Interactions Between Polyamines and Nucleotides
Studied by $^{31}$P and $^1$H NMR

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Abstract. The formation of complexes between poly- and mono-amines and some polyphosphate molecules such as ATP, ADP and tripolyphosphate was studied by $^{31}$P and $^1$H NMR. Evidence for formation of complexes was obtained by $^1$H NMR, while the values of dissociation constants of the complexes between nucleotides and spermine and spermidine, calculated from the shift of the $^{31}$P NMR, were in mM range.

1. Introduction

It has long been recognized that polycations have a great influence on conformation of polynucleotides. In fact, electrical fields of various intensities produced by positive charge bearing molecules seem to be responsible of structural changes of backbone conformation, since they act on the $-PO_4$ group orientation [1]. Among the cell components bearing positive charge, particular attention has been devoted to polyamines, because these compounds have been implicated in cell growth and proliferation [2, 3] and are known to stabilize the conformation of DNA through their binding, by electrostatic interaction, to the negatively charged larger groove of double helix [4].

The removal of polyamines in biological systems is regulated mainly by a copper-containing enzyme (amine oxidase), which catalyzes the oxidation of the amino group to aldehyde, decreasing the positive charge of these polycations [5]. Recently, it has been observed that some polyamines are substrates of amine oxidases also when bound to various polyphosphates, such as ADP or ATP [6], thus supporting an involvement of these enzymes in the control of DNA or RNA stabilization. However, in spite of the large interest devoted to the polyamine-polynucleotide interaction, only scattered quantitative values of the equilibrium constant for model complexes are reported [7–11].
In this paper we investigated the formation of complexes between poly- and monoamines and some phosphate containing molecules. Evidence for complexes formation was obtained by $^1$H NMR, while quantitative data on the affinity of the various phosphate-containing molecules for polyamines were obtained from the shift of the $^{31}$P NMR.

2. Materials and Methods

All chemicals were analytical grade and have been used without further purification. NMR experiments were carried out on a Bruker MSL 300 spectrometer, equipped with a variable temperature unit, operating at 300 and 121 MHz for $^1$H and $^{31}$P respectively.

To acquire fully relaxed $^{31}$P NMR spectra, the pulse angle was 60° and the pulse repetition time 12 s. Phosphoric acid in an external co-axial capillary was used as chemical shift reference.

$^1$H NMR spectra were obtained by 90° pulse angle and 8 s pulse repetition time; 1 mM 3,(trimethylsilyl)3,3,2,2-tetadeuteropropionate was used as internal standard. To avoid shifts of the resonances due to variation of pH [12], the pH of the solutions was carefully controlled before and after each experiment.

All the measurements were performed at 27°C in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 7. Unless otherwise stated, the ionic strength was adjusted to 90 mM, by suitable addition of NaCl.

A fitting program written in Basic, was used for the calculations, which were performed on an IBM PC/486.

3. Results and Discussion

ATP, ADP and tripolyphosphate (TPP) were used as model compounds for polyphosphates. $^{31}$P NMR spectra of the phosphate-containing compounds, were acquired under the conditions described in Section 2, in the presence of spermine (SPM), spermidine (SPD), benzylamine (BZA) and propylamine (PRA). The NMR spectra showed that the chemical shift of $^{31}$P nuclei is affected by the presence of a polyamine such as SPM or SPD, while it is insensitive to the presence of BZA or PRA which bear one positive charge. This result suggests an interaction between positive sites on polyamines and phosphate groups, which bear one or more negative charges at pH 7.

In Table 1 we reported the shift of the $^{31}$P resonance measured in the presence of spermine or spermidine, at [amine] $\gg$ [polyphosphate], that is when all the polyphosphate should be complexed by the polyamine. In the same Table we reported the shift of protons of the adenine ring of ATP (8H and 2H) induced either by SPM or SPD, as observed by acquiring $^1$H NMR spectra. The shift of these protons