O26

Mössbauer and EPR Evidence for Nickel and 3Fe Cluster in the Hydrogenases of *D. desulfuricans* and *D. gigas*

B. H. HUYNH

Emory University, Atlanta, Ga., U.S.A.

J. LEGALL, D. V. DERVARTANIAN, H. D. PECK, Jr., H.-J. KRÜGER

University of Georgia, Athens, Ga., U.S.A.

I. MOURA, J. J. G. MOURA and A. V. XAVIER

Universidades de Lisbon, Portugal

Hydrogenases from *Desulfovibrio desulfuricans* (27774) and from *Desulfovibrio gigas* have been studied by EPR and Mössbauer spectroscopy. As isolated, hydrogenase from *D. gigas* exhibits an 'isotropic' EPR signal at g = 2.02 and a rhombic EPR signal at g = 2.31, 2.20 and 2.02. Isotopic substitution of ^{61}Ni (I = 3/2) proves that the rhombic signal is due to Ni. A similar EPR spectrum was observed for *D. desulfuricans* hydrogenase, indicating that both enzymes contain an EPR active nickel center. Combining the Mössbauer and EPR data, the isotropic g = 2.02 EPR signal was shown to originate from a 3Fe cluster which may have oxygenous or nitrogenous ligands. In addition, the Mössbauer data also revealed two [4Fe–4S] clusters in each molecule of hydrogenase.

O27

Core Extrusion of Iron–Sulfur Proteins with Benzenethiol in Aqueous Urea/Triton X-100 Solutions: A Study on the Reactivity of Different Conformational States of Succinate Dehydrogenase

FRANCO BONOMI, SILVIA PAGANI and PAOLO CERLETTI

Department of General Biochemistry, Faculty of Agriculture, University of Milan, Milan, Italy

The aqueous DMF/Triton X-100/benzenethiol extrusion procedure developed by Kurtz [1] and investigated in detail in [2] has been modified by substituting urea for DMF as the protein-denaturing agent. This replacement allows for shorter reaction times, lower sensitivity to ionic strength [3] and for lower dilution factors. Preliminary experiments with spinach ferredoxin [4] demonstrated the identity of the extruded thiolate with respect to the synthetic analogue, and the feasibility of an easy and ready separation of the products of the reaction by ion-exchange chromatography. The thiolate does not bind to a cellulosic anion exchanger, whereas the bound apoprotein is eluted with high-ionic strength buffer and can be chemically [5] or enzymatically [6] reconstituted to its native form.

TABLE I. Relationships between the Conformational State, E_m^{S1}, and the Rate Constant of the Urea/Triton/Benzenethiol Extrusion Reaction for Succinate Dehydrogenase. E_m^{S1} is determined by potentiometric titration of the EPR signal at g = 1.94 at liquid nitrogen temperature. Reaction rates for the extrusion reaction are determined by monitoring the increase in absorbance at 474 nm resulting from the addition of the protein to a suitable reaction medium, whose final composition is: 9×10^{-6} *M* protein, 0.096 *M* benzenethiol, 4.73 *M* urea, 4.77 (% v/v) Triton X-100, 0.05 *M* Tris-HCl and enough effector or NaCl to give a final ionic strength of 0.0365 *M*.

Effector	E_m^{S1}, mV	Pseudo-first-order rate constant of the extrusion reaction, min^{-1}
None	−14	0.0769
Oxaloacetate	+80	0.2631
Succinate	+108	0.4444

We used this technique in our investigation on the molecular basis of the modulation of the redox potentials of the electron-transferring groups in the complex iron–sulfur–flavoprotein succinate dehydrogenase. This protein exists in three different conformational states, or conformers, depending on the binding of different effectors to a site different from the catalytic one [7]. We thus distinguish between: the active, ligand-free enzyme; the active, succinate-stabilized enzyme, and the inactive complex between the protein and oxaloacetate. Different catalytic activities of the conformers depend on the different midpoint potential of the covalently bound flavin, which is high in the active conformers and low in the inactive one [8].

Also the redox midpoint potential of the tetrahedral Fe–S center S1 (E_m^{S1}) is affected by ligand binding. This parameter is related in Table I to the reaction rates measured for the extrusion of Fe–S structures from different conformers. Although a possible non-selectivity of the extrusion must be considered, rate constants for different conformers exactly parallel the figures obtained for E_m^{S1}, increasing as this latter increases. The concentrations of both the denaturing agent (urea) and of the extruding one (micellar benzenethiol) being the same, the reaction rate only depends on the accessibility of the cluster to the hydrophobic extrudant. Thus binding of effectors which increase E_m^{S1} also increases the hydrophobicity of the environment of the cluster. This extends to different conformational states of the