1994 PROGRAM

A.M.

9:00 **Registration and Coffee - Room 137, Chemistry-Physics Building**

9:30 **Wecome by Dr. David Watt, Vice Chancellor for Research and Graduate Studies, University of Kentucky - Room 139, Chemistry-Physics Building**

9:40 **Introductory Remarks - Mark Meier, University of Kentucky**

9:45 **Dr. Douglas C. Rees, California Institute of Technology**
The Structure and Function of Nitrogenase

The conversion of dinitrogen to ammonia during biological nitrogen fixation is catalyzed by the nitrogenase enzyme system. Nitrogenase consists of two component proteins, the iron (Fe-) protein and the molybdenum-iron (MoFe-) protein. Three-dimensional structures of both proteins have been determined by X-ray crystallography. The MoFe-protein is an $\alpha_2\beta_2$ tetramer containing two different types of metal centers, the FeMo-cofactor and the P-cluster pair, while the Fe-protein contains a single 4Fe:4S cluster symmetrically ligated by two identical subunits. Aspects of the nitrogenase mechanism, as well as general structural similarities that exist between nitrogenase and other complex electron transfer systems, will be explored.

10:45 **Discussion**

10:55 **Dr. William E. Newton, Virginia Polytechnic and State University**
Probing Nitrogenase Catalytic Function Through Amino Acid Substitutions

The nitrogenase complex consists of a smaller Fe protein, which acts as an ATP-binding, specific electron donor to the larger $\alpha_2\beta_2$ MoFe protein, which contains the substrate-binding site. The MoFe protein contains two types of prosthetic group: two P-cluster pairs, each of which consists of two [4Fe-4S] clusters bridged by the γ-S of two cysteinyls (Cys) and a likely disulfide bond; and two FeMo-cofactors (or M centers), each of which has the composition, $MoFe_7S_9$(homocitrate). Our approach to assigning function to the individual prosthetic groups is through site-directed mutagenesis/gene-replacement techniques, then monitoring the consequences of these substitutions. Correlated changes in EPR spectra and catalytic properties occur on substitution of residues interacting with the FeMo cofactor, indicating the intimate involvement of this prosthetic group with the substrate-reducing site. A working model of nitrogenase function based on these and other results will be described.

11:55 **Discussion**

P.M.

12:15 **Buffet Lunch, Faculty Club (Please return card by April 1, 1994 for reservations. Cost $10.00 to be paid at registration.)**

1:40 **Dr. Robert R. Eady, University of Sussex**
The Role of Vanadium in Biological Nitrogen Fixation

Three related, but genetically distinct, nitrogenase systems are known to be present in *Azotobacter* species. One, which is found in all N_2-fixing bacteria and has been studied extensively for many years, is based on molybdenum and iron; a second (first isolated in 1986) contains vanadium and iron; and a third (first isolated in 1988) appears to contain only iron. Which of these systems is expressed depends on the availability of Mo and V to the organism. The results of studies on the regulation of the expression of these alternative systems by Mo and V and the structure-function relationships of V-nitrogenase in comparison with Mo-nitrogenase of *Azotobacter* will be reviewed.

2:40 **Discussion**

2:50 **Dr. Dimitri Coucouvanis, University of Michigan**
Synthetic Models of the Nitrogenase Fe/Mo/S Cluster

Recent X-ray structure determinations of the Fe/Mo protein of nitrogenase revealed the structure of the Fe/Mo/S active site. Neither the Fe_4S_3 nor the $MoFe_3S_3$ subunits have precedents among synthetic clusters with biologically acceptable terminal ligands. In this lecture we will report on the first observation of catalytic behavior by synthetic Fe/Mo/S clusters that structurally, albeit partially, resemble the Fe/Mo/S site of nitrogenase. Some of these clusters are effective in the catalytic reduction of hydrazine to ammonia in what could be the last stage in nitrogen fixation. Reactivity studies strongly implicate the Mo atom as the site directly involved in the reduction of N_2H_4. These studies also suggest that the catalytic process likely occurs by a single metal site mechanism. Possible pathways of these reactions will be presented.

3:50 **Discussion**

4:00 **Mixer - Room 137, Chemistry-Physics Building**

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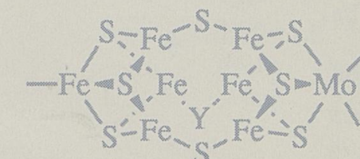
Twentieth Annual
Symposium on

Chemistry & Molecular Biology



established in the memory of
Anna A. Naff

NITROGENASE: THE CHEMISTRY OF BIOLOGICAL NITROGEN FIXATION

SPEAKERS
Dimitri N. Coucouvanis
Robert R. Eady
William E. Newton
Douglas C. Rees

Monday, April 11, 1994

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