Application of Poly (Ethylene Glycol)-Bound NAD in Model Enzyme Reactor

Itaru Urabe
Department of Fermentation Technology, Osaka University

Many enzymes require the participation of readily dissociable coenzymes as NAD for thir catalytic activities. The continuous utilization of the enzymes requires the retention and regeneration of the coenzymes. For this purpose, several kinds of macromolecular NAD derivatives have been prepared by covalently attaching NAD to watersoluble polymers. We have prepared poly (ethylene glycol)-bound NAD (PEG-NAD) by coupling N^6 -(2-carboxyethyl)-NAD to one terminal of γ ω -diaminoly (ethylene glycol) (Mr 3000) with water-soluble carbodiimide. PED-NAD thus obtained has one NAD moiety located at a terminal of the linear, flexible and hydrophilic chain of poly (ethylene glycol). PED-NAD has good coenzyme activity for various dehydrogenases and is applicable in a continuous enzyme reactor.

To use these macromolecular NAD derivatives in an enzyme reactor, it si necessary to understand the behavior of the system in which the reactions of dehydrogenases are coupled by the recycling of the NAD derivative. We investigated the kinetic properties of a continuous enzyme reactor containing lactate dehydrogenase, alcohol dehydrogenase and PEG-NAD. The steady-state behavior of the enzyme reactor is explained by a simple kinetic model.

As for a reaction using NAD(P)H, the efficient regeneration of NAD(P)H is important, because NAD(P)H has higher free energy than NAD(P). Glucose dehydrogenase (GDH) from *Bacillus megaterium* is a tetrameric enzyme that catalyzes the oxidation of D-glucose to Dglucono-1,5-lactone, which is spontaneously hydrolyzed to gluconic acid, using NAD or NADP as coenzyme. As the equiliblium of the overall reaction lies in much favor of NAD(P)H formation, this enzyme is useful as an NAD(P)H regenerator in enzymer reactors. GDH, however, shows very low activity for PEG-NAD(P): reducation rate of PEG-NAD is only 0.08% of that of NAD. We prepared a covalently linked GDH-PEG-NAD conjugate. GDH-PEG-NAD thus obtained has much higher reaction rate than GDH plus PEG-NAD: the ratio of the reaction rates of GDH-PEG-NAD and GDH+PEG-NAD is 10000-fold at the concentrations of GDH subunit and NAD moiety of 0.31 and 0.65 μM, respectively; the rate of the conjugate is even higher than that of GDH+native NAD. The NAD moety of GDH-PEG-NAD has similar coenzyme activity to PEG-NAD for other dehydrogenases, and is efficiently recycled by coupled reactions of the active site of GDH-PEG-NAD and other dehydrogenases. Therefore, GDH–PEG-NAD is a good NADH-regeneration unit for enzyme reactors.

Application of Biotechnology to Wastewater Treatments in Japan

Tadahiro Mori, D. Sc.
Professor, Biochemical Engineering
Laboratory.
Faculty of Agriculture
Shimane University

The biotechnology including genetic engineering is expected to be applied to various fields of wastewater treatments in order to promote biological reaction rate, to grade up the effluent quality and to advance the stability of microorganisms against temperature, pH and toxic substances.

The current topics in Japan on application of biotechnology to wastewater treatment will be reviewed at the beginning of the presentation.