STRUCTURAL CONTROL OF THE REDOX POTENTIALS AND OF THE PHYSIOLOGICAL ACTIVITY BY OLIGOMERIZATION OF FERREDOXIN

J. J. G. MOURA and A. V. XAVIER
Centro de Química Estrutural da Universidade de Lisboa, IST, Av. Rovisco Pais, Lisboa 1, Portugal

and

E. C. HATCHIKIAN and J. LE GALL
Laboratoire de Chimie Bactérienne, CNRS, 13274 Marseille, Cedex 2, France

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1. Introduction

Desulphovibrio gigas ferredoxin is active in the transfer of electrons in the phosphoroclastic reaction as well as in the transfer of electrons from molecular hydrogen, via hydrogenase and cytochrome c_3, to the reduction of sulphite [1,2]. As shown in fig.1 the electrons are transferred at very different potentials for each of the reactions.

After a complete purification of *D. gigas* 4-iron–4-sulphur ferredoxin, 3 distinct oligomeric forms of this protein were isolated [3]. These are 2 trimers (FdI and FdI') with different isoelectric points, and 1 tetramer (FdII). Each oligomer has the same monomer unit with mol. wt 6000, a single (4 Fe, 4 S) cluster and the same amino acid composition. The stabilization of ferredoxin in oxidation states with different redox potentials is controlled by modifications brought about by the oligomerization of the protein. This paper shows that the constraints imposed by the oligomerization of this metalloprotein stabilize different oxidation states for each oligomer [4,5] and that this is an important factor in determining their differentiated physiological activity.

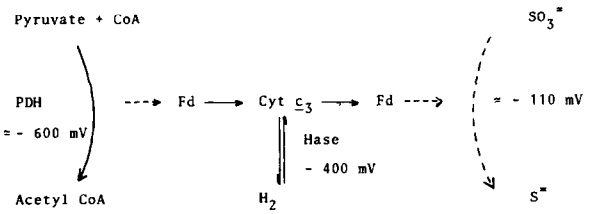

Fig.1. Two relevant electron pathways in *Desulphovibrio* sp., the phosphoroclastic reaction and sulphite reductase system. The central role of cytochrome c_3 and the coupling effect of ferredoxin are schematized. Since the trithionate pathway, which involves the existance of sulphur intermediates during the reduction of sulphite to sulphide [16], is still under controversy, the global value of E'_0 from SO_3^{2-} to S^{2-} is given here. This value has been computed from the data in [17–20]. *Abbreviations:* PDH, pyruvate dehydrogenase; Cyt c_3, cytochrome c_3 (4 haems); Fd, (4 Fe, 4 S) ferredoxin; Hase, hydrogenase 3x (4 Fe, 4 S).

2. Materials and methods

The 3-oligomeric forms of *D. gigas* ferredoxin were isolated and purified as in [3].

The pyruvate dehydrogenase activity and the coupling effect of the ferredoxin oligomeric forms was determined by measuring the hydrogen evolution by a manometric assay in the following conditions: Main compartment: 150 μmol phosphate buffer (pH 7.0), 100 μmol electron carrier (Fd), pure hydrogenase, 4 μmol CoA, 5 μmol TPP, 20 μmol Cl_2Mg, 10 mmol mercaptoethanol and the pyruvate dehydrogenase containing extract in final vol. 3.0 ml.

Side arm: 30 μmol sodium pyruvate.
Centre well: 0.05 ml 10 M NaOH.
The flasks were incubated for 30 min at 37°C.

The pyruvate dehydrogenase containing extract, devoided of ferredoxin and flavodoxin was obtained by passing the *D. gigas* acidic extract [2] (10 ml containing 30 mg/ml protein) on a small DEAE column (10 × 10 mm) equilibrated with 0.01 M Tris–HCl, pH 7.6. Of this extract, 27.7 mg were used in all the assays performed. Pure hydrogenase prepared from *D. gigas* as in [6] with spec. act. 30 μmol hydrogen consumed/min/mg protein and was added in all cases to the system to ensure an excess of this activity.

3. Results and discussion

Physico-chemical characterization, using visible spectroscopy [3], NMR and magnetic susceptibility [4] and in particular EPR [5], have shown that the 3 forms stabilize the (4 Fe, 4 S) cluster in different oxidation states of the 'three state hypothesis' [7]. FdI stabilizes almost exclusively the C^{2-}/C^{3-} states (E_0 = –455 mV), FdII stabilizes almost exclusively the C^{-}/C^{2-} states (E_0 = – 130 mV). FdI′ has an intermediate behaviour and will not be discussed here.

We have shown (see fig.3, in [3]) that FdII is more efficient than FdI in the reduction of sulphite. The tetramer FdII utilizes oxidation states more positive than those normally available for bacterial ferredoxins and is then more efficient in accepting electrons from cytochrome c_3.

Figure 2 shows that in the phosphoroclastic reaction (see fig.1) it is the trimer FdI which is more active in the transfer of electrons between pyruvate and molecular hydrogen, as it should be expected since only the C^{2-}/C^{3-} redox states have the necessary redox potential to be active in the indicated pathway at ≃ –600 mV to –400 mV. There is a lag phase after which the tetrameric form FdII starts to be active in the transport of electrons from pyruvate. The decrease of activity during this lag phase, as compared to the endogenous, is proportional to the initial concentration of FdII, suggesting that FdII blocks the transfer of electrons in this pathway. The inhibition may be due to the complexation of cytochrome c_3 by FdII

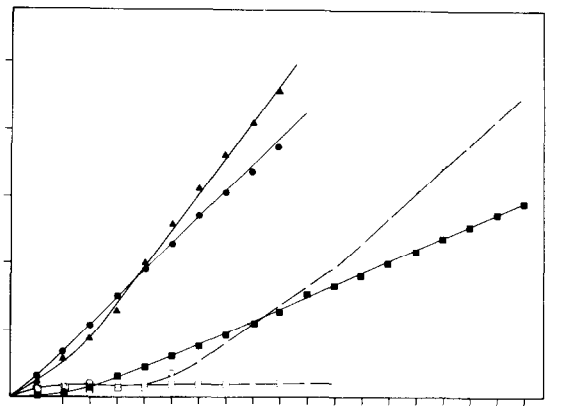

Fig.2. The pyruvate dehydrogenase activity and the coupling effect of the ferredoxin oligomeric forms. For sake of comparision, Fd concentrations are calculated per monomeric unit: (●) control (initial system); (▲) the pyruvate dehydrogenase-containing extract, devoided of electron carriers, plus 100 nmol *D. gigas* FdI; (○) the pyruvate dehydrogenase containing extract, devoided of electron carriers, plus 100 nmol of *D. gigas* FdII; (■) endogenous activity measured on the pyruvate dehydrogenase containing extract devoided of electron carriers; (□) control without pyruvate.

[8]. One possible explanation for the increase in activity after the initial lag phase is that the tetrameric form FdII is converted into the trimeric form FdI. This is supported by the fact that conversion of the tetramer to the trimer is made possible by reconstituting the apoprotein obtained from the tetrameric form [5]. However, even after the lag phase the activity for the same monomeric concentration is not fully recovered since the slope of the FdII curve is smaller than that of the FdI curve.

Flavodoxin can substitute for ferredoxin in the above reactions [1,9]. The redox potentials of the 2 available redox couples [10] of flavodoxin, Fld/FldH· (–150 mV) and FldH/$FldH_2$ (–440 mV) [11], are strikingly similar to those of FdII and FdI, suggesting that flavodoxin uses the 2 available redox couples in coupling the hydrogenase to the sulphite reductase and in the phosphoroclastic reaction respectively.

The 4-iron–4-sulphur cluster can use a wide range of redox potentials. This was tentatively explained by the 'three state hypothesis' which attributed this difference in potentials to the fact that different proteins utilize different oxidation states of the cluster. Our work, as well as [12] shows that the redox

potential of the (4 Fe, 4 S) cluster is not exclusively determined by the oxidation state. The structure of the protein plays an important role in the control of the cluster redox potential suggesting that a different 'entatic state' [13,14] is created. In *D. gigas* ferredoxin the quaternary structure modifications change the physico-chemical properties of the protein with important phyisiological consequences. Also, the great versatility of the (4 Fe, 4 S) cluster may be useful to understand the mechanism of action of more complex (4 Fe, 4 S) proteins, e.g., hydrogenase, for which a wide range of redox potentials have been observed [15].

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