STOICHIOMETRY OF LABILE SULFIDE, NONHEME IRON AND FLAVIN IN RECONSTITUTIVELY ACTIVE SUCCINATE DEHYDROGENASE FROM HEART MITOCHONDRIA

Tsoo E. King
Oregon State University, Corvallis, Oregon

Received June 18, 1964

Cardiac mitochondria and mitochondrial preparations have been found rich in nonheme iron (Green and Wharton, 1963; King et al. 1964). From these mitochondrial preparations is isolated the reconstitutively active, soluble succinate dehydrogenase which shows an unusually high content of nonheme iron (King, 1963a) and exhibits a distinct EPR signal at g = 1.94 (King, 1961). This signal is presumably due to nonheme iron (Beinert and Lee, 1961) linked with a protein in a special configuration. Recent studies mainly on ferredoxins of plants and microorganisms and hepatic aldehyde oxidase of presumably cytoplasmic origin have induced a generalization about the coexistence of nonheme iron in an amount essentially equivalent with the "labile sulfide" content (see, for example, Handler et al., 1964; San Pietro, 1964).

These facts prompted us to investigate the occurrence of labile sulfide, if any, and its stoichiometry with nonheme iron and flavin in succinate dehydrogenase. Results reported in this communication show that labile sulfide and nonheme iron do exist in approximate equivalence in the dehydrogenase. Thus, this finding reinforces the generalization about the coexistence of these two entities from a soluble, physiologically active enzyme of heart mitochondria.

Materials and Methods -- Reconstitutively active succinate dehydrogenase was prepared according to Method 3A previously described (King, 1963a) up to the stage of the first ammonium sulfate fractiona-

tion or according to the same method with minor modifications (to be published). A highly purified sample of photosynthetic pyridine nucleotide reductase (PPNR) from spinach leaf was kindly supplied by Dr. A. San Pietro.

Nonheme iron was determined colorimetrically (King, 1963a; King et al., 1964). The succinate dehydrogenase flavin which is acid non-extractable was estimated fluorometrically (King, 1963b; Wilson and King, 1964). "Labile sulfide" was determined by the method of Fogo and Popowsky (1949) as adopted by Fry and San Pietro (1962) and Lovenberg et al. (1963) with minor modifications; sodium sulfide standardized iodometrically was used as a standard.

Results and Discussion -- The absorption spectrum of the colored compound formed from sodium sulfide in the "labile sulfide" determination was virtually the same as originally reported by Fogo and Popowsky (1949). However, the spectra formed from succinate dehydrogenase and PPNR in the colorimetry were not exactly identical with the spectrum from inorganic sulfide. The main difference, between succinate dehydrogenase and PPNR on the one hand and inorganic sulfide on the other, was the absorbance ratio of 668-9 mμ (maximum) to 707 mμ (minimum), as illustrated in Fig. 1.

Contents of labile sulfide, nonheme iron and flavin for several batches of succinate dehydrogenase are listed in Table I. These preparations were completely free of acid extractable flavin and heme iron. It can be seen from the table that, although absolute contents of these constituents varied, their ratioes remained essentially constant. The average ratioes of flavin : nonheme iron : labile sulfide were found to be 1 : 8.5 : 8.1.

The labile sulfide in succinate dehydrogenase was completely sensitive to a mercurial compound. The dehydrogenase was mixed with an excess (with respect to its labile sulfide content) of Mersalyl, o-[(3-

Table 1. Contents of labile sulfide, nonheme iron and acid non-extractable flavin in succinate dehydrogenase*

Preparation	Content			Ratio
	(1) Sulfide	(2) Iron	(3) Flavin	(1) : (2) : (3)
1	22	23	2.9	7.6 : 7.9 : 1
2	20	19	2.4	8.3 : 7.9 : 1
3	25	30	2.8	8.9 : 10.7 : 1
4	24	26	3.0	8.0 : 8.7 : 1
5	28	30	3.6	7.8 : 8.3 : 1
6	27	26	3.5	7.7 : 7.4 : 1
Average				8.1 : 8.5 : 1

* The unit is in mμmoles per mg of succinate dehydrogenase protein for labile sulfide and flavin and in mμatoms per mg protein for nonheme iron. Preparations 5 and 6 were obtained from a modified method (see the text).

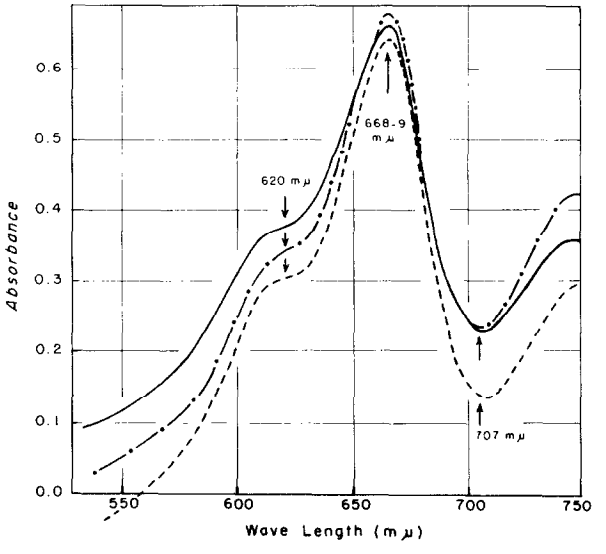

Fig. 1. Absorption spectra of the colored compound formed from sodium sulfide (.....), succinate dehydrogenase (————), and PPNR (—·—·—), in labile sulfide determination according to the method of Fogo and Popowsky (1949). A Cary spectrophotometer model 11, at 23°, was used in measurement.

hydroxymercuri-2-methoxypropyl)carbamyl/phenoxyacetate, for one minute at 4° and then passed through a Sephadex G-25 column to remove the excess reagent. The yellow eluate showed less than 1% of its original content of labile sulfide. No change of the labile sulfide content was observed after the dehydrogenase had been passed through a similar column of Sephadex but without prior treatment by Mersalyl.

The reconstitutive activity of succinate dehydrogenase is very labile; its half-life is less than 90 minutes at 0-4° (King, 1961; King, 1963a). However, the loss of labile sulfide of the dehydrogenase was found not parallel to the reconstitutive activity. Succinate dehydrogenase samples at the age of 24 hours showed practically no reconstitutive activity but retained more than 75% of its original content of labile sulfide.

Massey (1957) working on a reconstitutively inactive succinate dehydrogenase (succinate-phenazine reductase) has noted "a strong odor resembling H_2S" upon boiling or acidification of the enzyme. He has implied that the H_2S is degraded from protein-sulfhydryls. In contrast to the reconstitutively active enzyme, succinate-phenazine reductase does not react with the cytochrome system (King, 1961, 1963a) and contains, according to the Singer group (e.g. Bernath and Singer, 1962) only 2 or 4 atoms of nonheme iron per molecule of flavin.

Recently Pharo and Sanadi (1964) have reported that soluble DPNH-coenzyme Q reductase contains flavin : nonheme iron : labile sulfide at 1 : 2.3 : 3.9. From a cytochromes b-c_1 complex of a heart mitochondrial preparation, Rieske et al. (1964a,b) have isolated an insoluble, presumably a polymerized, nonheme iron protein free of flavin. This insoluble protein after chemical modification becomes soluble in aqueous media. Although no enzymatic function of this protein has yet been found, interestingly enough it also contains labile sulfide at 0.7-0.8 molecule per atom of nonheme iron. According to Rieske et al. (1964b) their pro-

tein occurs between cytochromes b and c_1. In view of the difference in the positions on the respiratory chain, it does not seem to be likely that the protein moiety containing nonheme iron and labile sulfide in succinate dehydrogenase as reported in this communication is identical with the protein of Rieske et al.

Acknowledgement -- Tireless advice from Drs. Bob Buchanan and A. San Pietro on labile sulfide determination and from Professor H. Freund on the intricacy of the method is gratefully acknowledged. Technical assistance by Joanne Potter is also acknowledged. This work was supported by grants from the National Science Foundation, the U.S. Public Health Service, the American Heart Association, and the Life Insurance Medical Research Fund.

References

Beinert, H., and Lee, W. (1961), Biochem. Biophys. Res. Communs., 5, 40.
Bernath, P., and Singer, T. P. (1962), in S. P. Colowick and N. O. Kaplan (Editors), Methods in Enzymology, Vol. 5, Academic Press, New York, p. 597.
Fogo, J. K., and Popowsky, M. (1949), Anal. Chem., 21, 732.
Fry, K. T., and San Pietro, A. (1962), Biochem. Biophys. Res. Communs., 9, 218.
Green, D. E., and Wharton, D. C. (1963), Biochim. Zeit., 338, 335.
Handler, P., Rajagopalan, K. V., and Aleman, V. (1964), Federation Proc., 23, 30.
King, T. E. (1961), Biochim. et Biophys. Acta, 47, 430.
King, T. E., Howard, R. L., and Mason, H. S. (1961), Biochem. Biophys. Res. Communs., 5, 329.
King, T. E. (1963a), J. Biol. Chem., 238, 4032.
King, T. E. (1963b), Nature, 198, 366.
King, T. E., Nickel, K. S., and Jensen, D. R. (1964), J. Biol. Chem., 239, 1989.
Lovenberg, W., Buchanan, B. B., and Rabinowitz, J. C. (1963), J. Biol. Chem., 238, 3899.
Massey, V. (1957), J. Biol. Chem., 229, 763.
Pharo, R. L., and Sanadi, D. R. (1964), Federation Proc., 23, 486.
Rieske, J. S., MacLennan, D. H., and Coleman, R. (1964a), Biochem. Biophys. Res. Communs., 15, 338.
Rieske, J. S., Coleman, R., and MacLennan, D. H. (1964b), Federation Proc., 23, 323; and discussion.
San Pietro, A. (1964), Abstracts, 6th Int'l Congress Biochemistry, 5, 369.
Wilson, D. F., and King, T. E. (1964), J. Biol. Chem., in press.