"Reagent Grade" Enzymes for Nitrate Determination

Ellen R. Campbell
NECi

Enzymes are proteins that function as catalysts. They tend to be large and complex molecules, often with attached "cofactors" that facilitate catalytic function. Some B vitamins are enzyme cofactors; others are metal complexes such as hemoglobin or chlorophyll. Enzymes are named and grouped by catalytic function. There may be hundreds of variations of an enzyme, differing by minor changes in amino acid sequence. These variations affect the stability, efficiency, or ability to function in the presence of specific inhibitors.

The Nitrate Reductase, or NaR, suited to nitrate analysis is found in plants, yeasts, and some fungi. These NaR forms are free within cells (not membrane bound) and function in the presence of air. These forms of NaR reduce nitrate to nitrite, using the biomolecule NADH (reduced β-Nicotinamide adenine dinucleotide) as the electron donor. NECi's founders have been working with plant nitrate reductase for over 30 years and were the first to purify the enzyme to homogeneity (Hyde et al 1989). This "native" NaR purified from corn seedlings by immunoaffinity chromatography was NECi's initial product; it was sold to biomedical research product companies for NO test kits and was the form used in Dr Charles Patton's work at USGS on enzyme-based nitrate determination (Patton et al 2002).

As demand for NaR increased, efforts to develop recombinant forms became a company focus. Recombinant protein technology enables tight quality control. Once a gene has been designed and inserted into a suitable protein production system, the gene does not change, or mutate, over time. The enzyme is produced by fermentation using reagent grade water and analytical or molecular biology grade salts and sugars. Temperature, agitation, oxygen level, pH and rate of carbon source feeding are controlled. The recombinant gene has a purification "handle", or tag, built in, allowing for one step purification using commercially available affinity chromatography gels. Recombinant enzymes truly are "reagent grade". NECi produces two forms of recombinant NaR: YNaR is suited for low temperature applications. AtNaR is the form used for EPA and ASTM round robin validation studies.