# THE FUNCTION OF INORGANIC IRON IN THE REDUCTION OF CYTOCHROME *C*[*]

by

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In the course of an investigation of the reduced diphosphopyridine nucleotide (DPNH) oxidase of crude cell-free extracts of *Clostridium kluyveri* it was found that ferric iron, in the presence of *o*-phenanthroline and DPNH, could be reduced. In addition it was also shown that cytochrome *c* could be reduced by the crude extract and DPNH. Further studies showed that cytochrome *c* could be reduced non-enzymically by ferrous iron in the presence of either sodium citrate or pyrophosphate[1]. In view of these results, and of the recent work by MAHLER AND ELOWE[2] in which the participation of iron in the DPNH animal cytochrome *c* reductase has been indicated, a study of the various flavoprotein systems was undertaken to determine the significance of ferrous iron in the reduction of cytochrome *c*.

Purified DPNH diaphorase from pig heart[3], purified reduced triphosphopyridine nucleotide (TPNH) cytochrome *c* reductase from liver[4], and crude extracts of *Pseudomonas fluorescens*[5] reduced iron aerobically in the presence of *o*-phenanthroline. In addition, anaerobic experiments indicated that ferrous iron accumulated in these systems in the absence of *o*-phenanthroline.

In view of the fact that the $E_0'$ of the ferric/ferrous system is $+0.771$ volts, and that of cytochrome *c* at pH 7.0 is $+0.262$ volts[6], it would appear improbable that ferrous iron is capable of reducing cytochrome *c*. However, MICHAELIS AND FRIEDHEIM[7] have demonstrated that iron complexes of anions such as oxalate and pyrophosphate, at wide pH ranges, have potentials close to 0.00 volts. Because of this shift in O–R potential the reduction of cytochrome *c* by ferrous iron in pyrophosphate became thermodynamically feasible. Although the $E_0'$ of the ferric/ferrous citrate complex has not been elucidated, it appears that the potential is more negative than that of cytochrome *c*. The results of the non-enzymic reduction of cytochrome *c* by varying concentrations of ferrous iron in sodium citrate are summarized in Table I.

TABLE I

NON-ENZYMIC REDUCTION OF CYTOCHROME *c* BY VARYING CONCENTRATION OF FERROUS IRON

Reaction mixture consisted of cytochrome *c* (0.06 $\mu$Moles), $Na_3$ citrate·$2H_2O$ (0.03 mMoles), $FeSO_4 \cdot 7H_2O$ (as indicated) in a total volume of 3.0 ml. Reduction of cytochrome *c* was measured spectrophotometrically at $\lambda$ 550 m$\mu$.

| | | | | | |
|---|---|---|---|---|---|
| $\mu$Moles $FeSO_4 \cdot 7H_2O$ | 0.015 | 0.030 | 0.045 | 0.060 | 0.150 |
| $\mu$Moles cytochrome *c* reduced | 0.015 | 0.030 | 0.035 | 0.042 | 0.056 |

The DPNH diaphorase was found to have no appreciable cytochrome *c* reductase activity under both aerobic and anaerobic conditions. Addition of ferric iron did not increase the rate of cytochrome *c* reduction. However, anaerobic preincubation of the enzyme with DPNH and $1.5 \cdot 10^{-3}$ *M* ferric iron in sodium citrate for increasing periods of time gave respective increases in the total reduction of cytochrome *c* added subsequent to the incubation period. Preincubation for one hour gave 90% reduction of cytochrome *c*. Heating the preincubated anaerobic reaction mixture to 100° C before the addition of cytochrome *c* gave a comparable reduction. These results, as summarized in Table II, indicate that the reduction of cytochrome *c* does not require the presence of the diaphorase if ferrous iron is present.

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TABLE II

EFFECT OF ANAEROBIC PREINCUBATION OF DIAPHORASE, FERRIC IRON, AND DPNH ON THE REDUCTION OF CYTOCHROME *c*

Reaction mixture, in a total volume of 4.0 ml, consisted of $Na_3$ citrate·$2H_2O$ (0.133 mMoles), DPNH (0.763 $\mu$Moles), $FeCl_3 \cdot 6H_2O$ (6.0 $\mu$Moles), and diaphorase (0.29 mg protein). Cytochrome *c* (0.08 $\mu$Moles) was added after the indicated time interval of incubation. Reduction of cytochrome *c* was measured spectrophotometrically at $\lambda$ 550 m$\mu$.

| Time of preincubation (min) | 5 | 15 | 30 | 45 | 60 |
|---|---|---|---|---|---|
| $\mu$Moles cytochrome *c* reduced | 0.023 | 0.032 | 0.041 | 0.065 | 0.072<br>0.074* |

* After preincubation, the anaerobic reaction mixture was heated to 100° C, cooled, and cytochrome *c* added.

In contrast to the DPNH diaphorase, the TPNH cytochrome *c* reductase does not require free inorganic iron for the reduction of cytochrome *c*. If, however, ferric iron is incubated anaerobically with the reductase in the presence of TPNH and citrate prior to the addition of cytochrome *c*, an increased rate in the reduction of the cytochrome *c* occurs. This increase is due to the non-enzymic reduction of cytochrome *c* by ferrous iron formed during the preincubation period.

The data presented above suggest that the reduction of inorganic iron may be a general property of flavoprotein systems. Although the DPNH diaphorase would reduce cytochrome *c* by preincubation with ferric iron, this does not imply that the diaphorase is a true cytochrome *c* reductase. The reduction of the cytochrome *c* is achieved by the enzymically reduced inorganic iron. This reduction of the cytochrome *c* itself occurs non-enzymically.

The significance of inorganic iron reduction by flavoproteins and non-enzymic reduction of cytochrome *c* by ferrous iron will be described in detail elsewhere.

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## BOOK REVIEWS

*Phosphorus metabolism. A symposium on the role of phosphorus in the metabolism of plants and animals.* Volume II. Edited by WILLIAM D. MCELROY AND BENTLEY GLASS. The Johns Hopkins Press, Baltimore, 1952, 930 pages. $ 11.00.

Le premier tome de cet ouvrage, paru en 1951 et examiné dans cette rubrique même — *Biochimica et Biophysica Acta*, 10 (1953) 201 — avait été consacré essentiellement à la synthèse des liaisons phosphorées riches en énergie et à leur utilisation au cours du métabolisme glucidique, à la structure et au métabolisme des coenzymes, et à quelques problèmes voisins d'intérêt biologique tels que la contraction musculaire et la bioluminescence. Le second tome est avant tout consacré à l'intervention des composés phosphorés dans la synthèse des protéines, dans le métabolisme des phospholipides et des acides nucléiques et dans la photosynthèse; l'assimilation du phosphore, la