

Short Communications

The Splitting of the Porphyrin-protein Bonds in Cytochrome c

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Unlike many other iron-porphyrin proteids, *e. g.* hemoglobin, myoglobin, horse radish peroxidase, and catalase, cytochrome c is not divided into its protein part and prosthetic group by acid acetone. This may be attributed to the unique arrangement with cysteine-sulphur bridges from the side chains 2 and 4 of the porphyrin to the protein¹. Because of that it has not been possible to examine cytochrome c by means of some of the methods which have proved to be valuable for the studies of heme-linked groups in those other proteids.

Salts of some heavy metals have been used to cleave thioether bonds in other compounds^{2,3}. Cytochrome c has been found to be affected in the same way by these salts, of which the silver salts seem to be the best. At faintly acid reaction and slightly elevated temperature the iron porphyrin is liberated, so that it can be extracted with ether-acetic acid or stays in the liquid phase upon the addition of an excess of acid acetone. The heat of activation is roughly constant (about 18 000 cal/mole) between + 20° C and + 80° C.

The prosthetic group can be crystallized

from aqueous butanol-acetic acid. It gives a pyridine hemochrome, which is spectroscopically indistinguishable from that of hematohematin and after its conversion to the free porphyrin by means of the pyruvic acid method⁴, it agrees with hematoporphyrin as regards hydrochloric acid number and spectrum.

The protein part is easily soluble in water after its previous precipitation with acetone in the cold. It is electrophoretically homogenous. A comparison of the titration curves of the intact cytochrome c and its protein part shows that the former consumes three equivalents more per mole below pH 4 than does the latter. Between pH 4 and 6, however, the protein residue takes up 2 equivalents more. The results seem to support the theory that histidines occupy two of the six coordination possibilities of the iron atom⁵.

A detailed report as well as some experiments on the position of the thio-ether bonds in the side chains 2 and 4 will be published in this journal. A micromethod for the determination of cytochrome c in tissues has also been worked out on the basis of these experiments and will be published.

1. Theorell, H. *Biochem. Z.* **298** (1938) 242.
2. Holmberg, B. *Arkiv Kemi, Geol. Mineral. A* **12** (1935).
3. Peters, R. A., and Wakelin, R. W. *Biochem. J.* **41** (1947) 555.
4. Paul, K. G. To be published.
5. Theorell, H., and Åkeson, Å. *J. Am. Chem. Soc.* **63** (1941) 1804.

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