ESR Study of the Mononuclear Non-heme Iron Center in Putidamonoxin, the Oxygenase of the 4-Methoxybenzoate-O-demethylase

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Putidamonoxin (PMO), the oxygenase of a 4-methoxybenzoate monooxygenase, contains besides an $Fe_2S_2^*$-cluster an additional non-heme iron center (1). The non-cluster iron atom is more firmely bound in the reduced PMO, and is lost within short terms under oxidizing conditions whereby the enzymatic activity decreases. This inactivation is prevented by the presence of substrate (2). As the non-cluster iron center is regarded as the binding site for dioxygen the properties of this iron complex are studied by ESR spectroscopy in the temperature range from 3.6 K to 80 K.

The reduced form is ESR-silent. The oxidized form shows a strong signal at $g_5 = 4.293$. Furthermore, up to four weaker signals (g_1, g_2, g_3, g_4) are observed which appear on the low-field position of the resonance at g_5.

The apparently isotropic signal at $g_5 = 4.293$ is indicative of a ferric high spin iron atom ($^6S_{5/2}$) in a rhombic ligand field. This signal arises from transitions between the two sublevels of the middle Kramers doublet. As the intensity of the signal decreases raising the temperature from 3.6 K to 80 K, we must assume that D is smaller than 1 cm^{-1} (4). This is reasonable for a ligand field which is set up mainly by oxygen and nitrogen atoms (4), although one sulfur atom is unconditionally necessary for the coordination of the iron atom (2). The parameter $\lambda = E/D$ reaches the value of 1/3. This means that the iron atom which produces this signal possesses a totally rhombic symmetry (3).

The low field signals are not clearly understood at present. The signal lowest in field ($g_1 = 9.4$-9.6) correlates in the λ-versus-g diagramme with a λ value which agrees with that found for the transition at $g_5 = 4.293$. This $g \approx 9$ transition can be attributed to the low-lying Kramers doublet.

The other signals, assigned as g_2, g_3 and g_4 vary in amplitude and g-factor as different substrates are bound to PMO (see Table 1).

Substrate	g_1	g_2	g_3	g_4	g_5
4-methoxybenzoate	9.39	7.15	5.88	5.05	4.293
4-hydroxybenzoate	9.55	8.58	-	5.18	4.293
benzoate	9.37	7.89	6.72	5.68	4.293

These three ESR signals also seem to be characterized by a common λ value which is nearly 0.05. We conclude that these

signals have to be attributed to an iron center with tetragonal symmetry. The tetragonal symmetry of this iron center is significantly influenced by the binding of different substrates to PMO.

Based on ligand binding studies we assume that both ESR-spectral species (with rhombic and tetragonal symmetry respectively) belong to the same mononuclear non-heme iron center.

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