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Detection of 9-methylhypoxanthine tetrad by electrospray ionization mass spectrometry

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ABSTRACT

The ability of 9-methylhypoxanthine (**MeHyp**) to form stable cluster ion was studied by electrospray ionization mass spectrometry (ESI-MS). It was found that 9-methylhypoxanthine (in contrast to hypoxanthine) forms stable magic number cluster tetramer, namely $[(\mathbf{MeHyp})_4 + \mathrm{Na}]^+$ ion, as detected by ESI-MS. Results obtained for alkali metals other than sodium, as well as for hypoxanthine nucleoside (inosine) are also discussed.

1. Introduction

Deamination of adenine, in nucleic acid molecules, leads to formation of hypoxanthine (hypoxanthine nucleoside is called inosine). Hypoxanthine has a structure similar to that of guanine, therefore in biochemical processes hypoxanthine may be interpreted as guanine [1]. It is interesting to check how far hypoxanthine/guanine exchange affects chemical properties of nucleic acid molecules, which has been studied in a number of papers [2–6].

A very important property of guanine is its ability to form a tetramer, called guanine tetrad, which is stabilized by alkali metal cations, sodium and potassium. Guanine (or guanosine) tetrad has been successfully analyzed by electrospray ionization mass spectrometry (ESI-MS) [7–10]. However, the attempts at detection of hypoxanthine tetrad by ESI-MS have been unsuccessful [11,12]. On the other hand, xanthine tetrad has been detected by ESI-MS [11–13]. Thus, the question is if hypoxanthine does not form tetramer in solution or the tetramer it forms is so unstable that it cannot survive even such soft conditions such as in ESI. It is obvious that guanine and xanthine tetrads are more stable than hypoxanthine tetrad because of the presence of amine and oxygen atom, respectively, at C2 position (Scheme 1). These substituents at C2 position make hydrogen bonds [14] which substantially increase the stability of guanine and xanthine tetrads in comparison to that of hypoxanthine tetrad (Scheme 1).

In this paper it is shown that 9-methylhypoxanthine (**MeHyp**) forms stable tetramer in solution, namely $[(\mathbf{MeHyp})_4 + \mathrm{Na}]^+$ ion, as detected by ESI-MS. Results obtained for alkali metals other than sodium, as well as for inosine, are also discussed.

2. Experimental

9-Methylhypoxanthine was prepared from adenine [15]. Adenine and inosine have been obtained from Sigma–Aldrich (Poznań, Poland).

In order to obtain ESI mass spectra, the sample solutions were prepared in methanol, the 9-methylhypoxanthine (and/or inosine) concentration was 10^{-4} mol/dm^3. Abundant sodium clusters were observed without adding sodium. In order to obtain abundant cluster of other metal cations, a respective salt was added (chloride or perchlorate) in a concentration of 10^{-5} mol/dm^3.

The full scan ESI mass spectra were obtained on a Waters/Micromass (Manchester, UK) ZQ2000 mass spectrometer (single quadrupole type instrument, Z-spray, software MassLynx V3.5). The sample solutions were infused into the ESI source using a Harvard pump, the flow rate was 80 (l/min. The ESI source potentials were capillary 3 kV, lens 0.5 kV, extractor 4 V and cone voltage (CV) 5–70 V. Cone voltage has the most profound effect on the mass spectra obtained. Increase in this parameter leads to the so called “in-source” fragmentation/dissociation but a too low cone voltage may cause a decrease in sensitivity. The

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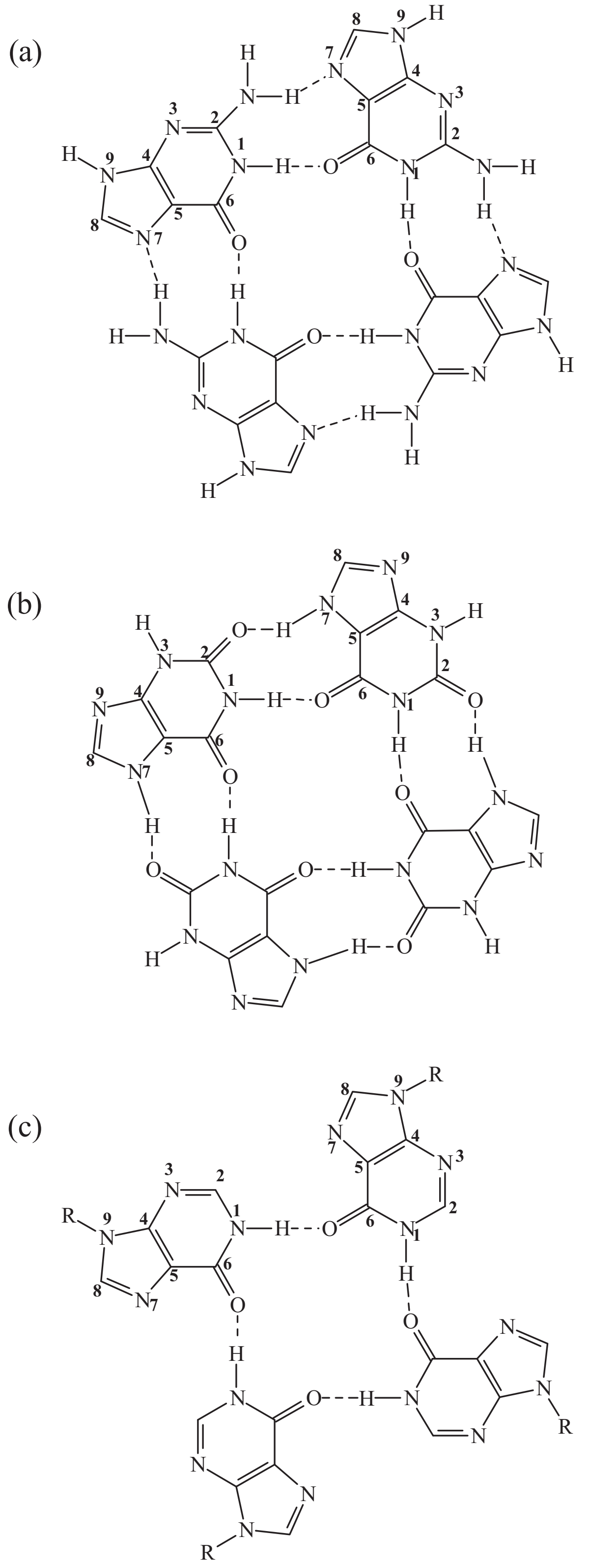

Scheme 1. Tetrads of purine bases, (a) guanine tetrad, (b) xanthine tetrad, and (c) R=H hypoxanthine tetrad, R=CH$_3$ 9-methylhypoxanthine tetrad.

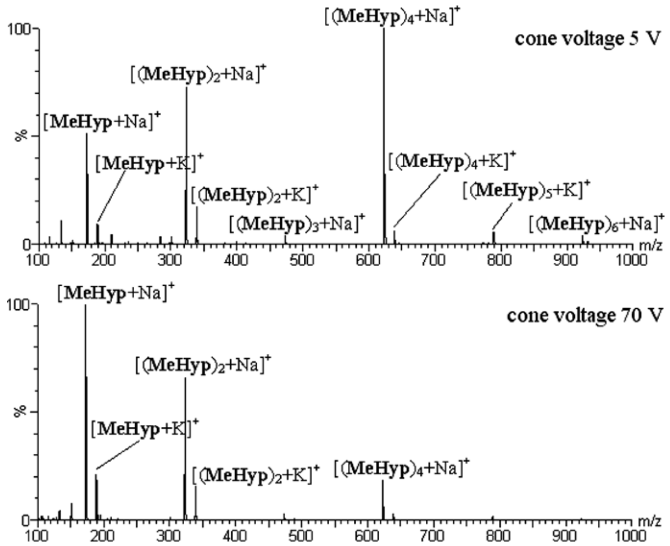

Fig. 1. ESI mass spectra of 9-methylhypoxanthine (**MeHyp**). The presence of magic number cluster ion [(**MeHyp**)$_4$ + Na]$^+$ at m/z 623 is evident.

source temperature was 120 °C and the desolvation temperature was 300 °C. Nitrogen was used as the nebulizing and desolvating gas at the flow-rate of 100 and 300 l h^{-1}, respectively.

3. Results and discussion

Fig. 1 shows ESI mass spectra obtained for 9-methylhypoxanthine solution. Although sodium cation was not added to the solution analyzed, the sodium adducts were formed, there are also less abundant signals of potassium adduct (sodium and potassium are common contaminants). At low cone voltages, the most abundant signal corresponds to [(**MeHyp**)$_4$ + Na]$^+$ ion at m/z 623 (tetramer), so to the 9-methylhypoxanthine tetrad. At low cone voltages, tetramer is a little more abundant than dimer and monomer and much more abundant than trimer and pentamer. Tetramer is more abundant than trimer and pentamer also at higher cone voltages. Thus [(**MeHyp**)$_4$ + Na]$^+$ ion can be called a magic number cluster, so unusually stable cluster compared with its neighbors. Thus 9-methylhypoxanthine forms tetrad as shown in Scheme 1.

The question is why hypoxanthine did not form an analogical tetrad. As shown in Scheme 1, hypoxanthine tetrad should exist thanks to the N1-H···O=C6 hydrogen bonds (by analogy to the xanthine tetrads [14]), whereas N9-H (or N7-H because of tautomerization) should not be involved in the formation of any hydrogen bond. However, it is obvious that N9-H of one hypoxanthine molecule is able to form hydrogen bond with any other hypoxanthine molecule. Formation of such hydrogen bonds (e.g., N9-H···O=C6) may disturb formation of the hydrogen bond N1-H···O=C6 required to produce a hypoxanthine tetrad. **MeHyp** contains N9-CH$_3$ instead of N9-H, therefore there is no possibility to form hydrogen bonds which would compete with formation of N1-H···O=C6 hydrogen bonds (it is known that sometimes a methyl group can make a hydrogen bond [16] but for 9-methylhypoxanthine it is very unlikely).

Summing up the above, 9-methylhypoxanthine forms a stable magic number cluster tetramer, whereas hypoxanthine does not. The question is if hypoxanthine nucleoside, inosine (hypoxanthine 9-β-D-ribofuranoside, **Inos**) is able to form a stable tetramer. Sugar moiety is able to make hydrogen bonds, which can disturb formation of the hydrogen bond N1-H···O = C6, thus it is expected that inosine would not make a stable tetramer. As shown in Fig. 2,