OXIDATIVE INACTIVATION OF THE Fe-PROTEIN FROM *CLOSTRIDIUM PASTEURIANUM* NITROGENASE

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1. Introduction

Nitrogenase, the enzyme responsible for the reductive breakdown of the N_2 molecule into ammonia, consists of two different (non-heme) iron–sulfur proteins, one of them containing molybdenum. Both components show an extraordinary sensitivity toward oxygen compared with other iron–sulfur proteins [1,2].

On the basis of the redox potentials and the kinetic properties of the two proteins, it is currently accepted that electrons are transferred for the reduction of the substrate according to the following simplified sequence [3]:

Fd → Fe-protein·ATP·Mg → MoFe-protein → Substrate

There is abundant evidence for the specific binding of ATP·Mg to the Fe-protein of nitrogenase [4–7] rather than to the MoFe-protein. Also the redox potential of the clostridial Fe-protein (–294 mV) becomes about 100 mV more negative when the measurement is made in the presence of ATP·Mg due, apparently, to a conformational change induced by the nucleotide [8,9]. For the MoFe-protein a relatively positive midpoint potential (–20 mV) was obtained which was unaffected by ATP·Mg [9]. However, it has been proposed that it may exist at a lower potential during the catalytic cycle of the enzyme [10–13].

Nevertheless, given the complexity of this enzyme system, in which two different proteins, ATP·Mg, and an electron donor are involved, the study of the redox and conformational states of each individual protein during the catalytic turnover presents great difficulty and many questions remain unanswered.

In the present note, data are presented showing that the Fe-protein exhibits different sensitivities toward oxidation, depending on the reaction components present. This finding suggests that substantial changes apparently occur in the redox state of the Fe-protein upon binding of ATP and Mg^{2+} and during the turnover when the MoFe-protein is present.

2. Materials and methods

The procedures for the isolation and purification of both nitrogenase components from *Clostridium pasteurianum* cells and for the assay of their enzymatic activities are in [14]. The Fe-protein had a specific activity of 1300 nmol acetylene reduced/min/mg protein.

The electrochemical cell used for the oxidative inactivation of the Fe-protein was essentially the same as in [14], but a motor-driven syringe was used, so that the oxidant could be added continuously and very slowly so as to avoid local accumulation of the oxidant. The following redox mediators were used, each at 50 μM: benzyl viologen ($E_{m7} = -363$ mV),

Abbreviations: MoFe-protein, molybdenum–iron protein; Fe-protein, iron protein; ATP·Mg, $MgATP^{2-}$

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anthraquinone-1,5-disulfonate ($E_{m7} = -175$ mV), anthraquinone-β-sulfonate ($E_{m7} = -225$ mV), 2-hydroxy-1,4-naphtoquinone ($E_{m7} = -137$ mV) and neutral red ($E_{m7} = -324$ mV). Deoxygenated potassium ferricyanide was used as the titrant. The potentials were measured with a gold electrode versus the Ag/AgCl reference electrode, and the latter was calibrated with saturated quinhydrone at selected pH values. All potentials discussed in this note are referred to the standard hydrogen electrode.

All reagents were of the highest purity available and were used without further purification.

3. Results

The Fe-protein from *C. pasteurianum* nitrogenase is inactivated by incubation at relatively low potentials. Figure 1A shows the course of inactivation of this protein by incubation at potentials ranging from −450 mV to +100 mV. The inactivation occurs over a potential range of approx. 300 mV and the activity is decreased to 50% at −125 mV.

When the same experiment was carried out in the presence of ATP, or Mg^{2+}, or both, the inactivation occurs at even lower potentials, since in all cases the protein was 50% inactive at about −275 mV (fig.1B). The potential dependence for the inactivation of the Fe-protein was not affected by the presence of Ca^{2+}, a cation that cannot replace Mg^{2+} in the nitrogenase reaction (see fig.1A).

When the Fe-protein was oxidized in the presence of all the components of the reaction, i.e., MoFe-protein, ATP, Mg^{2+}, and H^+ (which serves as the substrate), the inactivation occurs immediately after $S_2O_4^{2-}$ becomes exhausted (fig.1C), while the potential changed very little. Apparently, only the Fe-protein and not the MoFe-protein was affected, as when the incubation mixture was supplemented with fresh, untreated Fe-protein in the assay mixture full activity of the MoFe-protein was obtained. The same inactivation pattern was obtained in a similar experiment in which Mg^{2+} and ATP were deleted (fig.1C).

When the oxidative titration was carried out with the intact nitrogenase complex obtained in the early stages of the purification, when the two components were not yet separated, the Fe-protein showed a sensitivity similar to that when it was titrated alone.

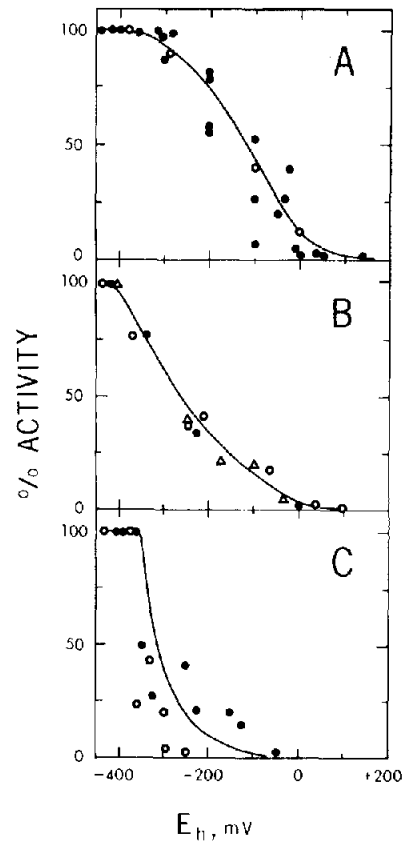

Fig.1A. Oxidative titration of the Fe-protein. 1.8 mg Fe-protein was incubated in the electrochemical cell in 1 ml 0.2 M Tris–Cl, pH 7.0, containing 0.2 M NaCl, 0.5 mM $Na_2S_2O_4$ and the mixture of redox mediators described in section 2. The potential was increased by addition of small amounts of a de-aerated solution of 0.1 M ferricyanide. After 5 min at the potential indicated (± 5 mV), 50 μl samples were withdrawn and the activity measured. The activities are expressed as % sample activity prior to ferricyanide addition. The results shown are a composite of 3 separate sets of experiments (•), and those titrated in the presence of 10 mM $CaCl_2$ (○).

Fig.1B. Oxidative titration of the Fe-protein in the presence of Mg^{2+} (•), or ATP (○), or ATP·Mg (△). The reagent concentrations were the same as in (A); Mg^{2+} and/or ATP were present at 10 mM.

Fig.1C. Oxidative titration of the Fe-protein in the presence of the MoFe-protein (•) or MoFe-protein plus the ATP-generating system (○). The conditions were the same as in (A), except MoFe-protein was present at 1 mg, 5 mg or 8 mg and the ATP-generating system was used at the same concentrations as in the standard assay.

4. Discussion

Sensitivity toward oxidation is a common phenomenon among iron–sulfur proteins. The difference in stability seems to be related to the protein structure, as urea and guanidine hydrochloride have been found to promote inactivation in many cases [15]. In the case of nitrogenase, the Fe-protein activity is very much dependent on the redox potential, the threshold being much lower, i.e., at a much more negative potential, than for MoFe-protein [14].

The monotonic course of inactivation suggests that the process probably occurs as the result of oxidation of protein groups with similar structure or redox potential, most likely the oxidation of the sulfide groups to sulfur zero, as has been found with other iron–sulfur proteins [1].

There are several reports on the effect of ATP·Mg on the oxygen sensitivity and on the accessibility to the iron atoms and sulfhydryl groups, although not all agree on the nucleotide and metal requirements [5,7,16]. We have observed that in the presence of ATP and Mg^{2+} the potential at which the Fe-protein is inactivated shifted toward more negative values, and it seems reasonable to attribute it to a conformational change that leads to an exposure of the Fe–S centers.

The markedly enhanced sensitivity of the Fe-protein toward oxidation produced by the MoFe-protein when it is present either alone or in combination with other reaction components, contrasts with the relative stability when the titration is performed with the crude preparation in which the MoFe-protein is still complexed to the Fe-protein. This peculiar behavior of the Fe-protein in its isolated state could be the result of some changes in its structure as it is removed from the native nitrogenase complex, which makes it more sensitive to oxidation not only in the presence of ATP and/or Mg^{2+}, but also the MoFe-protein [17].

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References

[1] Petering, D., Fee, J. A. and Palmer, G. (1971) J. Biol. Chem. 246, 643–653.
[2] Zumft, W. G. (1976) Struct. Bond. 29, 1–65.
[3] Winter, H. C. and Burris, R. H. (1976) Annu. Rev. Biochem. 45, 409–426.
[4] Bui, P. T. and Mortenson, L. E. (1968) Proc. Natl. Acad. Sci. USA 61, 1021–1027.
[5] Yates, M. G. (1972) Eur. J. Biochem. 29, 386–392.
[6] Tso, M.-Y. W. and Burris, R. H. (1973) Biochim. Biophys. Acta 309, 263–270.
[7] Thorneley, R. N. F. and Eady, R. R. (1973) Biochem. J. 133, 405–408.
[8] Zumft, W. G., Palmer, G. and Mortenson, L. E. (1973) Biochim. Biophys. Acta 292, 413–421.
[9] Zumft, W. G., Mortenson, L. E. and Palmer, G. (1974) Eur. J. Biochem. 46, 525–535.
[10] Smith, B. E., Lowe, D. J. and Bray, R. C. (1972) Biochem. J. 130, 641–645.
[11] Walker, M. and Mortenson, L. E. (1974) J. Biol. Chem. 249, 6356–6358.
[12] Evans, M. C. W. and Albrecht, S. L. (1975) Biochem. Biophys. Res. Commun. 61, 1187–1192.
[13] Orme-Johnson, W. H., Hamilton, W. D., Ljones, T., Tso, M.-Y. W., Burris, R. H., Shah, V. K. and Brill, W. J. (1974) Proc. Natl. Acad. Sci. USA 69, 3142–3145.
[14] Gomez-Moreno, C. and Ke, B. (1978) in preparation.
[15] Malkin, R. (1973) in: Iron–Sulfur Proteins (Lovenberg, W. ed) vol. II, pp. 1–26, Academic Press, New York.
[16] Walker, G. A. and Mortenson, L. E. (1974) Biochemistry 13, 2382–2388.
[17] Benemann, J. R., Smith, G. M., Kostel, P. J. and McKenna, C. E. (1973) FEBS Lett. 29, 219–221.