A Combined Electrochemical Process for the Regeneration of Co-enzymes

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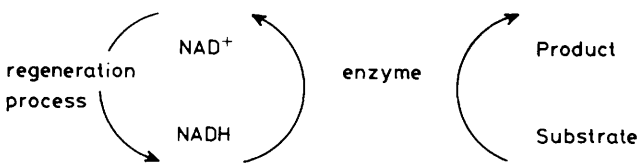
Cyclic regeneration of the co-enzyme nicotinamide adenine dinucleotide couple NAD^+/NADH has been achieved by reduction of NAD^+ using electrochemically produced potassium amalgam in a specially designed reactor, and by electrochemically oxidising NADH and biochemically inactive species from the reduction reaction back to active NAD^+ *via* a nickel foam anode; this regenerated co-enzyme can be used to drive a range of chemical reactions catalysed by oxido–reductase enzymes.

It has been an objective for some time to develop a process in which co-enzymes are cyclically regenerated and used to drive organic reactions catalysed by enzymes (*e.g.* Scheme 1). The theoretical scope of such a process is limited only by the range of enzymes available.[1]

Several methods, both electrochemical and non-electrochemical, have been explored towards this regeneration goal.[2] The electrochemical oxidation of NADH to NAD^+ has been extensively studied and accomplished with remarkable success.[3]

However, a major problem exists in the electrochemical reduction of NAD^+ to NADH whereby the adsorption of a layer of NAD^+ molecules on a static electrode surface leads, preferentially, to the dimerisation of adjacent incipient radicals formed by initial electron transfer, to produce a biochemically inactive species.

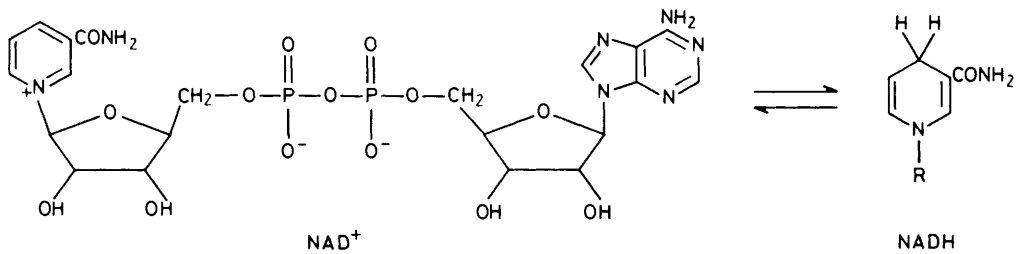


Scheme 1

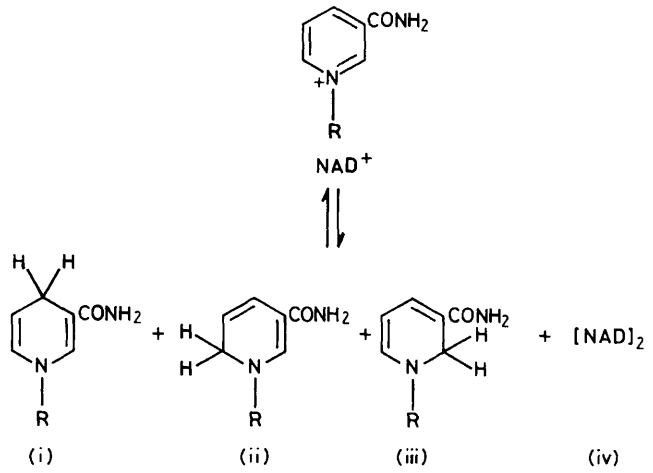
Here we make a preliminary report on the dynamic techniques developed in our laboratories designed to circumvent this problem. These have led to a significant improvement in control over the kinetics of the reduction reaction by presenting a constantly renewable interface between the reducing surface and the organic compound. We believe this has opened up the possibility of an electrochemical route of considerable importance.

NAD^+ was reduced to NADH in aqueous solution (1.0% w/w NAD^+; 0.5 M $K_4P_2O_7$ buffer solution, pH 10) by injecting the aqueous solution into the base of a column of electrochemically regenerated potassium amalgam (10 M KOH soln; 1500 mA) immediately below a high speed stirrer to ensure intimate contact. Proper regulation of potassium concentration, pH, column depth, stirrer speed, and contact times controlled the rate of NAD^+ reduction, and the amount of damage incurred.

In typical single pass experiments, complete conversion of NAD^+ was achieved with no detectable damage to the co-enzyme. However, when biochemically assayed, the reduced co-enzyme proved to be only 60—70% active. The reason for this was shown to be the presence of species other



NAD^+ NADH



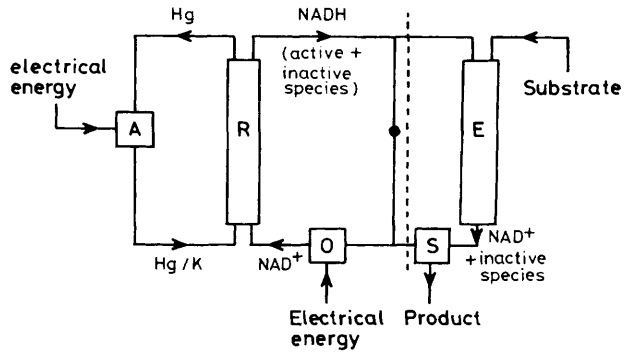
Scheme 2. (i) 4-dihydro, λ_{max} 340 nm (70%); (ii) 6-dihydro, λ_{max} 341 nm (27%); (iii) 2-dihydro, λ_{max} 345 nm (1%); (iv) mixed dimers (2%).

than the desired 4-dihydro-isomer of nicotinamide adenine dinucleotide (*e.g.* Scheme 2).

Obviously, a loss in activity of *ca.* 30% per pass would very quickly render a cyclic system totally biochemically inactive. A method of regaining the 'lost' activity needed to be found.

It has now been shown to be possible to oxidise these biochemically inactive species back to active NAD^+, in an electrochemical cell, equipped with alternating nickel mesh cathodes and high surface area nickel foam anodes. Proper control over pH, flow rates, and electrochemical parameters within this cell were necessary to minimise damage to the delicate co-enzyme structure. Thus, by combining both these reduction and oxidation techniques it was possible to establish the cyclic process illustrated in Scheme 3.

In just such an experimental arrangement, a typical bench scale operation was capable of processing 60 g of co-enzyme per hour, with a damage rate of *ca.* 0.4% min^{-1}, *i.e.* 99.6% of the co-enzyme being processed per minute was successfully



Scheme 3. A, Amalgam generator; R, reducer; O, oxidiser; S, separator; E, enzyme reactor.

passing through the regeneration cycle. Further, in model experiments, an enzyme reactor, packed with immobilised alcohol dehydrogenase, installed in the circuit (as shown to the right of the dotted lines) demonstrated that the co-enzyme electrochemically regenerated in this manner successfully reduced acetaldehyde to ethanol.[4]

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