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Nitrogen Heterocyclic Compounds: Electrochemical Information Concerning Energetics, Dynamics and Mechanisms *

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The information is summarized, which is currently available from the use of electrochemical approaches (primarily based on polarographic and voltammetric technics) to investigate the behavior in aqueous and non-aqueous media of biologically important compounds, where such approaches are advantageous for the study of certain aspects of the energetics and pathways involved in redox reactions, the reaction dynamics for charge-transfer and accompanying chemical reactions, and adsorption phenomena. Emphasis has been placed on three classes of nitrogen heterocycles (pyridines as nicotinamides, pyrimidines, and purines), whose sugar-phosphate derivatives (nucleosides, nucleotides, and polymeric nucleotides) are of major importance as nucleic acid components, coenzymes, and energy-transfer intermediates.

The specific compounds examined were selected on the dual basis of:

a. investigating in detail the fundamental processes in the parent heterocycles, which are the sites of oxidation and reduction,

b. investigating sequences of compounds in order to determine the basis for transferring information from simple to more complicated structures. Since the electrochemical behavior of the latter complex species is largely determined by that of the constituent bases, detailed knowledge of the behavior of the monomeric and dimeric units should facilitate meaningful evaluation and interpretation of results obtained with polymers and should, in turn, allow estimation of the effects of polymer secondary structure and sugar and sugar-phosphate moieties on the behavior of the parent bases, including nature of the species in solution and on adsorption. The study of adsorption phenomena is particularly relevant since many biological reactions in the living cell involve adsorption at charged boundaries such as membrane or ribosome surfaces.

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The entire text of the lecture, which is summarized here, will be published in the first volume of the series *Topics in Bioelectrochemistry and Bioenergetics* (G. MILAZZO, Editor; John Wiley and Sons, Publisher), which is scheduled for publication in July, 1976.

There is presented a *state of the art* report in the behavior of the compounds in solution and at a solution|electrode interface. Solution behavior includes :

a. the *shape* of the compounds as determined by their structure, conformation, and association including stacking,
b. intra– and intermolecular association,
c. orientation of the compound as it approaches the charged interface.

Behavior at the interface includes, *inter alia* :

α. adsorption of reactant, intermediate and product species,
β. association in the adsorbed state,
γ. mechanisms and kinetics of electron–transfer (redox) processes,
δ. chemical reactions (kinetics and mechanisms) involving reactant, intermediates (*e.g.*, free radical and carbanion) and product species, which precede, are concurrent with and follow electron–transfer.

Of particular concern are satisfactory descriptions of :

a. the kinetics of the individual electron–transfer and accompanying chemical steps composing the overall redox processes, and the exact sequence of these individual steps in the latter process,
b. the effects on the kinetics and energetics of these steps of orientation of the compound at the electron–transfer interface, and of its adsorption and that of other species in the redox process.

The discussion is based primarily on the investigations of the author and his collaborators. The principal reason for this is the fact that whole sequences of related compounds were examined in some detail under compatible conditions in aqueous and non–aqueous media, using a diversity of experimental approaches including perturbation technics such as cyclic voltammetry and *a.c.* polarography and emphasizing the chemical and spectrophotometric behavior of reactant, intermediate and product species. A few examples are given of the information obtained.

The initial one–electron (1 e^-) reduction characteristic of the nicotinamide, pyrimidine and purine systems in aqueous and non–aqueous media is inherently reversible, *e.g.*, as indicated by the appearance of complementary redox couples on cyclic voltammetry, and proceeds with a $k_{s,h}$ exceeding 0.1 to 1 cm s^{-1}. This allows the application of various approaches for measuring the rates of rapid chemical processes associated with the electron–transfer process. Thus, rate constants and, frequently, energies of activation and frequency factors were determined for many of the chemical reactions encountered, *e.g.*, radical dimerization, radical anion and carbanion protonation, and deamination.

Valid ways for correlating electrochemical behavior with structure and reactivity, particularly in respect to the initial energy–controlling 1 e^- step and the properties of the anion radical generated, were explored in a study of the reduction in acetonitrile of five azabenzenes (the monoazine (pyridine), the three diazines, and the symmetrical triazine; pyrimidine is 1,3–diazine).

The redox pattern for 1–substituted 3–nicotinamides in non–aqueous media involves initial 1 e^- addition to form a neutral free radical, whose further reduction requires proton participation ; nicotinamide itself un-

dergoes two successive 1 e^- additions to radical anion and dianion. In the presence of proton donors, the wave patterns are similar to those seen in aqueous media.

In aqueous media, the artifacts introduced into the observed electrochemical patterns, *e.g.*, for NAD^+, by coenzyme and reduction product adsorption at the solution|electrode interface can be removed by addition of a more strongly adsorbed species. An initial reversible 1 e^- addition to the pyridinium ring produces a free radical, which dimerizes. At more negative potential, the radical is reduced (1 e^-, 1 H^+) to a dihydropyridine, which is 1,4–NADH in the case of NAD^+ ; some 1,6–NADH is also formed, indicating the lesser specificity of electrochemical reduction as compared with enzymatic. At sufficiently positive potential, dimer and dihydropyridine are oxidized to NAD^+ ; the dimer is much more easily oxidized. The dimer is not directly reduced electrochemically within the available potential range. Both reduction products hydrolyze ; the rate increases with decreasing pH ; at a given pH, dimer is less stable than dihydropyridine.

An introductory study of the electrochemical oxidation of 1,4–dihydropyridines (NMNH, NADH and NADPH) showed that these compounds in both aqueous solution at carbon electrodes and DMSO solution at platinum electrodes undergo 2 e^- oxidation to generate the parent pyridine nucleotides, which, in turn, are reduced at considerably more negative potential in 1 e^- processes to free radicals which dimerize. Protonation of the dihydropyridines by protons liberated at the interface during their oxidation, or available from other solution species, leads to their decomposition, producing species which further decompose ; rate constants were determined.

In acetonitrile, pyrimidine is initially reduced in a reversible 1 e^- process to a radical anion, which is deactivated *via* competitive pathways: rapid dimerization and proton abstraction followed by further 1 e^- reduction. In a related study designed to clarify certain aspects of the electrochemical reduction of purines, *e.g.*, behavior of the initially produced radical anions and their dimers, and the effects of addition of weak and strong proton donors and of substitution, a series of 6–substituted purines (including purine and adenine) was investigated in non–aqueous media.

Such studies have resulted in understanding the basic pyrimidine redox pattern in aqueous media in terms of reduction of the 3,4 and 1,2 N=C bond systems and the variations introduced by substituents on the ring including imidazole fusion to form purines. For example, the similarities and differences seen in the polarographic behavior of a fundamental cytosine–based nucleoside–nucleotide series (cytosine, cytidine, cytidine monophosphate, and selected cytosine dinucleoside monophosphates) in aqueous solution could be considered in terms of the effect of structure on kinetics of intervening chemical reactions, association in solution, and adsorption at the interface and association in the adsorbed state. It was then possible to enlarge the systematic investigation of nucleic acid components by initiating a study of interrelated series of

adenine and cytosine–based dinucleoside monophosphates (which included guanine and uracil derivatives), and of adenine oligonucleotides in aqueous media, in which emphasis was placed on the use of electrochemical measurements as a means of correlating the component steps and features in the reduction mechanisms in terms of secondary structure.

The systematic approach indicated should eventually permit a more detailed understanding of how some biological processes such as coenzyme action proceed, a more meaningful interpretation of the electrochemical data being obtained on large biological species such as nucleic acids, and a clearer definition of the aspects of biological phenomena concerning which electrochemical approaches can yield useful information.