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FINAL SCIENTIFIC REPORT

KINETICS STUDIES ON HEMOPROTEINS BY FLASH PHOTOLYSIS

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Summary of the results.

The flash photolysis apparatus has been improved and a stopped flow system has been built in it. This latter device allows flash photolysis experiments to be performed on solutions prepared by rapid mixing of reagents, at short times after mixing.

The work has continued alogn the lines covered by the previous final report (Grant AF. EOAR 66-22) and also along new directions.

The main results are summarized below:

1) KINETIC EFFECTS OF THE REVERSIBLE DISSOCIATION OF HEMOGLOBIN INTO SINGLE CHAIN MOLECULES.

The kinetics of the reactions of human hemoglobin with carbon monoxide and oxygen have been studied in photochemical and rapid mixing experiments over a large range of hemoglobin concentration.

When the reaction is initiated by rapid removal of the ligand from ligand bound hemoglobin, the kinetics of combination of hemoglobin with CO shows a marked concentration dependence both in the photochemical and the rapid mixing experiments. In dilute hemoglobin solutions (below 10^{-5} M in heme) dissociation of the ligand from oxy or carbon monoxy hemoglobin is followed by slow changes (half time of the order of seconds) in the properties of the system.

These results lead to the following picture, which is also consistent with other as yet unexplained aspects of hemoglobin kinetics:

a) Ligand bound hemoglobin dissociates reversibly into single chain molecules at concentration below 10^{-5} M.

b) Deoxygenated hemoglobin has a much lower tendency to dissociate into single chain molecules and there is no appreciable dissociation even at concentration of the order of 10^{-7} - 10^{-8} M.

c) The speed of association of deoxygenated α and β chains is a relatively slow process. Therefore, after sudden dissociation of the ligand from dilute hemoglobin solutions the properties of the system, for a brief time, are those of a mixture of deoxygenated hemoglobin and deoxygenated α and β chains.

d) The properties of the simple chain molecules obtained by dilution of ligand bound hemoglobin are the same as those of isolated α and β hemoglobin chains as obtained by preparative procedures.

2) <u>REACTION OF ISOLATED αCO and βCO HEMOGLOBIN CHAIN TO FORM HEMOGLOBIN $(\alpha_2\beta_2)(CO)_4$</u>:

The rate of this reaction has been measured in stopped flow-flash photolysis experiments. The change in kinetic behaviour on flash photolysis has been followed with time after mixing carbon monoxide α chains with carbon monoxide β chains.

3) <u>KINETIC BEHAVIOUR ON FLASH PHOTOLYSIS OF THE HIGH MOLECULAR WEIGHT HEMOGLOBIN FROM THE EARTHWORM.</u>

This hemoglobin shows a different kinetic behaviour on flash photolysis as compared to mammalian hemoglobins.

In particular, there is no appearance of a rapidly reacting form on dilution of the protien, which may be correlated to the fact that earthworm hemoglobin does not show tendency to dissociate into subunits at low protein concentrations.

4) SPECTRAL DIFFERENCE BETWEEN THE DEOXYGENATED HEMOGLOBIN CHAINS AND HEMOGLOBIN.

As a complement to the flash photolysis studies, the spectral differences between the deoxygenated hemoglobin α and β chains and their reaction mixture (hemoglobin) has been measured over the entire range from 400 to 650 mμ.

BIBLIOGRAPHY.

Some of the results have been presented in a preliminary form at:

Annual meeting of the Italian Society of Biophysics and Molecular Biology) Pavia (Italy) May 1967.

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M. Brunori, E. Antonini, J. Wyman and S. Anderson.

J. Mol. Biol. in press.

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13. ABSTRACT

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