

Schrader L E, Ritenour G L, Eilrich G L & Hageman R H. Some characteristics of nitrate reductase from higher plants. *Plant Physiol.* 43:930-40, 1968. [Dept. Agronomy, Univ. Illinois, Urbana, IL]

NADH and FMNH₂ were equally effective as electron donors for nitrate reductase from leaves of maize, spinach, and marrow, but the apparent K_m for FMNH₂ was 40- to 100-fold higher than that for NADH. We concluded that nitrate reductase is a single moiety with the ability to utilize either NADH or FMNH₂, but that *in vivo*, NADH is the electron donor. [The SCI® indicates that this paper has been cited in over 135 publications since 1968.]

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"As a graduate student in R.H. Hageman's laboratory, my thesis research concerned the induction and characterization of nitrate reductase from leaves of higher plants. Leonard Beevers *et al.*¹ reported in 1964 that nitrate reductase from 16 species of higher plants had a specific or preferential requirement for NADH rather than NADPH as cofactor. In 1965, Paneque *et al.*² reported that free FMNH₂ and FADH₂ are the natural cofactors for nitrate reduction in higher plants. In a companion paper,³ they stated that the enzymatic machinery needed for reduction of nitrate to ammonium is contained in the chloroplasts. These two reports were inconsistent with earlier reports from several laboratories as well as many observations in our labo-

ratory. For example, Gary Ritenour had just shown, using nonaqueous techniques for chloroplast isolation, that nitrite reductase, but not nitrate reductase, was inside the chloroplasts. Thus, photochemically reduced flavins seemed unlikely to be the natural electron donors for nitrate reduction. This controversy stimulated many discussions in the laboratory, and led to several experiments to resolve this controversy. Coauthors G.L. Eilrich and Ritenour became involved in conducting experiments, but several others including Beevers, K.W. Joy, R.L. Warner, and Lowell Klepper participated in lively discussions about this controversy.

"Several difficulties were encountered in proving that nitrate reductase is a single moiety capable of utilizing either NADH or FMNH₂. The enzyme was quite unstable, and we therefore worked 20-hour days to obtain and use a partially purified enzyme, as about 50 percent of the activity was lost when the enzyme was frozen or stored on ice overnight. In order to prove that the two activities were not additive, techniques were developed for reducing the FMN with H₂ rather than dithionite, as NADH plus dithionite interfered with the assay. The oxidation of both NADH and FMNH₂ was then monitored in a spectrophotometer.

"This paper has been widely cited because it resolved a controversy about the electron donor for nitrate reductase, several techniques were developed (e.g., purification of nitrate reductase and NADP- and NAD-reductases, optimization of the FMNH₂-nitrate reductase assay, and determination of the half-life of nitrate reductase), and a model for nitrate reductase was presented. Beevers and Hageman⁴ discussed this topic in a recent review."

1. Beevers L, Plaster D & Hageman R H. Studies on the pyridine nucleotide specificity of nitrate reductase in higher plants and its relationship to methylglutaryl level. *Biochim. Biophys. Acta* 89:453-64, 1964.
2. Paneque A, Del Campo F F, Romero I M & Landa M. Flavin nucleotide nitrate reductase from spinach. *Biochim. Biophys. Acta* 109:79-85, 1965.
3. Landa M, Romero I M, Paneque A & Del Campo F F. Light and dark reduction of nitrate in a reconstituted chloroplast system. *Biochim. Biophys. Acta* 109:86-96, 1965.
4. Beevers L & Hageman R H. Nitrate and nitrite reduction. (Miflin B J, ed.) *The biochemistry of plants: a comprehensive treatise. Volume 5. Amino acids and derivatives.* New York: Academic Press, 1980. p. 115-66.