low-iron enzyme was under comparable conditions only 10–15% as intense as that observed in the isolated normal-iron enzyme. This unusual temperature-sensitive resonance has been reported in proteins with as least four iron-sulfide atoms per mole, e.g. ferredoxins from clostridial and sulfate-reducing bacteria, adenylate sulfite reductase and nitrate reductase A from Micrococcus denitrificans. The reaction of NADH with low-iron NADH dehydrogenase results at 15 °K in a “g = 1.9” type signal with g-values of 2.034 and 1.934, due to the appearance of a single iron-sulfide center. In contrast the reduction of normal-iron NADH dehydrogenase results in the appearance of two iron-sulfide centers with major g-values at 2.042, 2.034 and 1.940. Quantitation by double integration of the “g = 1.9” type resonances observed in the low-iron and normal-iron enzymes account for approx. 30% of the chemical iron content when NADH is the reductant. Recent studies by Gutman et al. have strongly implicated the role of iron-sulfide centers of different redox potentials in site I phosphorylation. It is noteworthy that respiratory membrane systems obtained from A. vinelandii on low-iron growth conditions show a lower P/O ratio with NADH as substrate than in the similar system obtained on normal-iron growth conditions. The possible role of the different iron-sulfide centers in normal derived NADH dehydrogenase in site I phosphorylation of A. vinelandii is under investigation.

Supported by NSF research grant GB-13242 and USPHS NIH research grant 1-R01-GM18895 and Research Career Development Award 1-K04-GM70,010.

4 P. Forget and D. V. DerVartanian, Biochim. biophysica Acta [Amsterdam] 256, 600 [1972].
5 M. Gutman, T. P. Singer, and H. Beinert, Biochemistry 11, 556 [1972].

On the Mechanism of Adenylyl Sulfate Reductase for the Sulfate-Reducing Bacterium, Desulfovibrio vulgaris

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Desulfovibrio vulgaris, sulfate reducing bacterium, Adenylyl sulfate reductase

The roles of enzyme-bound FAD and non-heme iron in the mechanism of adenylyl sulfate reductase have been investigated by inhibitor studies, stopped-flow techniques and EPR spectroscopy. The results indicate that the non-heme iron found in the purified reductase is catalytically active and that the turnover number of the enzyme-bound FAD is identical with the maximum turnover number for the enzyme.

Unusual abbreviations: EPR, electron paramagnetic resonance; APS, adenylate sulfite.

APS reductase catalyzes the oxidative activation of sulfite in the presence of AMP and an electron acceptor to yield APS and reduced electron acceptor as shown in the following equation.

\[
\text{SO}_3^{2-} + \text{AMP} + 2 \text{Fe(CN)}_6^{3-} \rightarrow \text{APS} + 2 \text{Fe(CN)}_6^{4-}
\]

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A maximum turnover number has been obtained with ferricyanide but a low level of activity can be observed with O_2 as the electron acceptor. The enzyme also catalyzes the reduction of APS to AMP and SO_3^{2-} in the presence of reduced methyl viologen. A mechanism of action has been postulated which involves an initial reaction of sulfite with FAD to form an N-5 adduct and subsequent transfer of the high energy sulfate to a mononucleotide acceptor such as AMP with the concomitant reduction of non-heme iron. The proposed scheme is shown in Fig. 1 where X represents the non-heme...
iron component. This reaction sequence was based largely on two observations. First, the addition of sulfite to mononucleotide-free reductase causes bleaching of the flavin component in the visible absorption spectrum and reduction of a small fraction of the non-heme iron. In addition, there is a concomitant increase in absorption at 320 nm which has been attributed to the formation of the N-5 adduct. Second, the subsequent addition of AMP effects a further bleaching of the enzyme which appears to be due to the reduction of non-heme iron. Aside from these spectral changes and the presence of both FAD and non-heme iron in the purified enzyme, no direct evidence for the involvement of these components in the catalytic activity of the reductase has been reported.

The turnover number for the bleaching of enzyme-bound flavin by sulfite in the presence of AMP has been determined by stopped flow techniques and found to be identical to that observed in the kinetic (ferricyanide) assay (18 μmole/sec/μmole enzyme). In the absence of AMP, the rate of flavin bleaching is only 1/10 that found in the presence of the mononucleotide. AMP thus appears to function as a positive effector in the oxidative direction and this observation may be related to a previously described effect of AMP on the association of enzyme molecules.

Previous EPR studies at 77 °K revealed no signals attributable to non-heme iron in oxidized (as isolated), sulfite-reduced, or dithionite-reduced APS reductase but recent measurements at 17 °K now show an asymmetric signal at \( g = 2.00 \) in oxidized APS reductase (Fig. 2, upper trace). This signal may be tentatively assigned to low spin ferric ion of unknown symmetry and has been reported in bacterial ferredoxins, homogenous metalloproteins such as nitrate reductase A, and metalloflavoproteins which contain at least 4 iron-sulfide groups. The addition of excess sulfite (50-fold) causes a decline of the initial oxidized asymmetric signal at \( g = 2.00 \) and is paralleled by the appearance of a typical “\( g = 1.9 \)" type resonance with 3 well-resolved \( g \)-values (2.094, 1.943, 1.910). This latter signal intensity is only about 30% of the intensity observed when APS reductase is reduced chemically with sodium dithionite (Fig. 2, lower trace). These changes in signal intensity are consistent with changes observed in the absorption spectrum of the enzyme on the addition of sulfite and dithionite; however the effect of AMP on this signal needs to be determined.

Further evidence for the involvement of non-heme iron in the catalytic activity of APS reductase has been obtained by inhibitor studies. PCMB (0.2 mM)
causes a partial bleaching of the absorption spectrum (Fig. 3; curves A and B) and the difference spectrum indicates that the non-heme iron centers have been destroyed. After removal of the PCMB by gel filtration, the enzyme is no longer active with ferricyanide as electron acceptor but the enzyme-bound FAD still retains activity with sulfite (Fig. 3; curve C) and can utilize O$_2$ as electron acceptor. The addition of AMP (Fig. 3; curve D) has a much reduced effect on the absorption spectrum and can be eliminated by careful titration of the non-heme iron with PCMB. Higher concentrations of the inhibitor completely inactivate the enzyme. After gel filtration, chemical analyses of the protein indicate that most of the non-heme iron has been eliminated from the enzyme.

The rate of reduction of the enzyme-bound flavin, the occurrence of the "$g = 1.9$" signal upon the addition of sulfite and the involvement of non-heme iron in the reduction of ferricyanide are consistent with electron flow proceeding from sulfite through FAD to non-heme iron as proposed in the reaction sequence for APS reductase.

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5. P. Forget and D. V. DerVartanian, Biochim. biophysica Acta [Amsterdam] 256, 600 [1972].