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PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Research Grant NGR-05-020-137: Structure and Function of Proteins and Nucleic Acid

Semi-annual Report for the Period July 1, 1967 to December 31, 1967

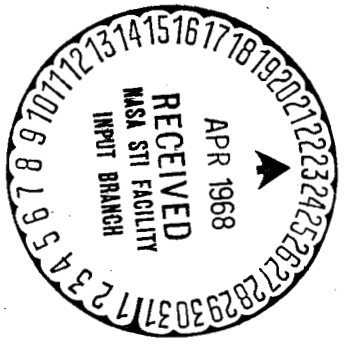
GPO PRICE $ _____

CFSTI PRICE(S) $ _____

Hard copy (HC) 3.00

Microfiche (MF) .65

ff 653 July 65



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N68-21139
(ACCESSION NUMBER)

4
(PAGES)

CR-93962
(NASA CR OR TMX OR AD NUMBER)

(THRU)

1
(CODE)

04
(CATEGORY)

FACILITY FORM 602

Lubert Stryer

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Principal Investigator

I. PUBLICATIONS

A. During the Year of 1967

1. Haugland, R. P., Stryer, L., Stengle, T. R. and Baldeschwieler, J. D., NMR Studies of Antibody-Hapten Interactions Using a Chloride Ion Probe, Biochemistry, 6, 498, (1967)

2. Stryer, L., Holmgren, A. and Reichard, P., Thioredoxin. A Localized Conformational Change Accompanying Reduction of the Protein to the Sulfhydryl Form, Biochemistry, 6, 1016, (1967)

3. Hundley, L., Coburn, T., Garwin, E. and Stryer, L., A Nanosecond Fluorimeter, Rev. Sci. Instru., 38, 488, (1967)

4. Haugland, R. P. and Stryer, L., A Fluorescent Probe at the Active Site of α-Chymotrypsin, in "Conformation of Biopolymers," ed. by G. N. Ramachandran, Academic Press, Vol. 1, pp. 321-335, (1967)

5. Stryer, L. and Haugland, R. P., Energy Transfer: A Spectroscopic Ruler, Proc. Nat'l. Acad. Sci., 58, 719, (1967)

B. During the Year of 1968

1. Epstein, H. F. and Stryer, L., Kinetics of Azide Binding to Normal and Mutant Ferri Hemoglobins as Evidence for Subunit Interaction, J. Mol. Biol., 31, (1968)

2. Stryer, L., Implications of X-Ray Crystallographic Studies of Protein Structure, Ann. Rev. Biochem., 38, (1968), in press

3. Galley, W. C. and Stryer, L., Triplet - Triplet Energy Transfer in Proteins as a Criterion of Proximity, Proc. Nat'l. Acad. Sci., (in press, 1968)

II. TRIPLET - SINGLET ENERGY TRANSFER IN PROTEINS

We have observed a new type of energy transfer in proteins. Triplet - singlet energy transfer was exhibited by a complex of α-chymotrypsin and proflavin, a chromophoric inhibitor. The triplet excitation energy of indole groups in the enzyme was transferred to the chromophoric inhibitors at the active site. The efficiency of transfer was about 75%. The transfer was expressed in terms of a delayed fluorescence of the proflavine in the millesecond time range. Triplet - singlet transfer should be a useful adjunct to singlet - singlet transfer in measuring distances in the 15 - 60 Å range.

III. CONFORMATION OF PYRIDINE NUCLEOTIDE COENZYMES

The conformation of the coenzymes β-DPN, β-DPNH, their isomers, analogues and related model compounds has been studied by optical rotatory dispersion (ORD) and circular dichroism (CD) measurements. In 1957, Weber first suggested the existence of a stacked structure for β-DPNH, to account for the fluorescence energy transfer properties, and the changes in absorption spectrum on pyrophosphatase hydrolysis. Reinvestigation of the latter shows that there is little hypochromicity of the 260 mμ band relative to the 340 mμ band in the intact structure, but that both bands are hypochromic relative to AMP and β-NMNH respectively. The interaction of the adenine and nicotinamide bases in β-DPN and β-DPNH is most clearly shown by ORD, and CD. The properties of the coenzymes in aqueous solution are not the sums of the separate parts. Marked changes occur on pyrophosphatase hydrolysis, and in propylene glycol. The release of hypochromicity by these treatments suggests that close-range, stacking interactions are indeed involved.

Similar results have been obtained with the oxidised and reduced forms of the α-isomers, and of the coenzyme analogues containing desamino-adenine, 3-acetyl pyridine, 3-pyridine aldehyde and thionicotinamide. These compounds do not in general show singlet - singlet energy transfer from adenine to the reduced nicotinamide moiety, implying that for β-DPNH the geometrical proximity of the two halves is not solely responsible for the effect.

From purely sterochemical considerations, the molecules could exist in many rotameric conformations. The characteristic exciton split seen in the CD spectra of the oxidised coenzymes suggests the existence of specific conformations, and relative orientations of the bases. This effect is even more pronounced in the coenzyme analogues, 5', 5' diadenosine pyrophosphate, and 5', 5' diadenosine triphosphates, Both show a classical strong exciton split in the CD in the region of the adenine absorption, characteristic of a specific asymmetric conformation.

IV. PROPERTIES OF MUTANT HEMOGLOBINS

The reaction of azide ion with ferrihemoglobin is 30-fold slower than with ferrimyglobin. The binding of azide ion to two mutant human ferrihemoglobins was investigated to determine whether the lower reaction rate of Hb A is due to subunit interactions. The abnormal hemoglobins studied were Hb M_1, in which the proximal heme-linked histidine residue of each α-chain is replaced by tyrosine, and Hb M_{HP}, in which the same change occurs in the β-chains. In these mutant hemoglobins, only the two normal subunits bind azide ion. Hb M_1, like myoglobin, reacts rapidly with azide ion, while Hb M_{HP} reacts at the slower rate characteristic of Hb A. In contrast, the four proteins have similar binding affinities for azide ion. The large difference in reaction rate between Hb M_1 and Hb A

IV. Properties of Mutant Hemoglobins - continued

shows that the binding kinetics of the β-chain depend on whether the adjacent subunit is normal or mutant. Differences in absorption spectra in the Soret region of the azide derivatives of Hb M_1 and Hb M_{HP}, as well as differences in their rates of reaction with azide ion, indicate that closely related structural changes in the α and β chains can have dissimilar effects.

V. SUBUNIT INTERACTION IN TRYPTOPHAN SYNTHETASE

Tryptophan synthetase is an enzyme composed of two types of readily separable protein subunits, α and β_2. The fully associated enzyme has the structure $\alpha_2\ \beta_2$. The β_2 protein contains two pyridoxal phosphate residues that serve as prosthetic groups in the reactions involving L-serine, one of the substrates. The distinctive spectral properties of this coenzyme make it a very useful probe of the active site of this enzyme. We have observed a highly specific fluorescence emission from the pyridoxal phosphate groups in a complex of the β_2 protein and L-serine. Related compounds such as D-serine, o-acetyl-L-serine, DL-homoserine, L-Threonine, and L-alanine are ineffective in eliciting the emission, showing that the interaction has a high degree of sterospecificity. Binding of indole, the second substrate, diminishes the fluorescence intensity. Most interesting, binding of the α subunit diminishes the fluorescence intensity; this spectroscopic change is correlated with a change in enzymatic activity. It appears likely that the emitting species is an intermediate in the reaction pathway leading to the deamination of L-serine.