THE SULFHYDRYL GROUPS OF CITRATE-CONDENSING ENZYME*

Paul A. Srere

Lawrence Radiation Laboratory, University of California

Livermore, California

Received October 23, 1964

Until recently it has been generally assumed that a sulfhydryl (SH) group is not involved in the active site of citrate-condensing enzyme. Stern (1960) has reported that citrate-condensing enzyme is fully active after treatment with 10^{-3}-M p-chlormercuribenzoate (pCMB). We have observed (Broder et al., 1962) that the citrate-condensing enzyme of pig heart contains SH groups that react in aqueous solution with pCMB with no great reduction of enzymatic activity. We also reported (Brazil and Srere, 1963) that other compounds which are known to react with SH groups do not inhibit the activity of the enzyme. In addition, we routinely assay the activity of the enzyme (Srere et al., 1963) in the presence of 5, 5'-dithiobis (2-nitrobenzoate) (DTNB) (Ellman, 1959), an SH reagent, with no apparent effect on enzyme activity. We have concluded that the SH groups on the protein do not participate in the enzymatic reaction.

Eggerer et al. (1964) on the other hand have reported that the SH group of citrate-condensing enzyme is essential for its activity and have proposed a mechanism which involves it in the catalytic reaction. Although Eggerer and his co-workers confirmed the observations that treatment of the enzyme with either iodoacetamide or N-ethyl maleimide does not cause an inhibition of activity, they report that

*Work done under the auspices of the U. S. Atomic Energy Commission.

1.3×10^{-3}-M pCMB incubated with the enzyme for 30 minutes at 0° resulted in loss of enzyme activity. Further, they interpret our results (Srere, 1963), which show that acetylation of citrate-condensing enzyme results in loss of activity, as indicating an involvement of SH.

I have, in addition, unpublished experiments which show that at a low molar ratio of iodine to enzyme a complete inhibition of enzyme activity is observed, which may also be evidence for SH involvement. In view of the possibility that the SH groups involved in the reaction might be unavailable to certain SH reagents, together with the discrepancies in the results of Eggerer and others, we have carried out more experiments to attempt to better understand the role of the SH groups in the reaction.

Amino acid analysis indicates the presence of about 6 mols of 1/2 cystine per mol of enzyme. When the enzyme is allowed to react in aqueous solution with DTNB no free sulfhydryl can be detected (Table 1); however, with DTNB plus urea 4 mols of SH can be titrated.

Table 1. The sulfhydryl groups of citrate-condensing enzyme.

Analytical procedure	Mols SH/80,000 g protein[b]
Amino acid analysis[a] (1/2 cystine)	6
DTNB in 0.1-M tris pH 8.1	0
DTNB in 0.1-M tris pH 8.1 4.0-M urea	3.8
pCMB in 0.1-M tris pH 8.1	1.9[c]
pCMB in 0.1-M tris pH 8.1 4.0-M urea	4.4

[a]Kindly performed by Dr. L. Levintow.

[b]Our recent studies on the sedimentation coefficient of the enzyme would indicate that this figure is a better value for the molecular weight of the enzyme than earlier figures.

[c]This value is obtained in 5 minutes before precipitation of the enzyme occurs. No precipitation occurs with pCMB in urea.

Their rate of appearance depends upon the urea concentration and is first-order for over 90% of reaction (Srere, 1964). In aqueous solution pCMB shows the presence of 2 mols of SH immediately (Fig. 1), but further titration is obscured because the pCMB enzyme begins to precipitate. With pCMB plus urea, however, 4 mols of SH groups can be titrated and no precipitation occurs (Table 1 and Fig. 1).

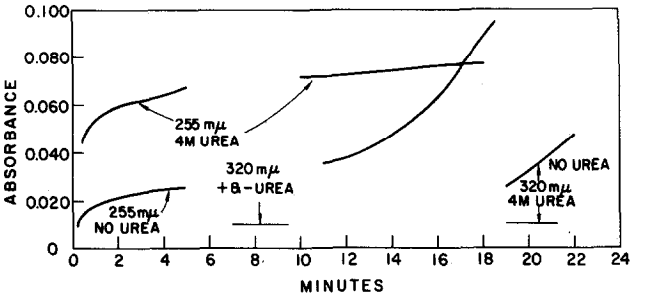

Fig. 1. The reaction of pCMB with citrate-condensing enzyme. Each cuvette contained 100 μmols tris-HCl, pH 8.1, and 200 μg (2.5 mμmols) citrate-condensing enzyme in a total volume of 1.0 ml. The reaction was started by the addition of 10 μl of 5×10^{-3}-M pCMB. An appropriate control without enzyme was run simultaneously.

If one looks at the activity of the variously modified proteins (Table 2), there is no correlation between SH groups and activity. When half of them are covered with pCMB, then 79% of the activity remains; when 94% of the activity is inhibited with acetic anhydride, little change has occurred in the SH content of the enzyme. Hg-enzyme has no sulfhydryl groups and retains 46% of its activity. If Hg-enzyme or pCMB-enzyme (or N-ethyl maleimide enzyme, not shown here) is allowed to stand for longer periods of time, then precipitation occurs and no activity can be detected. 10^{-3}-M iodoacetamide does not react with the enzyme (15 min at 0°) since no change in the enzyme SH content is seen, nor is there any observable loss of activity. DTNB-enzyme cannot be assayed for activity since it can only be formed in

urea and enzyme activity is lost in urea whether or not DTNB is present (Srere, 1964).

Table 2. Relation of SH groups to enzyme activity.

Preparation	% activity	Mols SH/80,000 g protein
Enzyme	100	3.9[a]
Acetyl-enzyme	6	3.6[a]
Mercuri-enzyme[b]	46	0[c]
pMB-enzyme	79[d]	(2)[e]

[a]Measured with DTNB in 6.8-M urea.

[b]5.0×10^{-4}-M protein SH incubated 15 min at 25° with 5.0×10^{-4}-M $HgCl_2$.

[c]Measured with pCMB with and without 4-M urea.

[d]This is 15 minutes after addition of pCMB and over half of the SH groups have been titrated; precipitation had proceeded (as judged by turbidity at 320 mμ) for 2-3 minutes.

[e]By difference.

Several other observations made by us in the course of other investigations are pertinent to this question. The SH content of preparations has varied from 1 mol per mol of protein to 4 mols per mol of protein, whereas the corresponding enzyme activities varied from about 70 to 100%. No activation of preparations has ever been seen with SH compounds (mercaptoethanol, cysteine, glutathione). We have reported the existence of two electrophoretically separable active proteins in a preparation which is homogeneous in the ultracentrifuge. The conversion of one form to the other seemed to depend upon an SH to SS conversion that did not affect the enzyme activity (Broder and Srere, 1963).

The available data are consistent with the idea that the SH groups of citrate-condensing enzyme are all involved in internal hydrogen bonding to maintain the proper conformation of the active site of the enzyme. It is unlikely that a subunit structure is involved since no

appreciable change was observed in the sedimentation of enzyme in urea or sodium dodecyl sulfate.

REFERENCES

Brazil. H., and Srere, P. A., Federation Proc. 22, 240 (1963).

Broder, I., Bhaduri, A., and Srere, P. A., Federation Proc. 21, 252 (1962).

Broder, I., and Srere, P. A., Biochim. Biophys. Acta, 67, 626 (1963).

Eggerer, H., Remberger, U., and Grünewalder, C., Biochem. Z, 339, 436 (1964).

Ellman, G., Arch. Biochem. Biophys. 82, 70 (1959).

Srere, P. A., Biochim. Biophys. Acta, 77, 693 (1963).

Srere, P. A., Brazil, H., and Gonen, L., Acta Chem. Scand., 17, S129 (1963).

Srere, P. A., unpublished observations (1964).

Stern, J. R., The Enzymes, 2nd Edition, Boyer, P. D., Lardy, H., and Myrbäck, K., editors, Volume 5. Academic Press, New York, p. 377 (1961).