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Photobiotechnology - from photosynthesis to cancer therapy

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Molecules comprised of native and/or chemically modified chlorophylls are utilized or constructed in our laboratory for studying photosynthesis, membrane protein functional assembly, metal catalysis and cancer therapy. Applied methodologies range from molecular biology and bioinformatics through chemical synthesis to quantum-mechanical calculations of relatively large molecules. Studies of cancer therapy with molecules designed in the lab involve pre-clinical and clinical studies with laboratories at the Weizmann Institute, U.S. and Europe.

Photosynthesis and membrane protein assembly (in collaboration with Ziv Reich, WIS and Haim Wolfson, TA University).

Research objectives: The reaction centers (RC) and antennas are membrane protein/chlorophyll complexes. Their combined structures are called photosystems (PS) and they convert solar energy to electro-chemical potential, driving carbohydrates synthesis in plants and bacteria. Studies in RC are expected to shed light on factors governing the functional assembly of membrane proteins, utilizing the electron transfer as a reporter mechanism to changes in the assembly process.

Research achievements: Hydrogen bonds between subunits of membrane proteins were suggested to be cardinal for the complex functional stability. Computer modelling and measurements of PSII activity under different perturbations, suggested such particular bond between D1-S212 and D2-G207. We constructed 13 photoautotrophic mutants at the D1-212 site utilizing combinatorial and direct mutagenesis. Utilizing the electron transfer in PSIIRC of the different mutants to report changes in the assembly process, we confirmed the significance of the aforementioned bond. Using Multiple Structure Alignment (MUSTA) of different RC, we showed that several structural motif are kept among evolutionary remote species. Atomic force microscopy (AFM) enables to monitor conformational changes in whole thylakoids in response to environmental perturbation (stress) at a nanometer resolution.

Metal centers in biocatalysis (In collaboration with Kim Baldridge, Department of Chemistry, UCSD, USA).

Research objectives: Enzymatic reactions mediated by metal centers frequently involve significant changes in the metal coordination sphere. This includes association or dissociation of substrate molecules or amino acid residues that define the catalytic site. We attempt to resolve the underlaying mechanisms of these processes and their role in the metal catalysis (i.e. metal mediated redox reactions). Our working hypothesis is that temporal changes in local energy modes and charge densities because of ligand association/dissociation may have significant contribution in helping the redox catalytic system to overcome the transition state barrier.

Research achievements: We have recently constructed a "molecular potentiometer" for estimating charge variations on metal atoms. The metal is incorporated at the molecule center and variations of the charge density are sensed by the bacteriochlorophyll (Bchl) -system. With this potentiometer we quantified charge migration from different amino-acid residues to Ni. These findings were compared with quantum mechanical calculations using the density functional theorem. On the ground of the experimental and theoretical work we could suggest a mechanism for the catalytic activity of F-430 as well as other catalytic metal centers in which ligand association and dissociation to and from a metal play a key-role.

Dismutation of super-oxide is a fundamental biological activity. Mn incorporated into Bchl provides an elementary model for the catalytic action of Mn containing superoxide dismutase SOD. Using this molecule we could demonstrate important steps of super-oxide dismutation *in vitro*. These steps seem to rely on a bifunctional role of the imidazolic and carboxylic residues. We propose a similar model for the Mn containing SOD *in vivo*. This model provides guidelines for future studies in vitro combined with examination of various models *in vivo*.

Photodynamic therapy of cancer (in collaboration with Prof. Yoram Salomon, Dept. of Biological Regulation).

Research Objectives: Current clinical application of photodynamic therapy (see Salomon Y. and Scherz A., Life Science Book 2000) is limited to a relatively shallow, or early stage small tumors and to palliative treatments. This is mainly because current PDT relies on preferential accumulation of the sensitizers in tumor cells and clinically used sensitizers have low selectivity. In addition, their exciting light does not penetrate deep into the tissue. Finally, the slow clearance rate of most current sensitizers (days to weeks) results in prolonged skin phototoxicity. Hence, we set out to synthesize new sensitizers

that override drawbacks of current PDT and enable treatment of large solid tumors.

Reseasrch achievements: We have synthesized novel photosensitizers based on native and metal substituted Bacteriochlorophylls. Together with Prof. Y. Salomon we have studied the phototoxicity of these sensitizers in cultured tumor cells and in the therapy of tumors in laboratory animals. We found that a Pd-substituted Bchl named TOOKAD, a hydrophobic PDT agent, clears from circulation in a bicompartmental fashion, and does not extravasate to other tissues at all measured times after injection. The lifetimes of the short ($t1_{1/2}$,~ 2-3min (95%)) and long ($t2_{1/2}$~25min (5%)) components suggest a complete clearance of the ensitizer in the blood at a few hours after injection. Because of the insignificant concentrations of TOOKAD in the skin, muscle and fat tissues, we anticipate no skin toxicity within 1 h after injection. Under these conditions the tumor vasculature becomes the initial treatment target. Based on these observations we designed treatment protocols that enable massive generation of reactive oxygen species (ROS) in the tumor vasculature, shortly after sensitizer administration. Differences between the vascular bed of tumors and normal tissue, are expected to allow minimization of the damage to the second one. Pre-clinical studies with TOOKAD, have supported this hypothesis. Application of the suggested protocols using this drug will enter phasel/II clinical trials on prostate and lung cancer patients at the end of 2001 and the second quarter of 2002 respectively. More recently we have synthesized water soluble Pd-bacteriochlorophyll derivatives that are easier for application. The combined action of the hydrophobic and hydrophylic classes on animal tumors is synergistic. At present and in the future we shall study the pharmacokinetics of the new drugs combinations in order to design novel treatment protocols. This work will involve evaluation of mathematical models that describes the drug biodistribution and their testing in a variety of animal models. The physico-chemical aspects of ROS generation by TOOKAD and other derivatives are crucial for the design of new protocols. Using electron paramagnetic resonance (EPR), time resolved optical spectroscopy, pigment analyses and other, complementary techniques we found out that excited TOOKAD generates both singlet oxygen and hydroxyl radicals at the illuminated site.

(See also Salomon et al., page 178).

Selected Publications

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