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ORIGINAL ARTICLE

**Luis A. Herrero · José Carlos Cerro-Garrido**
**María Cristina Apella · Angel Terrón-Homar**

# Interactions in solution of cobalt(II) and nickel(II) with nicotinamide adenine dinucleotide: a potentiometric and calorimetric study

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**Abstract** The equilibrium constants and the thermodynamic parameters enthalpy and entropy of the interaction between Ni(II) and Co(II) with $NAD^+$ in aqueous solution were determined by calorimetry and potentiometry methods (ionic strength adjusted to 0.1 with sodium nitrate at 25 °C). The macrochelation of the systems was also studied. All the data, including the protonation enthalpy data of $NAD^+$ (very similar to the protonation enthalpy of 5′-AMP) suggest a less restrictive model for the $NAD^+$ ''folded'' conformation without intramolecular stacking between the bases, in agreement both with recent theoretical calculations and with the X-ray structure of trimethylene-bisadeninium or the free acid form of $NAD^+$.

**Keywords** Cobalt · Nickel · Nucleotides · Nicotinamide adenine dinucleotide calorimetry

## Introduction

Nicotinamide adenine dinucleotide ($NAD^+$) acts as a coenzyme in proteins that are ubiquitously involved in redox processes [1], for instance liver alcohol dehydrogenase where Zn(II) is present at the catalytic site [2]. The fact that the metal ion may not be required for coenzyme binding does not preclude a strong interaction between these two cofactors [3]. Recent molecular dynamics simulation has shown that the predominant form of $NAD^+$ in water solution is a folded conformer characterized by a nicotinamide-adenine inter-ring distance of 5.2 Å and an angle of 148° between the aromatic ring planes [4]. In contrast, $NAD^+$ bound to enzymes adopts extended conformations in which the nicotinamide and adenine rings are separated by 12 Å [5]. Furthermore, the simulation predicted an analogous extended conformation in chloroform [5].

Extended conformation is also shown by X-ray diffraction in the structure of the Li-$NAD^+$ complex, where the metal is bound to N(7) of the adenine ring and to an oxygen atom of the pyrophosphate group [6, 7]. Crystal structures of enzyme-bound $NAD^+$ suggest that the unfolding of the dinucleotide is required for catalysis [8]. The X-ray structure of the free acid form of $NAD^+$ showed a structure with no intramolecular stacking between the bases, the distance between the two positively charged rings being 9.68 Å [9].

The information on M(II)-$NAD^+$ complexes in solution is limited ([3, 10] and references therein). The results of kinetic studies by Bidwell and Stuehr [3] for the system Ni(II)-$NAD^+$ were found to be consistent with a mechanism involving sequential formation of several different 1:1 metal ion complexes, including those in which the metal ion simultaneously coordinates the ring systems and phosphate groups.

Our research team has previously published a series of potentiometric and calorimetric studies about the interactions between divalent metal ions [Ca(II), Mg(II), Co(II), Ni(II) or Cu(II)] and mononucleotides (including 2′-, 3′- or 5′-AMP, 5′-GMP, 5′-IMP, 5′-CMP and 5′-UMP); equilibrium constants, enthalpies and entropies were determined [11, 12, 13, 14, 15]. Following these studies we have determined, by potentiometry and calorimetry, the thermodynamic magnitudes for the interactions of divalent metal ions [Co(II) or Ni(II)] and the dinucleotide $NAD^+$.

## Materials and methods

$NAD^+$ (β-nicotinamide adenine dinucleotide, sodium salt) (Fig. 1) was obtained from Sigma. Its aqueous solutions were freshly prepared by dissolving the required amount of the solid in water. The

L.A. Herrero · J.C. Cerro-Garrido · A. Terrón-Homar (✉)
Departament de Química, Facultat de Ciències,
Universitat de les Illes Balears,
07071 Palma de Mallorca, Spain
E-mail: dquath0@clust.uib.es

M.C. Apella
Centro de Referencia para Lactobacilos (CERELA-CONICET) and Facultad de Ciencias Naturales de la Universidad Nacional de Tucumán, Chabuco 145, 4000 S.M. de Tucumán, Argentina