per mg of protein) is comparable to that of other haloperoxidases that contain haem, the bromoperoxidase even at high protein concentration (10 mg/ml) does not exhibit a Soret peak typical of a haemoprotein. Recently, it was reported [6] that the bromoperoxidase activity, after inactivation of the enzyme at low pH, could be restored at high pH by addition of vanadium. This observation [6], which we could confirm, prompted us to determine the vanadium content of our preparation. Indeed, analysis demonstrated that a considerable amount of vanadium was present in our purified bromoperoxidase preparation. The amount of vanadium per mg of protein was high enough to be studied by EPR at low temperature.

In the oxidised state, the enzyme as isolated does not show signals that can be attributed to vanadium. Also, addition of $Br^-$ or $H_2O_2$ was without effect. However, upon reduction with dithionite, an EPR spectrum was observed which showed the typical hyperfine splitting of vanadium ($I = 7/2$). EPR experiments are in progress to determine whether the valence of this metal ion changes during catalysis. If this should be the case, a novel class of enzymes that contain vanadium as a prosthetic group has been discovered.

## *ACKNOWLEDGEMENTS*

*We gratefully acknowledge the support by DSM B.V., Geleen, and the grant support from the Foundation for Technical Sciences (S.T.W.) under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.).*

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PS1.17 — TU

VINCENT L. PECORARO
GERBEN ZYLSTRA
RONALD H. OLSEN
Department of Chemistry
University of Michigan
and
Department of Microbiology and Immunology
University of Michigan Medical School
Ann Arbor, MI 48109
U.S.A.

## ISOLATION OF THE DNA SEQUENCE CODING FOR *PSEUDOMONAS AERUGINOSA* AZURIN

The mechanism of electron transfer by metalloenzymes has been the basis of extensive research. One of the unanswered questions in the field is the importance of relative metal ion and protein orientations to the electron transfer rate. Important data have been accumulated for protein-protein interactions [1,2] and for protein--small molecule electron transfer reactions [3-5] with the "blue" copper proteins. Azurin, a "blue" copper protein isolated from *P. aeruginosa*, is an ideal donor/acceptor protein to study because: a) the protein sequence is known [6]; b) the X-ray structure is reported [7]; and c) electron transfer rates between azurin and other proteins and small molecules are tabulated [1-5].

Our entry into this field hopes to exploit the new biotechnology known as site specific mutagenesis. The single "blue" copper atom in azurin is coordinated in a distorted tetrahedral geometry by nitrogen atoms from His-46 and His-117 and sulfur atoms of Cys-112 and Met-121 [7]. We propose to develop a retroazurin in which residues 112 and 121 are changed to Met and Cys, respectively. This alteration of the protein sequence will result in a change in the long axis (Cu-Met) in the distorted tetrahedral environment of the copper. Besides generating a unique modification of the

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 [$\gamma$-$^{32}P$]. 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.

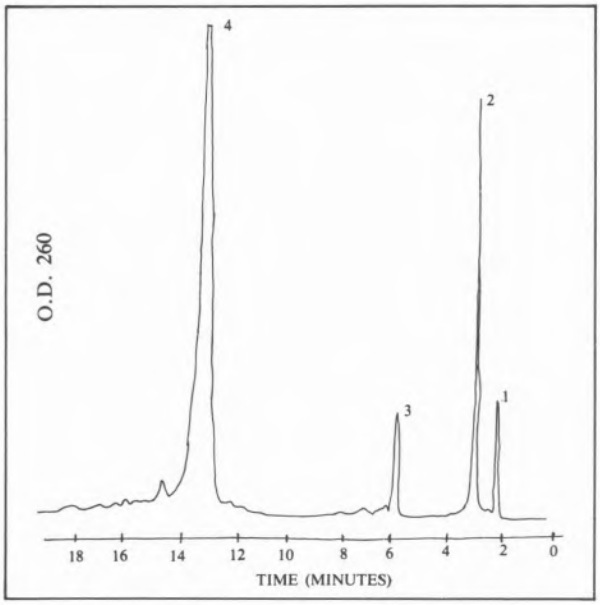


*Fig. 1*

*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

SERGIO PAULO SEVERO DE SOUZA DINIZ *
Department of Pharmacy and Biochemistry
Maringá State University
CP 331 — 87.100 Maringá — Paraná
Brazil

## 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