The Chemical Evolution of a Nitrogenase Model, XXIV. Correlational Analysis of Effects of Organic Acids on *in vitro* MoFe-Protein Substrate Reduction Activities

Gerhard N. Schrauzer and John G. Palmer

Department of Chemistry and Biochemistry, University of California, San Diego, Revelle College, La Jolla, CA 92093-0332

Reprint request to Prof. Schrauzer. Fax: 858 794 0212. E-mail: gschrauzer@ucsd.edu

Z. Naturforsch. **56b,** 1354–1359 (2001); received June 5, 2001

Nitrogenase, Organic Acids, Mechanism of Action

Among 29 organic acids whose effects on the *in vitro* enzymatic substrate reducing activity on the MoFe-protein of nitrogenase have been investigated, 12 acids stimulate the in vitro C_2H_2-reducing activities in proportion to the H^+-reducing activities. A second group comprised of eight acids significantly stimulates the H^+-reducing activity but has only modest stimulatory effects on C_2H_2- and N_2-reducing activities. A third group of nine acids causes only slight increases of MoFe-protein substrate reducing activities. The stimulatory effects of acids on MoFe-protein substrate reducing activity depend on their mode of interaction with molybdenum. Hydroxycarboxylic acids acting as bidentate ligands such as homocitric acid and its derivatives leave a sufficient number of molybdenum coordination sites available for interactions with the substrates, they have the highest stimulatory effects both on the C_2H_2- and N_2-reducing activities, and their H^+-reducing activities are not inhibited by CO. Acids acting as tridentate ligands, which include citric acid, have weaker stimulatory effects on the C_2H_2- and especially on the N_2-reducing activities, and CO inhibits their H^+-reducing activity. Whereas with the first group of acids the C_2H_2-reducing activities are linearly correlated with H^+-reducing activities, the N_2-reducing activities are directly correlated with H+-reducing activities *in the presence of CO*, and the association is exponential rather than linear. This exponential dependence is consistent with a stepwise mechanism of nitrogen reduction via diazene and hydrazine as the intermediates, the latter blocking one molybdenum coordination site prior to its reduction to NH_3. In the reduction of C_2H_2 to C_2H_4, no such blockage occurs as product C_2H_4 does not accumulate at the active site and is not reduced further.