Regulation of Nitrogenase Activity in *Rhodopseudomonas capsulata* AD 2

Kassem Alef

Lehrstuhl für Mikrobiologie, Universität Bayreuth, D-8580 Bayreuth, Bundesrepublik Deutschland

Z. Naturforsch. **38 c**, 436–438 (1983); received March 14, 1983

Nitrogenase, Regulation, *Rhodopseudomonas capsulata* AD 2

Regulation of nitrogenase activity in *Rhodopseudomonas capsulata* AD 2 differs in several respects from other Rhodospirillaceae:

1) Nitrogenase activity in this strain grown under severe N-starvation was fully derepressed, but in contrast to other Rhodospirillaceae, it was inhibited by ammonia *in vivo*.
2) Nitrogenase in extracts of glutamate grown cells was fully active and not further stimulated by Mn^{2+}.
3) Treatment of N-starved or glutamate grown cells with ammonia before harvest did not cause inactivation of nitrogenase *in vitro*.

Introduction

The nitrogenase system of the Rhodospirillaceae is reversibly inhibited *in vivo* by ammonia. The biochemical mechanism of this phenomenon in *Rhodospirillum rubrum* is due to the enzyme catalyzed attachment and removal of an adenine like moiety from the dinitrogenase reductase [1]. It has been also shown that, depending on the nitrogen source, nitrogenase can be found *in vitro* in either an active or an inactive form. The inactive form (R) was obtained from cells grown with glutamate or N_2 [2, 3], while the active form (A) was obtained from N-starved cells [4–6]. Yoch and Cantu [7] suggested a model for the regulation of nitrogenase in *R. rubrum* where the conversion of nitrogenase A to nitrogenase R was obtained by the addition of ammonia to N-starved cells. This model has been modified, since Preston and Ludden [8], Gotto and Yoch [9] showed that ammonia switch-off resulted in a change in the subunit composition of dinitrogenase reductase and incorporation of $^{32}PO_4$ into one subunit.

There are also some discrepancies in the literature as to whether nitrogenase activity of N-starved cells of the Rhodospirillaceae is susceptible to ammonia switch-off [7, 10, 11]. Very recently Alef and Kleiner [12] showed that this effect was not shown under N-starvation in several strains, *Rhodopseudomonas capsulata* AD 2 was an exception, since the nitrogenase of which was always susceptible to switch-off by ammonia. We report here studies on the regulation of nitrogenase activity in this strain and show the main differences to the other Rhodospirillaceae.

Reprint requests to Dr. K. Alef.

0341-0382/83/0500-0436 $ 01.30/0

Materials and Methods

Rhodopseudomonas capsulata AD 2 and B 10 were grown with thiamine as vitamine source [13] and illumination of 100 μ Einstein $m^{-2} \cdot S^{-1}$. As nitrogen source 10 mM glutamate, N_2 or 10 mM nitrate were used. N-starved cultures were grown with 1 mM ammonia, 1 mM glutamate or 1 mM nitrate (no gas phase) and collected after 67 h (total exhaustion of N-source). Nitrogenase in whole cells was measured using the C_2H_2 reduction technique, with 40 mM malate as electron donor and illumination of about 376 μ Einstein $m^{-2} \cdot S^{-1}$ and *in vitro* similarly as described [3] using cell-free extracts obtained after disruption by a Frensch press and centrifugation (20 min, 30000 × *g*). Protein was determined according to ref. [14].

Results and Discussion

In contrast to the other Rhodospirillaceae [12], Fig. 1 shows that nitrogenase activity in N-starved *R. capsulata* AD 2 cells was inhibited completely by ammonia, this switch-off was obtained in contrast to *R. rubrum* [11] – in high light (376 μ Einstein $m^{-2} \cdot S^{-1}$) without any lag phase. Fig. 1 shows also

Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung") beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

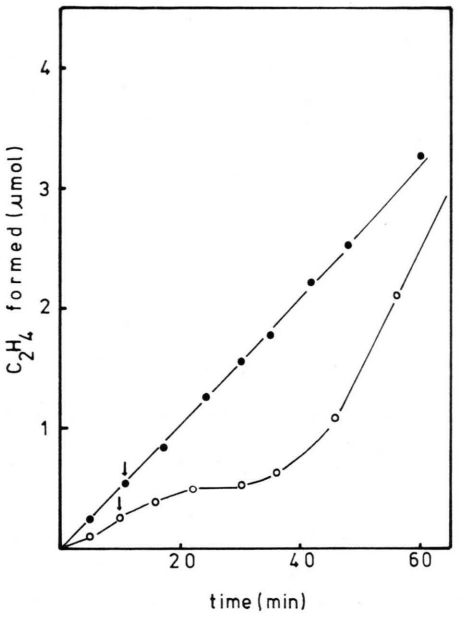

Fig. 1. The effect of ammonia on the light-dependent acetylen reduction by *R. capsulata* AD 2 (○) and B 10 (●) N-starved cells. The reaction mixtures contained cells equivalent to 3.5 mg/ml dry weight from *R. capsulata* AD 2 and 3.2 mg from B 10, 50 mM potassium phosphate pH 7.0 and 40 mM sodium malate. At time indicate by the arrow 2 mM NH_4Cl were added.

that the activity in *R. capsulata* B 10, as a comparison remained unaffected. These cultures had more than 5-fold higher nitrogenase activity than the nitrogen grown cultures. The switch-off effect was also found in *R. capsulata* AD 2 cells grown with glutamate, nitrate and nitrogen.

In contrast to *R. palustris* [15] the nitrogenase activity in N-starved *R. capsulata* AD 2 cells was also inhibited by glutamine. Glutamate (10 mM) and methylamine (5 mM) had no effect. Furthermore the addition of methionine sulfoximine (MSX) as glutamine synthetase inhibitor at concentrations between 0.2–5 mM to N-starved cultures of *R. capsulata* AD 2 caused almost a complete abolishment of the inhibition by ammonia but not of that caused by glutamine. This shows that the switch-off effect is caused by glutamine or another assimilatory product and not by ammonia.

We conclude that the switch-off effect in this strain is independent of the N-source, N-starvation and the degree of derepression of the nitrogenase in the cells.

Previously [13] we found that the transfer of *R. palustris* from gas phase of N_2 to one of argon was accompanied by a gradual increase in nitrogenase activity and gradual loss of switch-off. In *R. capsulata* AD 2, however, these effects were not observed. This may be due to the possibility that N_2 grown cells of *R. capsulata* AD 2 would be differently regulated at the genetical level than *R. palustris* or to inactivation of the nitrogenase which could be less stable than that in *R. palustris.*

In contrast to *R. rubrum* [6] and *R. capsulata* B 10 [16, 4], *R. capsulata* AD 2 cells grown with glutamate (10 mM) possess fully active nitrogenase *in vitro*, added Mn^{2+} had no stimulating effect, while the nitrogenase in extracts from nitrogen grown cells was largely inactive and siginificantly stimulated by Mn^{2+} (Fig. 2). The nitrogenase obtained from N-starved cultures of *R. capsulata* AD 2 and B 10 was always active and unaffected by the addition of Mn^{2+}.

Ludden (personal communication) proposed that the inactivation of nitrogenase from glutamate or nitrogen grown cells of *R. rubrum in vitro* occurred during the collection of the cells in the dark. Our results give an evidence that

1) the nitrogenase from *R. capsulata* AD 2 glutamate grown cultures was stable during the collection of the cells in the dark.

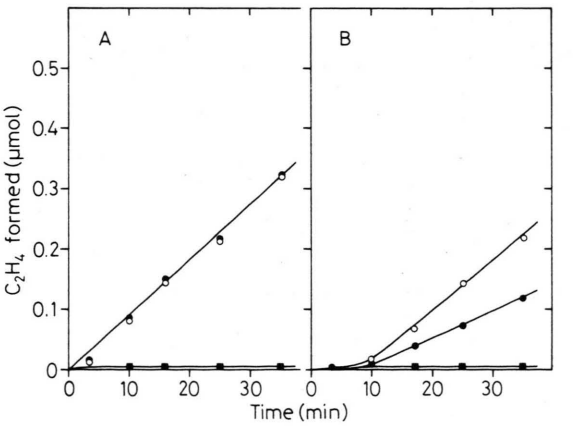

Fig. 2. Manganese requirement for nitrogenase of *R. capsulata* AD 2 in cell-free extracts, (○) 8 mM Mg^{2+} plus 0.4 Mn^{2+}, (●) 8 mM Mg^{2+}, (■) no addition. A) *R. capsulata* AD 2 grown with glutamate (10 mM). The reaction mixture contained 1.48 mg protein. B) *R. capsulata* AD 2 cells grown with nitrogen. The reaction mixture contained 8.9 mg protein.

2) the active form of nitrogenase is not necessary bound to N-starvation.

However the results obtained with cells grown with N_2 (Table I) indicate that the continued presence of N_2 seems to be important for the synthesis of the inactive form of the enzyme.

Gotto and Yoch [9] reported that upon addition of ammonium to N-starved cells of *R. rubrum*, the active form of nitrogenase was converted to the inactive form followed by a change in the subunit composition. Our data presented here (Table I) show that the enzyme in extracts of either glutamate grown or N-starved cultures of *R. capsulata* AD 2 was fully active, even if the cells had been treated with ammonium prior to harvest and had no *in vivo* activity. Furthermore when the enzyme was measured under activating conditions (added Mn^{2+}) or non activating conditions (no Mn^{2+}) no differences in activity was found (Fig. 2). Table I shows that the sensitivity of nitrogenase *in vivo* to inhibition by ammonia does not correlate with the enzyme activity *in vitro*. These observations differ from that found in *R. rubrum* by Gotto and Yoch [9].

Since the switch-off effect was completely independent of the two forms of the nitrogenase in *R. capsulata* AD 2, the biochemical mechanism of this phenomenon proposed by Ludden and Burris [1] cannot explain our observations.

Table I. Nitrogenase activity in *R. capsulata* AD 2 after *in vivo* treatment with ammonia.

Nitrogen source	Switch-off effect *in vivo*	Treatment *in vivo* with NH_4^+	Nitrogenase activity in cell-free extracts %
N_2	+	–	< 5
glutamate & N_2	+	–	< 5
N-starved	+	–	100 ± 5
		NH_4^+	105 ± 9
glutamate	+	–	80 ± 5
		NH_4^+	73 ± 6

+ = Nitrogenase activity was completely inhibited by ammonia; – = No treatment. For the ammonia treatment *in vivo* cultures were incubated with 4 mM ammonia for 30 min before harvesting. Within this time about 2 mM were assimilated. 10 nmole C_2H_4 $min^{-1} \cdot mg^{-1}$ protein = 100%.

Further studies are needed to provide insights into the mechanism of the short-term regulation of nitrogenase in this strains, specially a comparison of the amino acid pools in cells grown under different growth conditions, characterization of the dinitrogenase reductase and the activating system in cells grown with glutamate and under N-deficiency.

Acknowledgements

The author thanks D. Kleiner for helpful discussions. This work was supported by the Deutsche Forschungsgemeinschaft.

[1] P. W. Ludden and R. H. Burris, Proc. Natl. Acad. Sci. USA **76,** 6201–6205 (1979).
[2] S. Nordlund, U. Eriksson, and H. Baltscheffsky, Biochim. Biophys. Acta **462,** 187–195 (1977).
[3] D. C. Yoch, Biochem. J. **187,** 273–276 (1980).
[4] R. P. Carithers, D. C. Yoch, and D. I. Arnon, J. Bacteriol. **137,** 779–789 (1979).
[5] P. C. Hallenbeck, C. M. Meyer, and P. M. Vignais, J. Bacteriol. **149,** 708–717 (1982).
[6] P. W. Ludden, G. G. Preston, and T. E. Dowling, Biochem. J. **203,** 663–688 (1982).
[7] D. C. Yoch and M. Cantu, J. Bacteriol. **142,** 899–907 (1980).
[8] G. G. Preston and P. W. Ludden, Biochem. J. **205,** 489–494 (1982).
[9] J. W. Gotto and D. C. Yoch, J. Biol. Chem. **257,** 2868–2873 (1982).
[10] W. J. Sweet and R. H. Burris, Biochim. Biophys. Acta **680,** 17–21 (1982).
[11] D. C. Yoch and J. W. Gotto, J. Bacteriol. **151,** 800–806 (1982).
[12] K. Alef and D. Kleiner, Arch. Microbiol. **133,** 239–241 (1982).
[13] K. Alef, D. J. Arp, and W. G. Zumft, Arch. Microbiol. **130,** 138–142 (1981).
[14] J. Goa, Scand. J. Clin. Lab. Invest. **5,** 218–222 (1953).
[15] W. G. Zumft, D. J. Arp, and S. Neumann, Overproduction of nitrogenase in *Rhodopseudomonas palustris* under nitrogen limitation. In: Short Reports, 2nd European Bioenergetics Conference. EBEC Reports, **Vol. 2,** LBTM-CNRS ED., pp. 301–302 (1982).
[16] D. C. Yoch, J. Bacteriol. **140,** 987–995 (1979).