SHORT REPORTS

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THE INTERACTION OF H^+ AND Zn (II) WITH GUANOSINE

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ABSTRACT

Protonation and complex formation of guanosine with Zn(II) in aqueous solution have been investigated by means of potentiometry. Under biological conditions (pH ~ 7, 37°C) the dominant species are the neutral LH molecule and the zinc complex $ZnLH^{2+}$, with logarithmic stability constants of 2.12 ± 0.01, and 1.20 ± 0.01, respectively. These results are in accord with the previous experimental findings of nucleoside base-Zn(II) interactions in DNA.

INTRODUCTION

The effect of metal ions on DNA helices has been the subject of many investigations[1-5] due to its importance in the replication and transcription of DNA. The conditions under which the DNA double helix is unwound into single strands and rewound again in the presence of divalent metal ions have also been studied.[1,2] Among these ions, Zn(II) has been noted for its effective ability to bring about, reversibly, the unwinding and rewinding of DNA double helix on heating and cooling, respectively. This was attributed to the relative affinity of the metal ion for the ribose phosphate backbone and DNA base. Experimental determination of DNA melting curves as a function of metal concentration and the examination of UV spectra have indicated that at low metal concentrations Zn(II) stabilizes DNA by phosphate binding.[3] At elevated metal concentrations Zn(II) is supposed to undergo a second reaction with the nucleoside

base. Consequently, during 'denaturation' of DNA in the presence of Zn(II), the crosslinks between bases of the single strands through Zn(II) hold the two chains in close enough proximity so that the double helix is regenerated on cooling. It was also claimed[3] that the most favourable base binding site for Zn(II) was guanosine, forming a guanosine-Zn(II)-guanosine linkage. It was, therefore, worthwhile to further investigate the types and the stability of the complex species formed by Zn(II) and guanosine under conditions near to those realised in nature, and to thus obtain more evidence for the validity of the proposed reactions with DNA.

EXPERIMENTAL

Materials

Merck Analar (p.a) chemicals were used to prepare the solutions of appropriate concentrations. De-ionized distilled water was used to make up all solutions. Stock solutions of HNO_3 and KOH were prepared from Merck titrisol products. Guanosine was obtained from Fluka. Metal stock solution was prepared from $Zn(NO_3)_2 \cdot 4H_2O$ and the concentration was determined complexometrically. The ionic strengths of all solutions were adjusted to 0.15 M by adding KNO_3.

Method

Potentiometric titrations were performed using a Schott pH-meter CG 803 equipped with an Ingold microelectrode. Non-linear calibration of the electrode was employed for each acid-base titration. All investigations were carried out under N_2 atmosphere at 37 °C. The formation constants were evaluated from the titration data using the MINIQUAD programme[6] as previously described.[7] All calculations were performed on the CDC cyber 180-840 computer of the University of Innsbruck.

RESULTS AND DISCUSSION

Protonation

From the analysis of the potentiometric data (5 titrations, 150 points) of 0.005 - 0.03 M guanosine solutions in the pH range of 1.5 to 10, the following protonation and deprotonation equilibria were assessed :

$$(1)\quad LH + H^+ \overset{K_1}{\rightleftharpoons} LH_2^+ \qquad \log K_1 = 1.90 \pm 0.10$$

$$(2)\quad LH \overset{K_1'}{\rightleftharpoons} L^- + H^+ \qquad \log K_1' = -8.93 \pm 0.02$$

where LH refers to the neutral ligand molecule. The protonated species becomes significant below a pH of about 4, while the deprotonated one starts to appear beyond pH 7. It can be concluded therefore, that the major species in the aqueous solution of guanosine under biological conditions is the neutral LH molecule, and, under acidic conditions, also the singly protonated LH_2^+ species.

Zinc complexation

The results of the analysis of the potentiometric data (6 titrations, 190 points) of the solutions of guanosine (0.005 - 0.02 M) containing zinc ion with the metal-ligand ratio varying from 1:1 to 1:4 in the pH range of 1.5 to 7 deliver the following equilibrium constants :

$$(3)\quad LH + H^+ \overset{K_1}{\rightleftharpoons} LH_2^+ \qquad \log K_1 = 2.12 \pm 0.01$$

$$(4)\quad LH + Zn^{2+} \overset{K_2}{\rightleftharpoons} ZnLH^{2+} \qquad \log K_2 = 1.20 \pm 0.01$$

The small discrepancy between the value of the stability constant for the protonated species obtained here and that obtained from the protonation experiment could be due to the effect of the metal concentration on the variation of the ionic strength of the system. A slight variation in ionic strength is practically unavoidable during titration (even though all solutions were 0.15 M in KNO_3) when the metal ion is not strongly complexed. Besides, the result from the protonation experiment was obtained for the pH range up to 10 whereas the study of metal complexation is restricted to maximum pH 7.The zinc-complex stability constant is of the same order of magnitude as that found for Zn 9-methyl adenine complex.[8] Under biological conditions, (pH 7, 37 °C) the dominant species is therefore the neutral LH molecule and the zinc complex $ZnLH^{2+}$. Beyond pH 7, $Zn(OH)_2$ precipitation interferes with complexation. Owing to the small value of the stability constant of the metal complex, this equilibrium can be considered to be flexible, and strongly due to the Zn^{2+}, concentration present. This also provides evidence for the experimental finding of Eichhorn *et al*,[3] that small variations in Zn^{2+} concentrations can remarkably influence DNA stability. Taking into account previous results on the cation influence on DNA base-pair hydrogen bonds[9] and our data, a model can be proposed where Zn^{2+}, at elevated concentrations, influences the DNA stability by directly binding to the bases rather than by binding to the phosphate groups.

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บทคัดย่อ

ในงานวิจัยนี้ได้ศึกษาเกี่ยวกับโปรโตเนชั่นและการเกิดสารประกอบเชิงซ้อนของกวาโนซีนกับ Zn(II) ในสารละลายในน้ำด้วยวิธีโพเทนชิโอเมทรี ซึ่งพบว่าภายใต้สภาวะทางชีวภาพ (pH ~ 7, 37 °C) โมเลกุลของกวาโนซีนที่เป็นกลางคือ LH และสารประกอบเชิงซ้อนระหว่างกวาโนซีนกับ Zn(II) คือ $ZnLH^{2+}$ เกิดขึ้นในสารละลายมากที่สุด โดยที่ log K มีค่าเท่ากับ 2.12 ± 0.01 และ 1.20 ± 0.01 ตามลำดับ ผลของงานวิจัยนี้สอดคล้องกับผลการทดลองเกี่ยวกับแรงกระทำระหว่างเบสของนิวคลีโอซายด์กับ Zn(II) ของ DNA โมเลกุล