EXCHANGE BETWEEN DIVALENT METALLIC CATIONS AND SODIUM FIXED ON IONISABLE SITES OF DNA

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1. Introduction

As part of an investigation of the interactions of metal ions with desoxyribonucleic acid (DNA) in solution, we have studied the exchange between the counterion Na^+ with Mg^{2+}, Zn^{2+} and Cu^{2+} ions, using a Na-ion selective electrode.

Previous studies of the role of metallic ions with respect to DNA stability were based on absorbance measurements at 260 mμ, as a function of temperature [1, 2] : the "melting point" T_m shifted to higher temperatures, corresponding to a stabilisation of DNA upon addition of Mg^{2+} or Zn^{2+} ions.

Our method permits the observation of the exchange between counterions and metallic ions before the onset of thermal denaturation.

2. Materials and method

Experimental apparatus and methods have already been described [3, 4] . The free sodium ion concentration, C_{Na^+}, was determined potentiometrically by means of glass membrane electrodes selectively responsive to Na^+ (Beckmann type 39278), using a fiber junction reference electrode (Beckmann type 39170). The Nernst potentials were measured with a P 12 AOIP potentiometer followed by a CARY model 31 spectrometer, direct reading in pH units.

Light absorption data were recorded with a SAFAS Cadet 2000 spectrophotometer using thermoregulated quartz cells 1 mm long.

A Roussel Jouan digital spectropolarimeter with a two cm long cell permitted us to check the helicity of the substance.

Calf-thymus DNA (samples no. V 378 and V 464) were obtained from the Molecular Biophysics Center, Orléans. Stock solutions were prepared in a concentration of 0.4 g of Na-DNA in 1 l of 2×10^{-3} M NaCl (Merck ultrapure). The divalent cations were introduced as chlorides obtained from Merck (pro analysis).

The pH of the solutions remained close to 7 before and after denaturation. The theoretical Na-DNA molar concentration of ionisable sites was:

$$\frac{0.4}{331} \simeq 1.2 \times 10^{-3}\ \mathrm{M}.$$

3. Results

In fig. 1 the concentration of ejected Na^+ ions is plotted versus the concentration of added $MgCl_2$ and in fig. 2 versus added $ZnCl_2$.

A continuous exchange between the counterions Na^+ and the introduced ions Zn^{2+} or Mg^{2+} is evident in both cases. This process reaches a saturation level for a concentration of 3×10^{-3} M of divalent ions.

It is seen that in these two cases, the concentration of ejected Na^+ ions is only slightly inferior, or equal to the concentration of ionisable sites.

The presence of this saturation level allowed us to control the state of the DNA in the interval between the completion of the exchange process and the onset of thermal denaturation.

The extent to which the substance could be said to be in the native state was determined by measuring its absorbance as a function of the wavelength of the incident light.

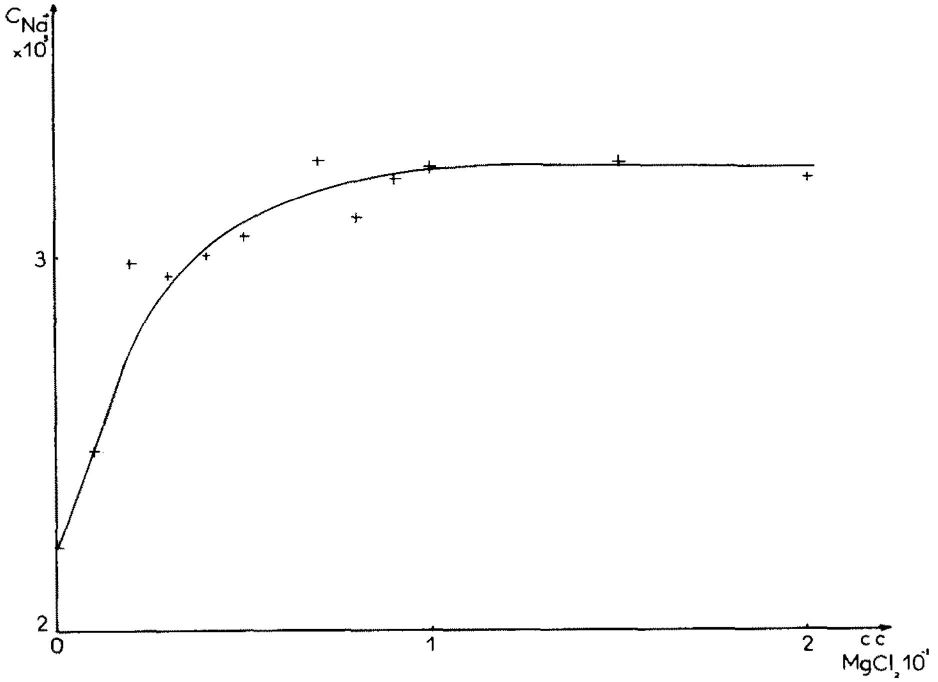

Fig. 1. Free sodium ion concentration in solution versus added concentration of $MgCl_2$.

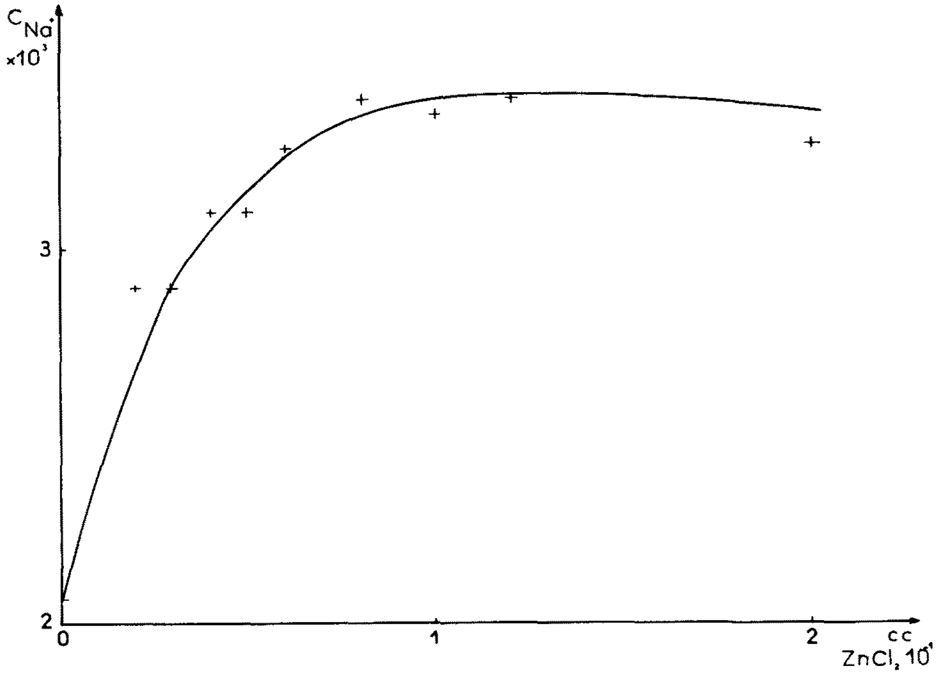

Fig. 2. Free sodium ion concentration in solution versus added concentration of $ZnCl_2$.

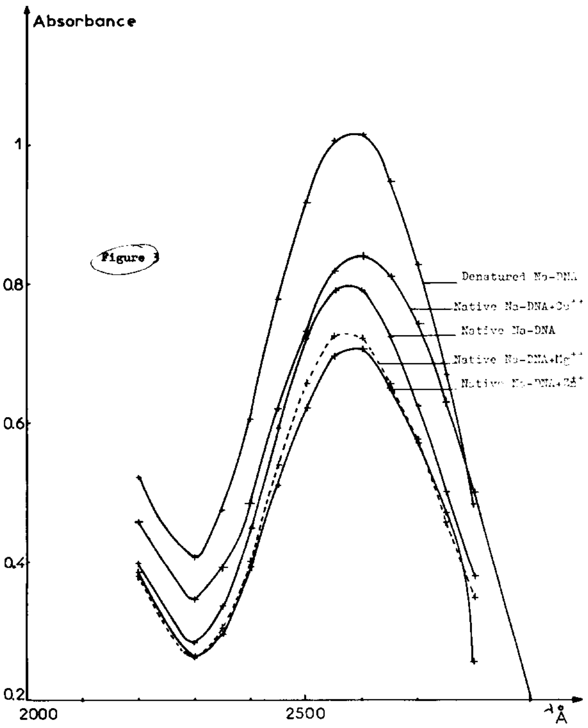

Fig. 3. Absorbance for different solutions of Na-DNA versus wavelength.

Results for Na-DNA, Na-DNA + $MgCl_2$, Na-DNA + $ZnCl_2$ and for DNA + $CuCl_2$ are shown in fig. 3. The absorbance values observed indicate that the product is in its native form.

In agreement with Eichhorn and Shin, stability is increased by addition of $MgCl_2$ or $ZnCl_2$ [1, 2].

The absorbance at 260 mμ, for Cu^{2+} at our concentration level, remains essentially unchanged. It is known that Cu^{2+} has a stabilizing action because it acts on the periphery of the molecule; at higher concentrations, however, it tends to become a de-stabilizer because of its interactions with the bases.

Experiments at lower concentrations of Cu^{2+} were not possible since the Na^+ ejection rate then is too low to yield a change in the Na^+ concentration detectable with our method.

On the other hand, it was possible to measure the product helicity for different concentrations of added $MgCl_2$. Table 1 gives the specific rotations obtained at 20° for the D spectral line of sodium, $(\alpha)_D^{20°}$.

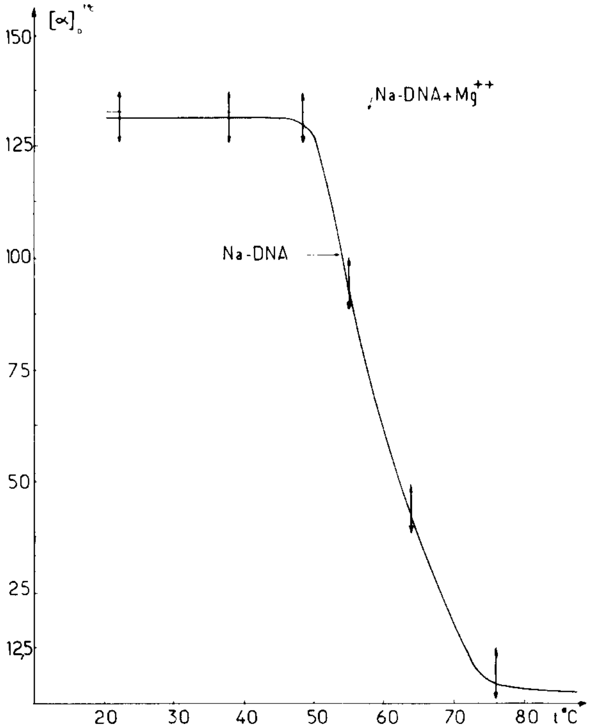

Fig. 4. Specific rotations $(\alpha)_D^{t°}$ versus temperature for a Na-DNA solution and for a Na-DNA solution with added $MgCl_2$ corresponding to the saturation regime.

It is seen that this rotation is not modified by adding $MgCl_2$.

The same quantity $(\alpha)_D^{20°}$ is plotted in fig. 4 versus temperature, for a Na-DNA solution only and for a Na-DNA solution with $MgCl_2$ added at a concentration corresponding to saturation.

Having ascertained that the DNA remained structurally in the double-helix form after ion exchange, it was important to verify that all the sites had indeed exchanged their Na^+. If not, further Na^+ ejection during thermal denaturation would be likely.

We have followed this phenomenon with Na^+ selective electrodes in steps of 5° to 70°, without obtaining any evidence for further ejection of Na^+. We then completely denatured the product by heating it for one hour at 100° followed by rapid cooling to 20° by means of immersion in liquid nitrogen to avoid any renaturation. The concentrations of ejected ions for native and for denatured Na-DNA are given in table 2.

Table 1

Added $MgCl_2$ ionic strength	0	0.062×10^{-3} M	0.125×10^{-3} M	0.187×10^{-3} M	0.25×10^{-3} M	0.5×10^{-3} M
$(\alpha)_D^{20°}$	+125°	+125°	+125°	+125°	+125°	+125°

Table 2

Solutions	Native Na-DNA	Denatured Na-DNA	Native Na-DNA $+MgCl_2$	Denatured Na-DNA $+MgCl_2$	Native Na-DNA $+ZnCl_2$	Native Na-DNA $+CuCl_2$
C_{Na^+}	2.2×10^{-3} M	2.7×10^{-3} M	3.2×10^{-3} M	3.2×10^{-3} M	3.2×10^{-3} M	3.2×10^{-3} M

During thermal denaturation of Na-DNA 0.5×10^{-3} M of Na^+ are ejected, while for Na-DNA with sites exchanged by Mg^{2+}, there was no variation of free C_{Na^+} in the solution upon thermal denaturation. There are more ions ejected during the exchange process than during denaturation.

Thus, not even denaturation at 100° can liberate all the sodium ions in in interaction with ionisable sites.

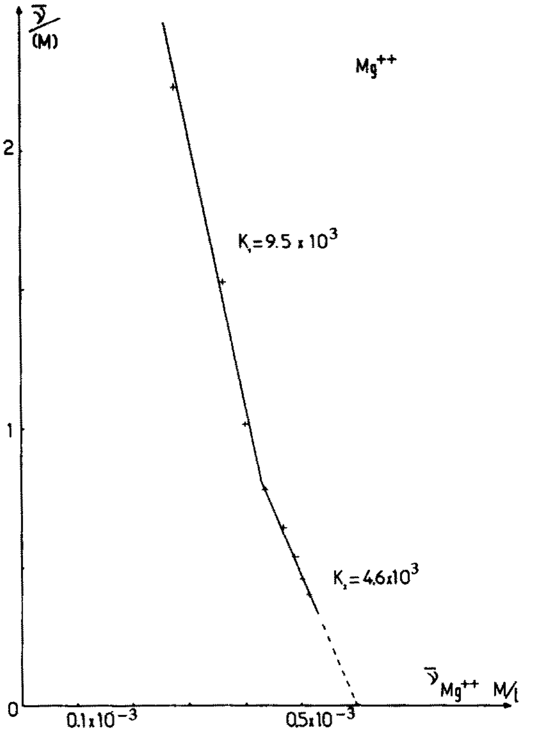

Fig. 5. Scatchard's plot $\bar{\nu}/(M)$ as function of $\bar{\nu}$: $\bar{\nu}$: molar concentration of fixed Mg^{2+}; $\bar{\nu} = C_{Na^+} - C_0/2$; C_{Na^+}: free sodium measured; C_0: initial free sodium; $(M) = C_{MgCl_2} - \bar{\nu}$; C_{MgCl_2}: introduced $MgCl_2$ concentration.

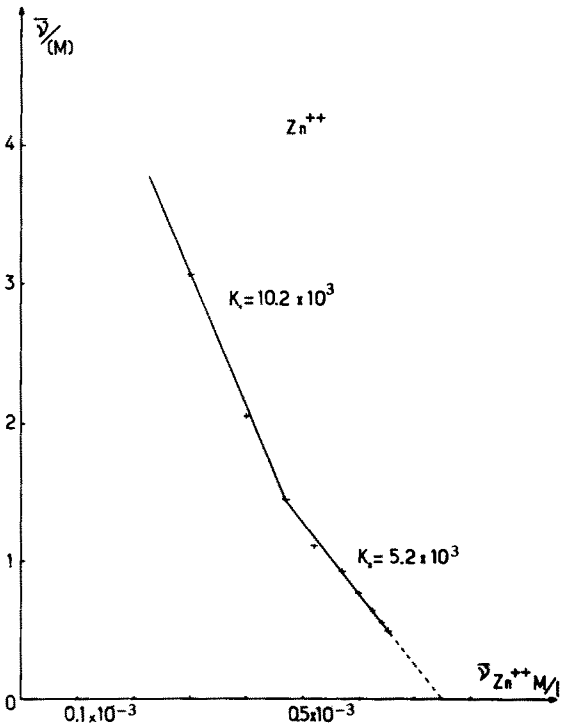

Fig. 6. Scatchard's plot $\bar{\nu}/(M)$ as function of $\bar{\nu}$: $\bar{\nu}$: molar concentration of fixed Zn^{2+}; $\bar{\nu} = C_{Na^+} - C_0/2$; C_{Na^+}: free sodium measured; C_0: initial free sodium; $(M) = C_{ZnCl_2} - \bar{\nu}$; C_{ZnCl_2}: introduced $ZnCl_2$ concentration.

Assuming that all the sites are equivalent and independent, one can follow the ion exchange phenomenon by means of a Scatchard plot, as shown in figs. 5 and 6.

We note that the two association constants for Mg^{2+} and Zn^{2+} are comparable. However the number of exchange sites with Mg^{2+} or Zn^{2+} is about equal to half the theoretical number of ionisable sites; this would confirm the hypothesis that two sodium ions are exchanged for each Mg^{2+} or Zn^{2+} ion.

4. Summary

Employment of ion selective electrodes for the measurement of free Na^+ yields evidence for the exchange between the metal ions Mg^{2+}, Zn^{2+} and Cu^{2+} and the Na^+ counterions of DNA. At 20° the exchange process saturates and there is no indication of further ejection of Na^+ after thermal denaturation at 100°.

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