PROGRESS REPORT

to the

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STRUCTURE AND FUNCTION OF PROTEINS AND NUCLEIC ACIDS

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1. Use of homopolymer templates to facilitate replication of transforming factor

A polythymidylate oligomer was linked to cellulose and then hybridized to denatured B. subtilis DNA to which a polyadenylate oligomer had been added. Subsequent replication with DNA polymerase of the B. subtilis DNA yielded a product which is denaturable. However, the size of the product is considerably smaller than that of the B. subtilis DNA template and current studies are designed to find what steps in the procedure have led to this reduction in the size of the product. We are hopeful that with the circumvention of this difficulty in transforming factor, future assays may identify a net enzymatic synthesis of biologically active DNA.

2. Excited state proton transfer reactions

A large deuterium isotope effect on fluorescence emission spectra and quantum yields has been observed in a number of chromophores that contain proton donor groups. In the absence of a proton donor or acceptor group, there is no appreciable isotope effect. When both the protonated and unprotonated excited species are fluorescent, the shape of the emission spectrum is different in H_2O and D_2O. When only the protonated form is fluorescent, the quantum yield is higher in D_2O than in H_2O. These effects are due to different rates of proton transfer in H_2O and D_2O in the reaction $RH + H_2O \longrightarrow R^- + H_3O^+$.

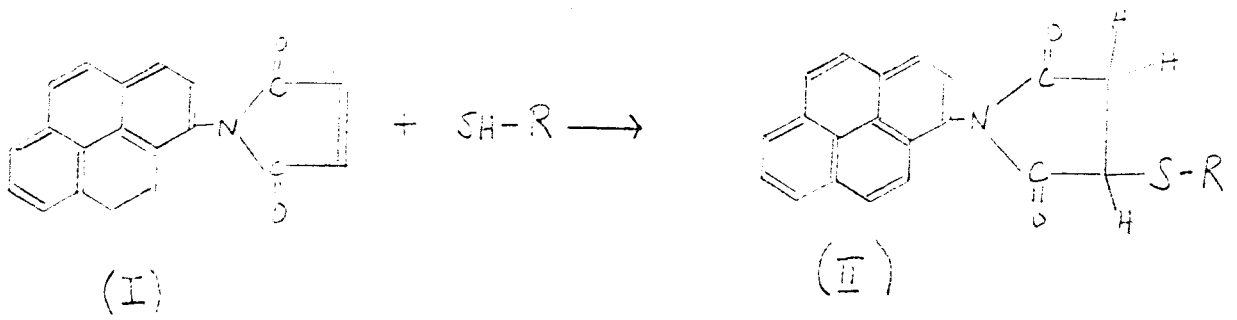
Submitted for publication: Stryer, L., Excited State Proton Transfer Reactions. A Deuterium Isotope Effect on Fluorescence.

3. A conformational change accompanying activation of an enzyme

Thioredoxin, an enzyme from E. coli, occurs in an inactive oxidized form ($T\text{-}S_2$) and an active reduced form ($T\text{-}[SH]_2$) which participates in the enzymatic conversion of ribonucleotides to deoxyribonucleotides. We have carried out fluorescence and other physical-chemical studies of thioredoxin. There is a striking change in the fluorescence quantum yield on reduction of thioredoxin. This indicates that a structural change accompanies activation of the enzyme. Since the optical rotatory and hydrodynamic properties of thioredoxin are unaltered on reduction, it is evident that the conformational change is localized to the active site of the enzyme. In addition, one or both tryptophan residues are near the sulfhydryl groups at the active site of thioredoxin.

4. Selective fluorescent labeling reagents

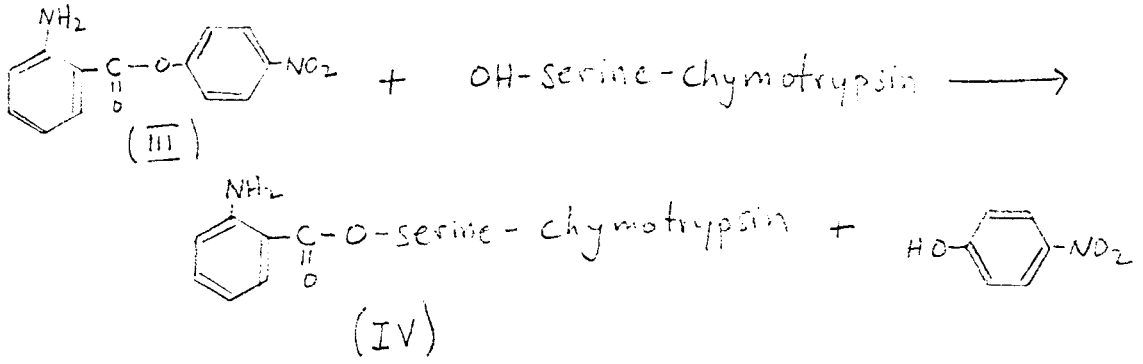
The availability of a series of fluorescent labeling reagents having selective affinity for single sites on proteins would greatly enhance the scope of fluorescence techniques. The fluorescent labeling reagents currently in use, such as dansyl chloride and various isocyanates and isothiocyanates usually react at several sites on proteins. We have recently synthesized two fluorescent reagents which appear to be more selective. One of these chromophores reacts with sulfhydryl groups in proteins, while the other labels the active site of α-chymotrypsin. The sulfhydryl reagent is a pyrenyl maleimide (I) which forms a fluorescent mercaptide derivative (II):



(I) (II)

The derivative (II) has a high extinction coefficient and a high quantum yield, in contrast to the maleimide (I), which is non-fluorescent. Its absorption and emission maxima differ from those of the aromatic amino acids, allowing selective excitation of the pyrenyl emission in a labeled protein.

The second fluorescent labeling reagent is the p-nitrophenyl ester of anthranilic acid (III), which reacts stoichiometrically with α-chymotrypsin to form a stable fluorescent anthranilate acyl enzyme (IV):



(III) (IV)

The ester (III) does not react with chymotrypsinogen or disopropylophosphoryl-chymotrypsin. It reacts more slowly with trypsin, suggesting that (III) may also label the active site of other serine proteases. We are now carrying out spectrofluorimetric studies of the fluorescent acyl enzyme (IV) to determine the rigidity of the active site of chymotrypsin as well as its accessibility to solvent molecules.

5. Energy transfer, a spectroscopic ruler

Energy can be transferred between chromophores 20 Å. Förster's theory predicts an r^{-6} distance dependence for the transfer. This suggests that energy transfer may be of potential use as a spectroscopic ruler. As a first step, we have synthesized oligomers of L-proline as "rigid sticks" to separate energy donor-acceptor pairs by defined distances. Poly-L-proline exists in either of two helical conformations: type I, 1.9 A/residue, is stable in alcohols, while type II, 3.12 A/residue, is stable in water. Prolyl oligomers from n = 2 to 10 were synthesized stepwise by the solid-state method. Fluorescent chromophores were then coupled to the amino and carboxyl ends of the oligomers to form a dansyl-(L-proline)-naphthylamide system. The energy donor is either 1-naphthylamide or 2-naphthylamide, while the acceptor is the dansyl (dimethylamino-naphthalene sulfonamide) chromophore. With 1-naphthylamide as the energy donor, the transfer efficiency is 100% for n = 1, 70% for n = 2, 30% for n = 3, and nearly 0% for n = 4. Work is in progress on the 2-naphthylamide system, which exhibits energy transfer at longer chain lengths. Optical rotatory dispersion studies of these oligomers are being carried out to establish their conformation.

6. Nanosecond fluorescence kinetics

Direct observation of the kinetics of fluorescence emission in the 10^{-9} second range is important in studies of energy transfer, rotational mobility, and reactions involving short-lived excited species. A nanosecond

fluorimeter has been designed and constructed in collaboration with Mr. Lee Hundley of the Genetics Department Instrumentation Laboratory. A modified xenon arc yields short light pulses at a repetition rate of 10 kc. The spectral output of the lamp is suitable for excitation of virtually all fluorescent chromophores. The light pulse has a rise time of 2.5 nsec and a decay time of 8 nsec. Since these times are comparable to the excited state lifetimes of interest, it is necessary to analyze the observed emissions in terms of a convolution integral. The fluorescence output from the sampling oscilloscope goes to a LINC computer, which stores the data, plots it, and writes a magnetic tape for detailed analysis on the IBM 7090. In addition, an on-line preliminary analysis of the data is obtained on the LINC. Lifetimes longer than about 2 nsec can be accurately measured on our apparatus. It has furnished excited state lifetimes that were essential in interpreting data relating to the mobility of the active site on an antibody (see Stryer, L. and Griffith, O. H., A Spin-Labeled Hapten, Proc. Natl, Acad. Sci. 54, 1785 (1965)).

A promising recent development is our observation that oxygen arcs can yield light pulses shorter than 1 nsec, though less bright than the pulses obtained with our xenon source. We are collaborating with Dr. Edward Garwin of the Stanford Linear Accelerator Center in search for higher intensity subnanosecond light sources.

7. X-ray crystallographic studies

We have initiated an x-ray study of crystals of the α-subunit of tryptophan synthetase, an enzyme involved in the biosynthesis of tryptophan.

The crystals grown by Dr. C. Yanofsky of the Biology Department have been examined, and are so far unsuitable for detailed x-ray analysis because of disorder along one axis. The translations along the other two axes are 71 and 40 A. Essentially identical patterns were obtained for a mutant which binds indoleglycerol phosphate but does not catalyze its conversion to indole. An effort will be made to obtain better crystals by varying some of the conditions of crystallization, such as pH and type of salt.

8. Potential applications in planetary explorations

Some of the research techniques and findings summarized in this report may prove valuable in planetary explorations:

(a) Nanosecond fluorimetry. The sensitivity of fluorescence as an analytical tool may be enhanced by the use of time-resolution as a complement to spectral resolution. For example, fluorescence could be distinguished from Rayleigh and Raman scattering, since the latter processes occur in a time less than 10^{-14} sec, while fluorescence lifetimes are typically 10^{-9} sec.

(b) Selective fluorescent labeling reagents for the detection of macromolecules.

(c) Computer processing of spectroscopic data.

(d) Subnanosecond light sources for fast kinetic studies and for evaluation of response times of photomultiplier tubes.

(e) Enzymatic synthesis of genetically active DNA will create the possibility for modifications of genetic material hitherto impossible in intact cells.

The influence of strange or fraudulent purine or pyrimidine substituents on gene function will be better understood and new insights into the evolution of genetic substance may emerge.