CHANGES IN UV-ABSORBANCE ARE DUE TO SLOW AND FAST CONFORMATIONAL REARRANGEMENTS DURING DNA-PLATINATION

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INTRODUCTION

Kinetics of DNA platination involve at least two conformational rearrangements (1). The fast one is observed by ethidium bromide-dependent fluorescence, and its nature is unknown. The slow one refers to partial melting of DNA and is seen by circular dichroism and Tb$^{3+}$-dependent fluorescence. We report here that the rearrangements are responsible for UV-hyperchromic effects known to accompany DNA platination (2).

MATERIALS AND METHODS

Salmon testis DNA was purchased from Sigma and dialysed against several changes of a solution of 10 mM KNO$_3$ pH 5.5 before use and stored at -20°C. The racemate of 1,2-bis(4-fluorophenyl)ethylene-diamineplatinum(II) sulfate (i) was a gift of Dr Schönenberger/Regensburg, cis-diamminedichloroplatinum(II) (ii) was a gift of Degussa/Frankfurt, and all other chemicals were of highest available grade from Merck/Darmstadt. Compound (i) reacted in solution as its diaqua complex. Cis-diamminediaquaplatinum(II) (iii) was prepared by the reaction of (ii) with AgNO$_3$ in a 1:2 stoichiometry over night at 37°C. All reaction solutions were pH 5.5 at 37°C and contained 10 mM KNO$_3$. Concentrations of DNA were determined spectrophotometrically and are given in concentration of nucleotide bases as described (3). Platination of DNA was carried out directly in photometric quartz cells and was continuosly under spectrophotometrical or fluorometrical observation (in the presence of ethidium bromide) as described (1).

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RESULTS

Platination of DNA exhibited a hyperchromic effect in the UV-range 255 nm - 310 nm. Kinetics were the sum of two superimposed exponentials similarly as in previous cases (1). Rate constants and reaction amplitudes of the exponentials were measured as a function of DNA concentration (Figure 1). Reaction amplitudes increased proportional to DNA concentrations, while values of the rate constants became independent at high concentrations in the case of the faster of the two superimposed reactions. Rate constants of the slow reaction were independent over the whole range of concentration investigated. In the case of (i) (Figure 1), the rate constant for the slow reaction was 0.04 - 0.06 min\(^{-1}\) and that for the fast reaction in the plateau 0.5 min\(^{-1}\). The initial portion of the concentration dependence was interpreted to follow a second-order rate constant of \(1.5 \times 10^3\) M\(^{-1}\) min\(^{-1}\). The corresponding values for (iii) were 0.02 - 0.03 min\(^{-1}\), 0.25 min\(^{-1}\) and \(1.6 \times 10^3\) M\(^{-1}\) min\(^{-1}\), respectively. In contrast, the reaction of cis-diammineaquachloro-platinum(II) (prepared by 12 h incubation of 2 mM (ii) at 37°C (1)) was monophasic following a first-order rate constant of 0.005 min\(^{-1}\).

Fig. 1. Reaction of salmon testis DNA with 1,2-bis(4-fluorophenyl)ethylenediamineplatinum(II) sulfate (i). Rate constants of the two superimposed exponentials as a function of DNA concentration. The ratio of concentrations of platinum to DNA nucleotides was 0.1. Open circles, the slow reaction. Filled circles, the fast reaction.