoporphyrin IX	10.0	45
Haemin	0.20	40
Haemin	0.15	46
Haemin	0.10	73
Haemin	0.05	78
Haemin	0.01	83

Incubation mixture was described in Methods. The activity of the system in the presence of these compounds is expressed on the basis of the amount of succinyl hydroxamate formed by the control as 100%. The effect of haemin and protoporphyrin IX in the colour development of succinyl hydroxamate was checked.

showed an homothropic cooperative effect; at concentrations above 10^{-2} M, ATP was inhibitory; ADP and AMP (final concn. 5 mM) inhibited the enzyme 30%. From a Hill plot [8] not shown here, an average value of $n = 1.7$ was obtained. These results suggest that two molecules of ATP react with one molecule of soybean synthetase. Ramaley [9] and Grinnell and Nishimura [10] found that two phosphoryl groups were incorporated per mole of *E. coli* succinyl-CoA synthetase. This suggests that the soybean and the bacterial enzyme might have a similar structure, with at least two different sites for ATP per mole of enzyme.

3.4. *Haemin and protoporphyrin effects*

An inhibitory effect of protoporphyrin and haemin at different concentrations was observed (table 2). As these are final metabolites in tetrapyrrole biosynthesis, it is possible that they exert a feed-back inhibition; Burham and Lascelles [11] described a similar effect in *R. spheroides*, taking into account their inhibitory effect on Δ-aminolaevulinate synthetase, an enzyme which catalyses the formation of Δ-aminolaevulinate from glycine and succinyl-CoA. Also, Granick and Levere [12] have considered these effects in their hypothetical scheme on the control regulation of haem biosynthesis, in which haemin is thought to be an example of coordinate repression on Δ-aminolaevulinate acid synthetase and Δ-aminolaevulinate acid dehydratase (5-aminolaevulinate hydrolyase, EC 4.2.1.24); therefore we can assume that it has a similar effect on succinyl-CoA synthetase. In addition, Labbe et al. [13] in 1965 suggested that this enzyme isolated from mammalian tissues might play a role in the control of porphyrin biosynthesis, because they found that porphyrinogenic drugs influence succinyl-CoA synthetase *in vivo*. We have confirmed such observations, using hexachlorobencene as a porphyrinogenic drug (unpublished results).

Finally, considering the ATP, ADP and AMP inhibition shown above, these compounds might also play a role in the regulation of this reversible enzyme and on the whole pathway of tetrapyrrole biosynthesis, as was also described by Gajdos et al. [14]. As a final conclusion, the most important fact which has emerged from our experiments is that succinyl-CoA synthetase showed a porphyrin feed-back inhibition, not reported for other synthetases suggesting that this enzyme might also be involved in the regulation of tetrapyrrole biosynthesis.

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