研 究 速 報

特 集 4
研 究 速 報

Biological Implication of Some New Iron-Sulfur Complexes — Nitrate/Nitrite Reductase Activity

新規な鉄-硫黄錯体の機能 — nitrate/nitrite reductase 活性

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Introduction

Since the isolation of ferredoxin from *clostridium pasteurianum,* iron-sulfur proteins have been the subject of immense activity. These proteins contain either 2Fe-2S, 4Fe-4S or 8Fe-8S active centres along with amino acids. Structurally, these proteins are metal complexes with an elaborate frame and are present in various plants and mammals involved in electron transfer reactions[1)~6)]. Synthetic and structural studies on the complexes of the type$Fe_4S^*_4(SR)_4^{n-}$ bearing R as different aliphatic and aromatic groups have been the focal point of research. However, there is lack of literature on the syntheses and structural studies on above type of clusters bearing five or six membered bidentate heterocyclic groups.

Thus, keeping the above facts in view and in view of recent reports[7)~10)] on the complexes of heterocyclic compounds with transition metals, we synthesised some iron complexes as active site of iron-sulfur clusters bearing derivatives of biologically active[9)~10)] oxadiazol, triazole, benzimidazole and benzthiazole as terminal ligands. Furthermore, the active site of the various iron-sulfur proteins have already been exploited in nitrogen assimilation studies[11)], it was therefore found worthwhile to carry out the nitrate/nitrite reductase activity of the synthesized products, as these reductases are the initial steps involved in the nitrogen assimilation process in the plants[12)].

Chemistry

The different heterocyclic compounds used as terminal ligands[Fig. 1] were prepared by the methods reported in the literature[13),14)] except 2-mercaptobenzimidazole (L^3H) and 2-mercapto-benzothiazole (L^4H) which were commercially available.

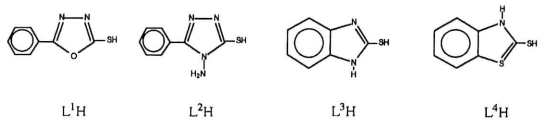

Fig. 1 Ligand Structures

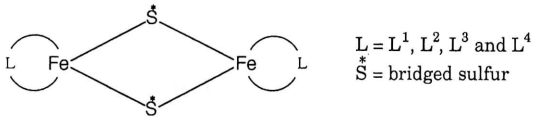

Fig. 2 Structures of the Complexes

The desired iron complexes were prepared using the method of Holm[15)] in an extremly dry condition and in an atmosphere of dry nitrogen gas and were characterized by their elemental analyses, I.R, NMR and UV/VIS spectroscopic studies. Structural assignments were made by comparing our data with the reported one[16)] which were found to be consistent with the active site of 2Fe-2S clusters[16)].

Thus, the synthesised complexes were tentatively assigned the structure as shown in Fig. 2.

Biochemistry

The complexes shown in Fig. 2 along with the free heterocycles (L^1H, L^2H, L^3H and L^4H) in addition to $FeCl_3$ were tested for their nitrate and nitrite reductase activity using dialysed enzyme preparations from shoots of 15 days grown rice plants of *cv. Ratna* according to the reported procedure[12)]. The nitrate reductase activities of all samples dissolved in phosphate buffer (50 μmol, pH 7.2) and DMSO

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(dimethylsulfoxide) respectively giving 0.4 mM concentrations in each case were assayed. Protein concentration in enzymic preparations were 0.372 mg ml^{-1} and specific activities of enzymes are expressed as μmol nitrite produced min^{-1} mg^{-1} protein (for nitrate reductase) and μmol nitrite reduced min^{-1} mg^{-1} protein (for nitrite reductase). Additionally we also investigated the nitrate reductase activity of $[Fe_2\ S^*_2\ (L^2)_2]$ in buffer and DMSO at various concentrations which showed highest activity compared with other complexes above 4mmol in DMSO. Nitrite reductase activity of the same compound in DMSO has also been studied.

In the buffer medium, the activity trend was found as $FeCl_3 > [Fe_2\ S^*_2\ (L^2)_2] < L^2H = [Fe_2\ S^*_2\ (L^4)_2] > L^3H = L^4H > L^1H = [Fe_2\ S^*_2 (L^1)_2] = [Fe_2 S^*_2\ (L^3)_2]$, whereas the activity trend in DMSO at the same concentration was found as $[Fe_2\ S^*_2\ (L^2)_2] > [Fe_2\ S^*_2\ (L^1)_2] > [Fe_2\ S^*_2\ (L^4)_2] > [Fe_2\ S^*_2\ (L^3)_2] > L^2H = L^1H > FeCl_3 = L^3H = L^4H >$ DMSO.

Thus it could be noticed from the above trend that $FeCl_3$ showed higher nitrte reductase activity in buffer medium whereas in DMSO it showed least activity. This could be possible due to its complexation with DMSO. However, it could further be seen that complex $[Fe_2\ S^*_2\ (L^2)_2]$ was found to be the best compound showing highest activity in DMSO and the activity is found to increase with respect to increase in the concentration of the complexes. At 2.5 mM concentration, the nitrate reductase activity of this complex is comparable to the activity shown by NADH (control) at the same concentration. Above 2.5 mM concentration in DMSO, the $[Fe_2\ S^*_2\ (L^2)_2]$ showed highest activity than the standard NADH. Furthermore, it was again noticed that the activity was very high in DMSO whereas free DMSO was inactive. Thus the role of DMSO appears to be very significant perhaps due to coordination and thereby changing the geometry of the complex. However, the encouraging results shown by $[Fe_2\ S^*_2\ (L^2)_2]$ led us to study it's Michaelian kinetics. Interestingly, a Lineweaver-Burk[17] plot of 1/[v] vs 1/[s] showed straight line (Fig. 3a) hence this compound follows the Michaelis kinetics. Lower K_m value (3.33 mM) obtained using $[Fe_2\ S^*_2\ (L^2)_2]$ as a cofactor (Fig. 3b) for the enzyme nitrate reductase and still lower K_m value (0.06 mM) for nitrite reductase (Fig. 3c) indicates the ideal behaviour of this compound for determination of such enzymatic activities. Thus on a preliminary level, this compound could be considered as an ideal substitute for

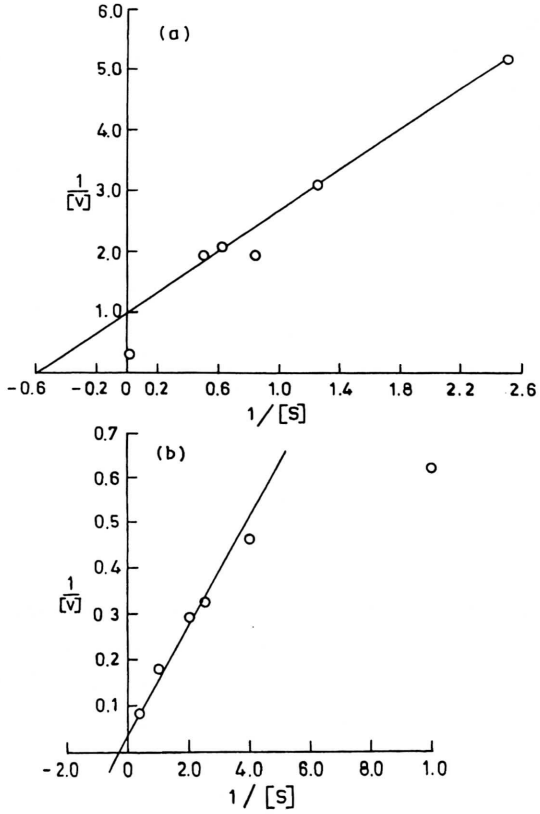

Fig. 3 Nitrate reductase activity of $[Fe_2S^*_2\ (L^2)_2]$ in (a) buffer, Km=17.24 mM^{-1} (b) DMSO, Km=3.33 mM^{-1}.

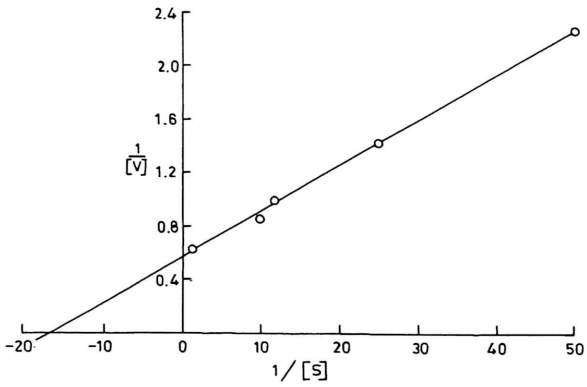

Fig. 3(c) Nitrite reductase activity of $[Fe_2S^*_2\ (L^2)_2]$ in DMOSO, Km=0.06 mM^{-1}.

NADH or other proton donors for measuring nitrate reductase activity in plants. One can easily understand the easy availability of protons from the NH_2 group of the coordinated ligand (L^2). Such situation is unfavourable in other complexes hence they did not show appreciable activity. The significant nitrate reductase activity shown in

presence of $[Fe_2 S^*_2 (L^2)_2]$ encouraged us to study the activity of the enzyme involved in the next step of nitrogen assimilation viz. nitrite reductase. The nitrite reductase activity was assayed by the reported procedure using KNO_2 as substrate[12]. Similar to nitrate reductase, again the compound $[Fe_2 S^*_2 (L^2)_2]$ showed very high activity above 0.4 mM concentration and a plot of 1/[v] vs 1/[s] is a straight line giving K_m value as 0.06 mM. The graphical presentation again indicated that $[Fe_2 S^*_2 (L^2)_2]$ could be employed as a suitable model for the assay of nitrite reductase activity.

Thus, on the basis of the preliminary observation it could be inferred that this new product could serve as a future candidate to be used as fertilizer. However, this surmise calls for deeper investigations.

Conclusion

The activities of 2Fe-2S type complexes bearing some five membered heterocycles of biological significance as a terminal ligands have been synthesized and characterized. Complex containg triazole ring as a terminal ligand was found to act as a better substitute for NADH in catalysing the nitrate/nitrite reductase activity.

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