The interactions mentioned can not so far be expressed in quantitative ways nor can their contributions to equilibrium constants of macromolecular complex formation be written explicitly, therefore the way of describing polymeric complexing systems is through the use of averaged values.

Various examples of the interactions of metal ions with macromolecular chains of DNA and RNA, pH-dependence, conformational changeability and reversibility, as well as the possibility of modelling the macromolecules under study are given.

---

## T19

## A Rapid Kinetic Study of Divalent Metal Interactions with Flavin Coenzymes

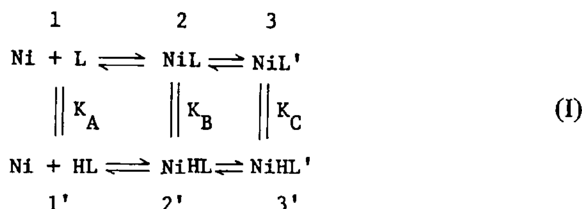
JOHN STUEHR

*Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106, U.S.A.*

Research in these laboratories has focussed in recent years on the kinetics of divalent metal ion interactions with coenzymes. The principal kinetic tool has been temperature-jump relaxation spectroscopy. A large amount of kinetic information is now available for several nucleotides (*e.g.* AMP) and inorganic phosphates.

The purpose of this paper is to report the first rapid kinetic study of the mechanism of divalent metal ion interactions with the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The two compounds are structurally related to each other as well as to other coenzymes and phosphates that we have previously studied. FAD, for example, is structurally a combination of riboflavin phosphate and adenosine monophosphate (AMP). Ni(II) was chosen as the metal ion for these studies because of the large body of kinetic information that is already available for that ion. It can serve as a useful representative of divalent transition metal ion interactions with these and related coenzymes.

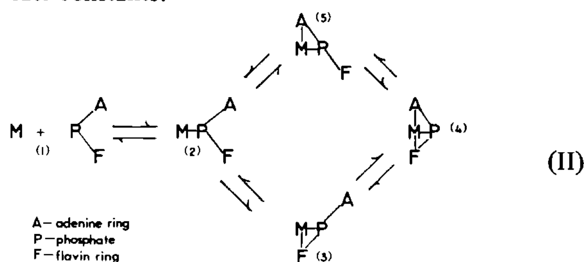
*The Ni–FMN system.* Two relaxation effects were observed in the kinetic experiments: one ($\tau_1$) on the order of 0.2 msec, the other ($\tau_2$) at about 2 msec. The detailed concentration and pH dependencies of $\tau_1$ and $\tau_2$ are quite similar to those for the relaxation times found in the Ni-ribose phosphate and Ni–AMP systems respectively. The mechanism consistent with these observations is a dual-pathway, back-bound complex mechanism, shown schematically as I:




(I)

in which NiL is the phosphate-bound complex, NiL′ the phosphate + base bound complex.

*The Ni–FAD System.* FAD presents a number of different binding sites in the ionized phosphate bridge and the base nitrogens on both the adenine and isoalloxazine rings. The flexibility of this molecule facilitiates both individual and simultaneous ring interactions with the phosphate-bound metal. The Ni–FAD system is unique in that *four distinct* relaxation times, $\tau_1$–$\tau_4$, were found and characterized. The relaxation times ranged from 90 μsec to 20 msec and were found to be only slightly pH and concentration dependent. Based on the large body of prior data from our laboratory on simple nucleotide systems, we were able to associate specific relaxation times with reaction steps in scheme II and to determine the rate constants.




(II)

The mechanism shown as II quantitatively accounts for the number and behavior of all the relaxation steps.

In this scheme, F, P and A refer to the flavin, phosphate, and adenine moieties of the FAD molecule, respectively. The first step (1–2) involves bridging to the phosphate moiety only, followed by species involving interactions with the phosphate plus the flavin (5) or adenine moieties (3). The final complex (4) involves simultaneous interactions with all the components of the molecule.

---

## T20

## The Copper(II) Promoted Hydrolysis of Salicyl Phosphate (2-Carboxyphenyl Dihydrogen Phosphate)

ROBERT W. HAY and ARUP K. BASAK

*Chemistry Department, University of Stirling, Stirling FK9 4LA, U.K.*

One of the striking observations of biological phosphate chemistry is that much of it appears to be