original site in the protein which is accessible to physical studies, the effect of the metal ion orientation on the electron transfer process can be addressed.

The first step in developing a new protein is to isolate and sequence the native DNA for the azurin gene. This report describes our progress in this endeavor.

We have used a DNA synthesizer to construct an oligonucleotide which is complementary to the mRNA encoding for amino acids 11-17 of azurin. The probe (shown below) is 20 nucleotide bases long and 32 fold redundant.

CTP-GTA-TAC-GTQ-AAP-TTP-TG
(where P = A or G and Q = C or T)

Purification of the probe was accomplished with a 0.1 M triethylammonium acetate pH 7.1/acetonitrile gradient which varied linearly from 15 to 40% acetonitrile over a 20 minute period. The probe eluted from the C-18 reverse phase column after 13 minutes (Fig. 1). The probe was then labelled with ^{32}P using T4 polynucleotide kinase and ATP [γ-^{32}P]. This radioactive probe is being used in southern blotting experiments against gene banks of the *P. aeruginosa* chromosome [8] to determine which group of clones contain the azurin gene. In addition, colony blotting experiments will allow isolation of the cloned gene. With these data in hand, we will isolate and sequence the DNA coding for the azurin gene. Our progress in the isolation of the proper clone, the determination of the DNA sequence of the azurin gene and determination of the sequence homology between the native DNA sequence and that predicted from the amino acid sequence will be reported.

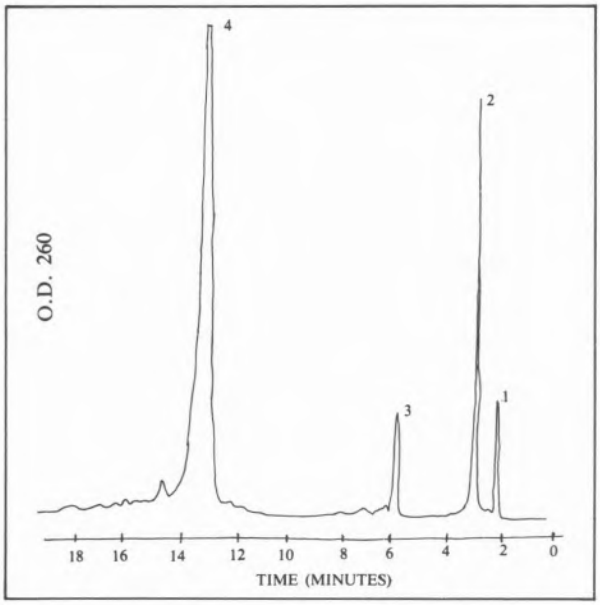

Fig. 1

HPLC elution profile of oligonucleotide probe used in southern blotting experiments. The probe elutes after 13 minutes. Peak 1, solvent front; Peak 2, failure sequences (i.e., probes of improper length); Peak 3, benzamide; Peak 4, desired oligonucleotide probe

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PS1.18 — TH

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THE EFFECT OF MOLYBDENUM ON NITROGEN FIXING ORGANISMS

Biological nitrogen fixation is carried out by prokaryotic organisms and blue-green algae, mediated by the enzyme nitrogenase. Molybdenum is a

constituent of the MoFe protein of the nitrogenase complex. The absence of Mo has been shown to prevent the synthesis of nitrogenase, but it is not clear how Mo regulates or how MoO_4^{2-} accumulation is regulated.

Nitrogenases from different bacteria have very similar properties. The enzyme consists of the two oxygen-labile proteins — component I (which has two copies each of two different subunits and contains iron and molybdenum atoms) and component II (which has two copies of a single subunit and contains the site of substrate binding and substrate reduction). The active site of nitrogenase resides in an iron-molybdenum cofactor (FeMo-co) that is a part of component I. The role of component II is to reduce component I.

MoFe proteins are complex metalloproteins with MW around 220,000, containing two molybdenum atoms with about 30 iron atoms and a slightly smaller number of acid-labile sulphur atoms per molecule. Mössbauer studies have resulted in identification of three types of metal centre named "P" clusters (16 Fe), the FeMo centres (12-16 Fe) and "S" atoms (2 Fe).

Mo-uptake is energy-dependent and is repressed by NH_4^+ (2 mM) and high concentrations of MoO_4^{2-} (1 mM) in *Clostridium pasteurianum*. In *Azotobacter vinelandii*, Mo-uptake was not inhibited in the presence of NH_4^+, although the rate of uptake of MoO_4^{2-} was slower. During derepression of *Klebsiella pneumoniae* both uptake and nitrogenase activity were maximal at 1 μM MoO_4^{2-} and uptake was inhibited by NH_4^+. *Azospirillum brasilense* showed the maximal nitrogenase activity *in vivo* at a MoO_4^{2-} concentration of 2 μM. *C. pasteurianum* and *A. vinelandii* have Mo-storage proteins which bind MoO_4^{2-} transported into the cell, but *K. pneumoniae* does not. The steps in the transformation of MoO_4^{2-} to the Mo-S environment of FeMo-co may be expected to be very similar in all nitrogen fixing organisms.

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PS1.19 — TH

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SPECTROSCOPIC STUDIES OF NONHEME IRON ACTIVE SITES

Nonheme iron active sites are involved in the interactions with O_2 in oxygen transport (hemerythrin, Hr), superoxide dismutation (iron superoxide dismutase, FeSD) and lipid peroxidation (soybean lipoxygenase, SBL). We have used a combination of variable temperature absorbance, CD, MCD, and EPR spectroscopies to obtain information on active site electronic structure relating to biological function of these iron sites in both ferric and ferrous oxidation states.

The structure of the binuclear iron active site in Met<Fe(III)Fe(III)>Hr is known to high resolution from X-ray crystallographic studies, which show two irons bridged by an oxo anion as well as aspartate and glutamate carboxylates. One iron is hexacoordinate, bound by three histidyl residues in addition to the bridging ligands; the other iron is pentacoordinate in the Met protein (three bridging ligands plus two histidyl residues) and binds exogenous ligands such as N_3^-, or O_2^{2-} in the oxygenated protein. The results of variable temperature absorbance and CD spectroscopy allow us to distinguish for the first time electronic transitions associated with each iron in the active site and to observe the changes which occur at each iron with temperature and exogenous ligand binding. This, coupled with polarized single crystal absorbance data leads to detailed insight into the electronic structure of the site and perturbation by binding of peroxide.

In contrast to the well defined geometric structure of the Met Hr iron site which has emerged, the