NUCLEAR MAGNETIC RESONANCE EVIDENCE FOR A Zn_2–ATP COMPLEX

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1. Introduction

It has been suggested on the basis of kinetic studies that enzymes may be inhibited at a high Mg: ATP ratio because a Mg_2ATP complex forms [1, 2]. It seems unlikely that enzymatic reactions would be affected by small changes in concentration of metal ion or nucleotide if only the 1:1 complex were formed. The stability constants for such complexes vary between 30000–100000 M^{-1} and a fairly large concentration change would be needed to affect the system. If, on the other hand, M_2L complexes are formed and are inhibitory, then an enzymatic reaction might be greatly affected by a relatively small change in concentration of metal ion or nucleotide. Previous reports involving the use of potentiometric [3, 4] and spectrophotometric [5] techniques have described the formation of a 2:1 metal–ATP complex involving Ca^{2+}, Mg^{2+}, and Ni^{2+}, respectively. None of these complexes nor any other 2:1 metal–ATP complex, has been examined by nuclear magnetic resonance (NMR) although this technique has been very useful with the 1:1 complex in showing that, in the case of Ni^{2+}, Co^{2+}, and Mn^{2+}, the metal ion interacts with the adenine ring of ATP at the N-7 position and that the interaction is via a bridging water molecule in an outer sphere complex [6, 7]. These paramagnetic metal ions have a very large effect on the NMR spectrum of ATP and at ratios of metal ion to ATP close to one there is so much broadening of the peaks that specific signals can no longer be identified. When the diamagnetic ions Ca^{2+} and Mg^{2+} were tested there was no detectable effect on either the proton or ^{15}N NMR spectra of ATP [8, 9]. Recently, however, Kuntz and Swift [10] studied the T_1 relaxation rates of the base protons and were able to demonstrate an effect of Mg^{2+} on the H-2 proton of ATP. The problem with using NMR to establish the existence of a 2:1 metal ion–ATP complex therefore is that the effect of the paramagnetic ions is too strong while that of the diamagnetic ions Mg^{2+} or Ca^{2+} are either weak or cannot be demonstrated. A more favorable situation exists however with the diamagnetic Zn^{2+} ion. In this case there is a strong interaction at the N-7 position of ATP resulting in a downfiled *shift* of the H-8 peak and an upfield *shift* of the H-2 and H-1′ peaks [8]. Since the effect of the Zn^{2+} on the NMR spectrum of ATP is predominantly on shift and only very slightly on line width it should be possible to obtain spectra at Zn^{2+}: ATP ratios greater than one. We have done this and obtained NMR evidence to demonstrate the existence of a 2:1 Zn^{2+}–ATP complex.

2. Materials and methods

Solutions in D_2O of 4.5×10^{-3} M ATP containing varying amounts of $Zn(NO_3)_2$ were prepared as previously described [6]. The final pH was 5.5 ± 0.2 Measurements were made with a Varian XL-100 spectrometer with tetramethylammonium ion as an internal standard.

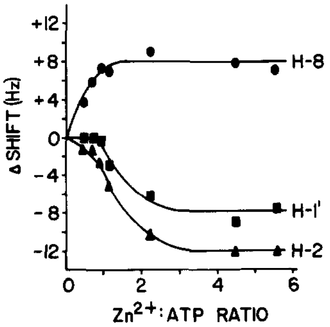

Fig. 1.

3. Results and discussion

Fig. 1 shows the relationship between the ratio of Zn^{2+}: ATP to the change in chemical shift of the H-8, H-2, and H-1′ protons of ATP. The change in chemical shift for H-8 is linear up to a ratio of approximately one, following which it seems to plateau. In contrast, the change in chemical shift of H-2 and H-1′ is only about 25% completed at a ratio of one and does not reach a plateau until the ratio exceeds 2. It is especially noteworthy that there is no change in shift for the H-1′ proton until the Zn^{2+}: ATP ratio reaches 1. This experiment is consistent with the conclusion that: (a) at ratios of Zn^{2+}: ATP up to one a 1:1 complex is formed with the metal ion interacting predominantly at the N-7 position and primarily affecting the chemical shift of H-8; (b) as the ratio of Zn^{2+}: ATP is increased above one a 2:1 complex is formed and the second metal ion interacts with or is near N-1 and/or N-3 leading to an effect on H-2 and H-1′. This data provides the first direct evidence that both metal ions in a M_2ATP complex are reacting with the adenine ring. This same conclusion was also reached indirectly in the case of the Ni_2ATP complex [5].

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