^{1}H and ^{31}P NMR study of ADP and GDP spontaneous synthesis from 2-methylimidazole-activated AMP and GMP nucleotides and inorganic phosphate

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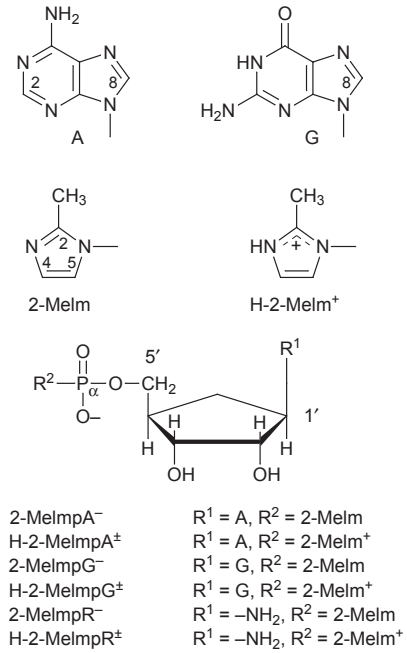
^{1}H and ^{31}P NMR data show that adenosine 5′-(2-methylimidazol-1-ylphosphonate) (2-MeImpA) and guanosine 5′-(2-methylimidazol-1-ylphosphonate) (2-MeImpG), known as possible prebiotic precursors of polynucleotides, produce the corresponding diphosphonucleotides in sodium phosphate solution at pD 7.6. Phosphate ions also enhance the hydrolysis of these molecules by activating a water molecular associated as a weak complex with the phosphoryl moiety of 2-MeImpA and 2-MeImpG. A kinetic study was done by quantitative ^{1}H NMR spectroscopy and mechanistic hypotheses were tested by semiempirical PM3 modelling.

Introduction

Imidazole-activated nucleotides can be formed in simulated prebiotic conditions and have been used as precursors for *in vitro* synthesis of various kinds of oligoribonucleotides[1–4] and oligodeoxyribonucleotides[5] using as templates pre-existent polynucleotides. Other lines of work study the effectiveness of inorganic catalysts or templates, more likely to be present in prebiotic conditions, like metal ions[6] or montmorillonite clays and other minerals.[7] Detailed mechanistic and kinetic analysis of nucleotide imidazolide hydrolysis and polymerisation is a fundamental step to understand the action of these particularly reactive oligonucleotide precursors. The general reaction of polymerisation occurs through a nucleophilic substitution on the phosphoryl moiety of the activated nucleotide by another nucleotide hydroxy group, imidazole being the leaving group. The Mg^{II} ion has an important role in the process,[8] further activating the phosphoryl moiety. Depending on the conditions (templates, catalysts, pH, temperature) several kinds and lengths of oligonucleotides may result, even dimers like NppN (pyrophosphoryl bridge between two nucleotides). The activated phosphoryl group is in fact sensitive to any nucleophile, suggesting a method for the prebiotic synthesis of nucleotide polyphosphates by reaction with inorganic phosphate or pyrophosphate in solution. In this work, the effect of phosphate concentration on 2-methylimidazole-activated nucleotides was studied in aqueous systems containing sodium phosphate and adenosine 5′-(2-methylimidazol-1-ylphosphonate) (2-MeImpA) or guanosine 5′(2-methylimidazol-1-ylphosphonate) (2-MeImpG) (Scheme 1).†

NMR was chosen as the qualitative and quantitative analytical technique in this study as an alternative to the commonly used HPLC because it provides an equally accurate and more straightforward experimental method. It also has the advantage of providing structural insight into the reaction products. The pD value of 7.6 was chosen for the experiments because it is low enough to avoid reaction of 2-MeImpN with OH^- and large enough to limit the reaction rate so that NMR spectra acquisition times are negligible. It is also inside the presumed pH values of prebiotic sea water.

† In the text 2-MeImpA stands for 2-MeImpA$^-$ and H-2-MeImpA$^\pm$, 2-MeImpG stands for 2-MeImpG$^-$ and H-2-MeImpG$^\pm$, 2-MeImpN stands for 2-MeImpA and 2-MeImpG. The word phosphate or the symbol P_i designate $H_2PO_4^-$ and HPO_4^{2-}.



Scheme 1

Results and discussion

Analysis and interpretation of 2-MeImpG and 2-MeImpA hydrolysis NMR spectra

Typical ^{1}H and ^{31}P spectra of 2-MeImpG and 2-MeImpA in phosphate buffer are presented in Fig. 1 after some of the reaction has occurred. The spectra taken in 0.2 M TRIS buffer instead of phosphate buffer are identical to the spectra in Fig. 1, but the signals assigned to ADP or GDP species are absent.

The chemical shifts presented in Fig. 1 are constant for all experiments reported in this work with respect to buffer type, buffer concentration and time and so correspond to a full ^{1}H and ^{31}P NMR spectral characterisation of 2-MeImpA and 2-MeImpG and their hydrolysis products, under the standard conditions used in all experiments: 0.02 M 2-2-MeImpN, 0.5 M NaCl, pD 7.6, 308 K (see Experimental).

The assignment of the 2-MeImpG spectrum was performed by comparing with spectra of GMP, guanosine and