

## LABORATORY OF PHYSICAL CHEMISTRY OF ENZYMES

Head: Dr. Tatsuo Ooi

In 1966, when nineteen research sections were set up in this Institute on the basis of the Ordinance No. 4 of the Ministry of Education, Dr. Keiichi Sisido, who had been the Head of Sisido Laboratory in this Institute since 1946, moved to this section, and continued his research works besides his principal responsibility of the Department of Industrial Chemistry, Faculty of Engineering of Kyoto University until his retirement in 1971. With the collaboration of the staff members of the Industrial Chemistry, the activities of his laboratory to cover the chemistry of natural products and organo-metallic compounds as a part of Organic Chemistry had been focused mainly on the synthesis of fragrant materials and related compounds as well as organotin. Also, the fundamental aspects of organic reactions were investigated. Papers published after 1967 till the end of his laboratory are given in the end of this description.

The Institute moved to Uji campus in 1968, when Dr. Tatsuo Ooi was appointed as professor of Kyoto University and established his own laboratory of Physical Chemistry of Enzymes in this Institute. The research projects since then have been to elucidate the mechanism of protein functions on the molecular basis, or how specific functions of protein molecules could be explained by the three dimensional structures. There are several approaches to this important problem in one of the research fields of Molecular Biology and Biophysics, and the problem has been studied experimentally and theoretically with various methods.

One of the projects is concerned with the folding of a polypeptide chain into the native structure which is definitely determined by the primary structure encoded from the genetic code in DNA. Renaturation experiments on ribonuclease A and T<sub>1</sub> have been performed to look for the folding pathway in addition to the physico-chemical studies on the stability of the tertiary structures of these proteins. Theoretical works on conformational analyses by utilizing the known atomic coordinates of proteins determined by X-ray crystallography have been another approach to the problem. The postulate that the native three dimensional structure of a protein is thermodynamically stable will be examined by computations of the sum of atomic interactions.

Second, architecture of assemblies of protein molecules is an interesting problem related to the generation of higher functions such as regulation of the enzymatic activity. Since the contractile system of muscle proteins is a good example, the research project on this aspect has been concentrated to one of the muscle proteins, tropomyosin which is a rod-like molecule of 400 Å in length. Studies have been done on the stability of this molecule and the binding to actin and troponin, other muscle proteins capable to interact specifically with tropomyosin. Efforts are now paid to identify the location of the interacting sites in the molecules.

Finally, studies on synthetic polypeptides, the basic materials of proteins, have been carried out, since the importance of such studies has been illustrated by the formation of specific secondary structures,  $\alpha$ -helix and  $\beta$ -structure depending on the species of amino acids. The synthesis of regular copolypeptides of L-alanine and glycine and the characterization of the polymers have been achieved. Recently the synthesis was in success on copolymers of a given polymerization degree, physico-chemical investigations of which are now in progress.

Publications in this laboratory during the period from 1968 to 1976 are listed below.

### Publications

(\* indicates an article published in Japanese)

1. T. Ooi: Interactions between Protein Molecules, *Bull. Inst. Chem. Res., Kyoto Univ.*, **46**, 148 (1968).
2. T. Ikkai and T. Ooi: The Effects of Pressure on Actomyosin Systems, *Biochemistry*, **8**, 2615 (1968).
3. S. Higashi-Fujime and T. Ooi: Electron Microscopic Studies on the Crystal Structure of Tropomyosin, *J. Microscopie*, **8**, 535 (1969).
4. S. Iida and T. Ooi: Titration of Ribonuclease T<sub>1</sub>, *Biochemistry*, **8**, 3897 (1979).
5. H. Hotani, T. Ooi, H. Kagawa, S. Asakura, and S. Yamaguchi: Biochemical Evidence for Identical Primary Structure of P-Filament and Flagellin, *Biochim. Biophys. Acta*, **214**, 207 (1970).
6. K. Nishikawa, M. Oobatake, and T. Ooi: Tertiary Structures of Proteins: Analysis of Conformations, *Bull. Inst. Chem. Res., Kyoto Univ.*, **48**, 103 (1970).
7. S. Takahashi and L. A. Cohen: The Facilitation of NaBH<sub>4</sub>: Reduction of Esters of Phenols and of Acidic Alcohols, *J. Org. Chem.*, **35**, 1505 (1970).
8. T. Iio and S. Takahashi: Conformations of Sequential Polypeptides Containing L-Alanyl and Glycyl Residues, *Bull. Chem. Soc. Japan*, **43**, 515 (1970).
9. T. Iio, S. Takahashi, and S. Kobayashi: The Behaviour of the Copolymer of L-Proline and O-Benzyl-L-Tyrosine in a n-Propanol and Water System, *ibid.*, **43**, 3853 (1970).
10. T. Ikkai and T. Ooi: Effects of Pressure on ATPase of Myosin A, Heavy Meromyosin, and Subfragment I, *Biochim. Biophys. Acta*, **234**, 190 (1971).
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14. T. Iio and S. Takahashi: Regular Polypeptides of Glycine and L-Alanine, *Bull. Inst. Chem. Res., Kyoto Univ.*, **49**, 80 (1971).
15. Y. Hiragi and H. Kadota: Fiber-Structured Material in a Fraction of *Bacillus subtilis*, *J. Bacteriol.*, **108**, 579 (1971).
16. K. Nishikawa, T. Ooi, Y. Isogai, and N. Saito: Tertiary Structure of Proteins. I Representation and Computation of the Conformations, *J. Phys. Soc. Japan*, **32**, 1331 (1972).
17. K. Nishikawa and T. Ooi: Tertiary Structure of Proteins II. Freedom of Dihedral Angles and Energy Calculation, *ibid.*, **32**, 1338 (1972).
18. K. Nishikawa and T. Ooi: Energy Calculations on Di- and Poly-L-Proline, *Bull. Inst. Chem. Res., Kyoto Univ.*, **50**, 94 (1972).
19. M. Oobatake and T. Ooi: Determination of Energy Parameters in Lennard-Jones Potentials from Second Virial Coefficients, *Prog. Theor. Phys.*, **48**, 2132 (1972).
20. Y. Hiragi: Physical, Chemical, and Morphological Studies of Spore Coat of *Bacillus subtilis*, *J. Gen. Microbiol.*, **72**, 87 (1972).
21. Y. Hiragi: Molecules and Structure of Regular Molecular Assemblies. I. Ring Forming Ellipse, *Bull. Inst. Chem. Res., Kyoto Univ.*, **50**, 584 (1972).

22. T. Ooi and K. Nishikawa: One Approach to Computing the Tertiary Structure of Proteins, *The Jerusalem Symp. Quant. Chem. and Biochem.*, **5**, 173 (1973).
23. W. L. Mattice, K. Nishikawa, and T. Ooi: Conformational Properties of Poly(L-proline) Containing a Flexible Pyrrolidine Ring, *Macromolecules*, **6**, 443 (1973).
24. F. T. Hesselink, T. Ooi, and H. A. Scheraga: Conformational Energy Calculations. Thermodynamic Parameters of the Helix-Coil Transition for Poly(L-lysine) in Aqueous Salt Solution, *ibid.*, **6**, 541 (1973).
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26. S. Takahashi and T. Ooi: Thermally Induced Denaturation of Intramolecularly Cross-Linked Bovine RNase A, *ibid.*, **51**, 153 (1973).
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28. S. Takahashi and T. Ooi: Renaturation of Intramolecularly Cross-Linked Bovine RNase A, *ibid.*, **51**, 329 (1973).
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39. H. Ueno, Y. Tawada, and T. Ooi: Properties of Non-Polymerizable Tropomyosin Obtained by Carboxypeptidase A Digestion, *J. Biochem.* **80**, 283 (1976).
40. T. Kontani, K. Nishikawa, T. Iio, S. Takahashi, and T. Ooi: Unidirectional Stability of  $\alpha$ -Helix. Theoretical Calculation and Attempt for Synthesis of Block Copolypeptides, *Bull. Inst. Chem. Res., Kyoto Univ.*, **54**, 128 (1976).
41. K. Nishikawa and H. A. Scheraga: Geometrical Criteria for Formation of Coiled-Coil Structures of Polypeptide Chains, *Macromolecules*, **9**, 395 (1976).
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#### Reviews

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6. T. Ooi: Interactions between Proteins, *Kobunshi*, **22**, 396 (1973).
7. S. Takahashi: Folding and Renaturation of Proteins, *Tanpakushitsu, Kakusan, Koso (Protein, Nucleic Acid, Enzyme)*, **18**, 410 (1973).

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8. T. Ooi: Non-Covalent Bonds in Proteins, "Tanpakushitsu Kagaku" ("Higher Structure of Proteins") Kyoritsu, Tokyo (1973), p. 349.
9. T. Ooi and S. Takahashi: Physical and Chemical Basis of Life, "Gendai Seibutsu Kagaku" ("Modern Biology") Iwanami, Tokyo (1975), p. 105, p. 153.
10. T. Ooi and S. Takahashi: "Kinosochi no Kozo", Asakura, Tokyo (1976), p. 1, p. 9.

#### Publications

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2. K. Sisido, K. Inomata, T. Kageyama, and K. Utimoto: Conformations of Iridolactones and the Stereochemistry in the Synthesis, *J. Org. Chem.*, **33**, 3149 (1967).
3. K. Sisido, S. Kurozumi, and K. Utimoto: Synthesis of Methyl Dihydrojasmonate, *Perfumary and Essential Oil Record*, London, **60**, 267 (1968).
4. K. Sisido, S. Kurozumi, and K. Utimoto: Synthesis of Methyl dl-jasmonate, *J. Org. Chem.*, **34**, 2661 (1969).
5. K. Sisido, S. Kurozumi, K. Utimoto, and T. Isida: Fragrant Flower Constituents of *Osmunthus fragrans* II., *Perfumary and Essential Oil Record*, London, **58**, 212 (1967).
6. K. Sisido, S. Kurozumi, and K. Utimoto: Fragrant Flower Constituents of *Daphne odora* Thunberg, *ibid.*, **58**, 528 (1967).
7. K. Sisido, N. Hirowatari, and T. Isida: Synthesis of Racemic Phytosphingosine and the lyxo Isomer, *J. Org. Chem.*, **34**, 3539 (1969).
8. K. Sisido, N. Hirowatari, H. Tamura, H. Kobata, H. Takagishi, and T. Isida: Synthesis of All of the Racemic Diastereoisomers of Phytosphingosine, *ibid.*, **35**, 350 (1970).

##### II Syntheses of Organotin Compounds

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2. K. Sisido, S. Kozima, and T. Tuzi: Direct Synthesis of Organotin Compounds V. Di- and Trialkyltin Chlorides and Bromides, *ibid.*, **9**, 105 (1967).
3. K. Sisido, S. Kozima, and T. Isibasi: Reaction of Alkyl Halides with Dialkyltins, *ibid.*, **10**, 439 (1967).
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