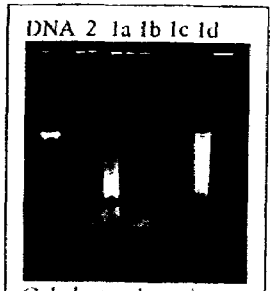
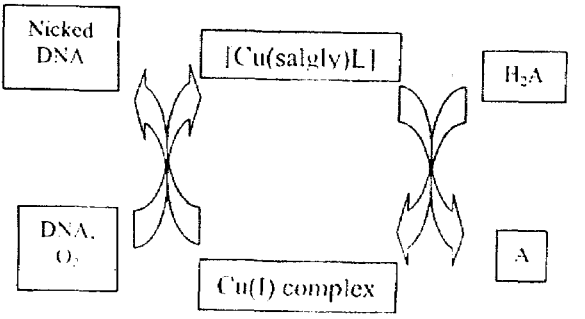


Gel electrophoresis using pUC19 DNA, Tris-HCl NaCl buffer at 37°C.



Scheme 1: Catalytic Reaction
H_2A=ascorbic acid, A=dehydroascorbic acid

References

1 Sigman D S, *Acc chem Res*, 19 (1986) 180.
2 Erkkila K E, Odom D T & Barton J K, *Chem Rev*, 99 (1999) 2777.
3 Reddy P A N, Datta R & Chakravarty A R, *Inorg Chem Comm*, 3 (2000) 322.

Heterogeneity in the folding intermediates of barstar detected by time-resolved fluorescence resonance energy transfer

G S Lakshmikanth, Jayant Udgaonkar & G Krishnamoorthy*

Department of Chemical Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005, India

Heterogeneity of folding intermediates has been proposed as a hallmark of protein folding process. A triple mutant of barstar having a single tryptophan (W53) in its core and a single cysteine sidechain has been prepared. The sulphhydral sidechain of the cysteine has been coupled to 5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB). The TNB group quenches the fluorescence of W53 to the extent of ~95% by fluorescence resonance energy transfer (FRET) in the native state. The quenching gets relieved in the denatured state.

Fluorescence decay kinetics of W53 has been analyzed as a distribution of lifetime by the unbiased maximum entropy method (MEM). The distribution of lifetimes has been translated into a distribution of distance between the W53 (the donor) and the TNB group (acceptor). Such distance distributions could reveal the structural heterogeneity of the intermediate species involved in the folding process. This FRET-based method has also been used in detecting small changes in the structure under various native-like and denaturing conditions.

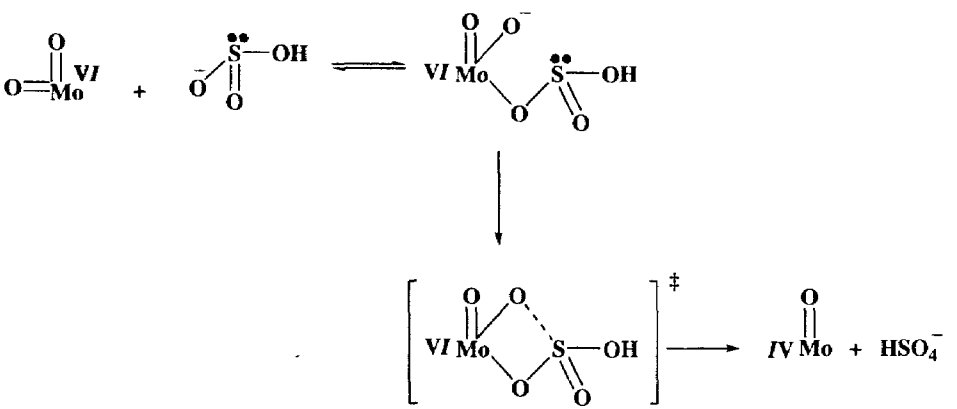
Reaction of a model sulphite oxidase with dimethylsulphite

P K Chaudhury[#], K Nagarajan[‡], B R Srinivasan[+] & S Sarkar[‡*]

[#]Department of Chemistry, University of Pune, Pune; [‡]Department of Chemistry, Indian Institute of Technology, Kanpur; India
[+]Department of Chemistry, Goa University, Goa, India

Sulphite oxidase catalyzes the oxidation of sulphite to sulphate, the last step of degradation of sulphur containing amino acids. The fundamental chemistry in relevance to the initial reaction of sulphite with sulphite oxidase has been rigorously pursued with the help of model compound using phosphine as model oxo acceptor species. The only model compound $\{(Bu_4N)_2MoO_2(mnt)_2\}$(mnt = maleonitriledithiolate) **(1)** has been found to be able to

perform biologically relevant reaction of oxidizing hydrogen sulphite to hydrogen sulphate both in terms of saturation kinetics as well as anionic inhibition. Nordlaner and co-workers have proposed a direct attack of the sulphur atom of sulphite on one of the oxo groups of molybdenum based on rate of phosphine oxidation towards **1** correlated to the relative basicity of phosphine[1]. An alternative proposal which involves direct co-ordination of oxoanion of bisulphite to the molybdenum center in the case model compound has been put forward by us[2] based on the work by Bray and co-workers. The feasibility of direct co-ordination of sulphite to the molybdenum centre has also been suggested recently by Rajagopalan and co-workers[3]. A very important experiment performed by Brody and Hille with the native enzyme utilizing dimethylsulphite as the substrate laid the importance of lone pair in such oxo-transfer reaction[4]. In view of the fact that our model complex(1) functionally mimicked the native sulphite oxidase is of interest its reactivity towards dimethylsulphite to gain further insight into the mechanism of the reduction of molybdenum centre of this enzyme. The reactivity of anionic bisulphite and of dimethylsulphite towards **1** is consistent with direct co-ordination of bisulphite to the molybdenum site of **1** as proposed earlier[2] which is Schematically shown below:



References

1 Lorber C, Plutino M R, Elding L I & Nordlaner E, *J chem Soc Dalton Trans*, (1997) 3997.
2 Das S K, Chaudhury P K, Biswas Dulali & Sarkar S, *J Am chem Soc*, 116 (1994) 9061.
3 George G N, Garrett R M, Graf T, Prince R C & Rajagopalan K V, *J Am chem Soc*, 120 (1998) 4522.
4 Brody M S & Hille R, *Biochim Biophys Acta*, (1995) 133.

Preliminary voltammetric characterization of *Clostridium pasteurianum* Fe-only hydrogenase I

Sean J Elliott[†]*, Brian J Lemon[#], John W Peters[#] & Fraser A Armstrong*[†]

[†]Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, England

[#]Department of Biochemistry and Chemistry, Utah State University, UT, USA

Email: sean.elliott@chem.ox.ac.uk

The iron only hydrogenase from the anaerobic microorganism *Clostridium pasteurianum* (CpI) is a soluble enzyme responsible for the interconversion of molecular hydrogen and protons. Hydrogenase enzymes of several types are known in a variety of organisms, though the function of hydrogenase activity may be tuned toward either hydrogen oxidation or proton reduction. CpI is a complex metalloenzyme, containing several iron sulphur clusters, including a multi-nuclear active site, termed the H-cluster. The crystal structure of CpI has recently been solved, as has a homologous protein from *Desulfovibrio desulfuricans*[1].