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PROTON PUMP MECHANISM DEDUCED FROM HIGH RESOLUTION STRUCTURES OF BOVINE HEART Cytochrome C Oxidase

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Cytochrome c oxidase, a terminal respiratory enzyme complex, accepts electrons from cytochrome c and reduces dioxygens to waters coupling with pumping protons across the membrane. X-ray structures of bovine heart enzyme at the fully oxidized, reduced and several ligand-binding states at 280 K have been determined (1-4). The enzyme consists of two copies of 13 different subunits. The transmembrane part of each monomer consists of 28 α-helices, including metal centers of hemes a and a_3 and Cu_B at the same level in the membrane. Another metal center, Cu_A, is located in the intermembrane part. Possible electron and chemical proton transfer pathways, as well as a proton pumping mechanism, have been proposed (3). The crystals diffracting X-rays up to 1.65 Å were recently obtained by using ethyleneglycole as a cryo-protectant. X-ray diffraction data were collected on the image plate of DIP2040 (MAC SCIENCE) at BL44XU of the SPring-8. Structures were determined by the molecular replacement method. Now, we have 1.8 Å oxidized and 1.9 Å reduced structures at 100 K, as well as 2.3 Å oxidized and 2.35 Å reduced enzyme structures at 280 K. Several novel water arrangements functioning in proton translocation were detected by inspecting these structures at both 100 K and 280 K

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Keywords: 'PROTON TRANSLOCATION' 'HEME PROTEIN' 'X-RAY CRYSTALLOGRAPHY'

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CRYSTAL STRCUTURE OF CodW IN *BACILLUS SUBTILIS*-THE FIRST N-TERMINAL SERINE PROTEASE

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CodWX, encoded by the cod operon in *Bacillus subtilis*, is a member of the ATP-dependent protease complex family, and is homologous to the eukaryotic 26S proteasome. It consists of two multimeric complexes: two hexameric ATPase caps of CodX and a protease chamber consisting of CodW dodecamer. Prior structural studies have shown that the N-terminal threonine residue is solely functional as a proteolytic nucleophile in ATP-dependent proteases such as HslV and certain beta-type subunits of 20S proteasome, which have a primary sequence similarity of -50% and -20% with CodW respectively. Here we present a 3.0 Å resolution crystal structure of CodW, which is the first N-terminal serine protease among the known proteolytic enzymes. In spite of the same fold and the conserved contacts between subunits with HslV in E. coli and H. influenza, this structure shows the five additional residues extending from conserved Thr1 among the other ATP-dependent protease and extraordinary basic proteolytic chamber.

Keywords: CodW BACILLUS SUBTILIS HSLVU

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THE SEARCH FOR A BETTER ANALGESIC

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The opiate morphine is still the most widely used analgesic for the treatment of acute pain. However, if the dosage is only a little too great its side effects may be life threatening. Even at routine dosages side effects can include nausea, vomiting, dizziness and lowered blood pressure. When its use is repeated, the analgesic effects wane and the dose must be increased and, worst of all, the patient can become addicted to its use. Over 100 years of research into developing replacements for morphine without its adverse and addictive side effects has not yet been successful. However, during the past decade new insights have been gained into how the various opioid receptors are involved in antinociception and addiction.

This paper will describe our recent work with morphine derivatives including enantiomeric analogs of m-hydroxyphenyl-N-phenylethyl- phenylmorphan and other related compounds designed to interact with specific opioid receptor sites while blocking action at others. It will also address our current work with derivatives of the novel alkaloid epibatidine, isolated by John Daly of the National Institutes of Health from the skin of a South American frog. Epibatidine is about 200 times more potent than morphine and its action appears to be non-opioid. Unfortunately it is also lethal so there is a great deal of interest in developing analogs that will retain only its analgesic properties. Structural information can facilitate modifications leading to a therapeutically useful analgesic.

Keywords: CNS AGENTS, ANALGESICS, SAR STUDIES

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CHARGE DENSITY STUDIES AS A TOOL IN DRUG DESIGN

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Recent developments in crystallography and high-performance computing make possible the determination of charge densities on a reasonable time-scale. Consequently, it is now feasible to carry out systematic studies of the charge distribution in drug molecules. Thus structure-activity relationships may be determined with this final and critical piece of information included.

The total experimental charge distribution in a number of flavonoids has been determined via high-resolution X-ray diffraction. Flavonoids are polyphenolic compounds found extensively in plants. They are widely known as antioxidants but also influence immune function, gene expression, platelet aggregation and enzyme activity. Recently, it has become clear that some flavonoids have effects on the central nervous system and it is this group that will be the subject of the work outlined here. GABA (γ-aminobutyric acid) is the major inhibitory transmitter in the brain and it has been shown that the anxiolytic flavone apigenin has a novel mode of action on GABA receptors in the brain.

The work detailed here relates the structural and electronic characteristics of this novel mode of action, and how flavonoids may influence actions of other molecules in order to design and develop potentially useful therapeutic agents for CNS disorders. An electrostatic “fingerprint” of these compounds has been generated using the electrostatic potential (EP). The varying inductive effects of the differing functional groups has been assessed via the experimentally determined EP, and clear conclusions about these inductive effects have been drawn, and correlated with activity.

Keywords: CHARGE DENSITY DRUG DESIGN SMALL MOLECULE