Chromosome Damage and Repair

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Chromosome Damage and Repair

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PREFACE

The NATO - EMBO Advanced Study Institute - Lecture Course on "CHROMOSOME DAMAGE AND REPAIR" was held at Godøysund Fjord Hotel outside Bergen, Norway, from May 27th to June 5th, 1980. This book represents the proceedings of this meeting. In addition to the formal lectures, a number of short contributions presented in the discussion sessions following the lectures are also included. The papers have been divided into different groups according to topic, essentially in the same way as they were presented during the meeting. The editors have made few alterations in the manuscript submitted and these were mostly confined to typing style and correction of typographical errors.

We would like to express our appreciation to all the persons who helped in making this meeting possible. Special thanks are due to Tomas Lindahl, Alan R. Lehmann and Erik Boye, who served in the advisory program committee. We would also like to thank our Danish friends and colleagues and others who provided invaluable assistance in an emergency situation. The editors are also grateful for the financial support provided by a number of organizations and institutions. First and foremost of these were the NATO Scientific Affairs Division and the European Molecular Biology Organization. In addition to these the following Norwegian institutions also gave financial assistance to the meeting: The Ministry of Foreign Affairs, The Ministry of Church and Education, The Norwegian Cancer Society, The Norwegian Research Council for Technology and Science, The City of Bergen, The Norwegian Defence Research Establishment and the University of Bergen.

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DAMAGE TO DNA CAUSED BY HYDROLYSIS

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INTRODUCTION

The covalent structure of DNA is unstable in aqueous solution. It tends to hydrolyze to its monomeric components, and they themselves are subject to various hydrolytic reactions. These processes are slow, when compared to most familiar chemical reactions. However, a reaction that is slow by these standards may still have great biological significance, if it occurs within the genetic material of an organism. A single base transformation within a DNA molecule may be sufficient to cause a mutation, or inactivate the DNA. Consider a reaction, for example, with a rate constant of 10^{-10} sec^{-1} at pH 7.4, 37°; it will have a half life of 220 years. Assume that, within a DNA, it affects two of the four bases. It will take place once every three hours per million base pairs of DNA, and thus be a significant source of damage.

The rates of such reactions cannot conveniently be followed, however, by conventional chemical methods. They have usually been examined under conditions where they are greatly speeded up - by elevated temperatures and acidic or basic catalysis. If reactions have been run at several elevated temperatures, it is usually not difficult to extrapolate down to 37°. Caution must only be observed when a nucleic acid, rather than a nucleotide, is the substrate, as changes in secondary structure may take place within the temperature range considered. An extrapolation from acidic or basic conditions to neutral solution is more difficult. The simplest assumption is that the rate will be proportional to the concentration of $H^{(+)}$ or $OH^{(-)}$ Thus a reaction is assumed to take place, at pH 7, at a rate 10^{-6} of that measured at pH 1. In some

cases, this treatment will give an approximately correct result; see, for example, the pH-rate profile for the depurination of deoxyadenosine, in Figure 4. Often, however, this approximation may greatly underestimate the reaction rate at pH 7, as in the deamination of cytidine in acid. For a better understanding of the effects of acid and alkali, the dissociation properties of the reactants must be taken into account.

The function of acid in a hydrolysis reaction is to convert the substrate to a more reactive protonated form. In the case of the deamination of cytidine, for example, this takes place as one decreases the pH from 6 to 3. Once protonation is complete, the addition of more acid does not speed up the reaction further. The rate remains constant over the pH range 1-3, affording a plateau area in the pH-rate profile. Deoxyadenosine and deoxyguanosine, like deoxycytidine, are converted to cations by aqueous acid, with pK_a values, respectively, of 3.8,2.5, and 4.3 at 25°. In concentrated sulfuric acid, or other strongly acidic media, these substances are converted to dications, and thymidine is protonated as well.

Alkali, like acid, enhances rates of hydrolysis. It does not do this, however, by increasing the reactivity of the substrate. In fact, it has an opposite effect upon two of the four deoxynucleosides in Figure 3, thymidine and deoxyguanosine. It converts them to a less reactive anionic form. The pK_a values are 9.8 and 9.3 respectively. The enhancement of rates observed in alkaline solution is due to the substitution of an effective nucleophilic reagent, hydroxide ion, for a relatively ineffective one, water. For the reason given above, deoxyadenosine and deoxycytidine are much more susceptible to degradation by alkali than thymidine and deoxyguanosine. Again, this is a result which can not be extrapolated to solutions of pH below 9, as the latter nucleosides will no longer be in anionic form.

DEAMINATION OF CYTOSINE, ADENINE AND GUANINE

Amino substituents on the bases of nucleic acids hydrolyze, with loss of ammonia, under acidic and alkaline conditions. The reaction is called hydrolytic deamination to distinguish it from the oxidative deamination produced by nitrous acid treatment. The hydrolytic deamination of cytosine is the most rapid one at neutral pH, and the only one whose kinetics have been studied in detail. The rate of deamination of cytosine derivatives is greatly affected by the presence of buffers (1). However, by extrapolation at each pH to zero buffer concentration, we can obtain a rate for catalysis by water alone (2). The variation of this rate with pH for cytidine is given in Figure 1. Four distinct areas should be noted: a plateau in acid, below pH 3; a sloping area in weak acid; another plateau in neutral solution (pH 6-8)

and a sharp rise as the solution becomes progressively more alkaline. Although several kinetically equivalent possibilities exist to explain each of these areas, the simplest scheme is probably the following: The rate in the first two areas reflects the attack of water on protonated cytidine, with the observed decrease due to its dissociation (the pK_a for cytidine at 80° is 3.6). This pathway declines in importance as the concentration of protonated cytidine falls. In the plateau area, pH 6 to 8, the rate depends on the concentrations of protonated cytidine and of hydroxide ion. One term falls as the other rises, and their product is constant. In the alkaline range, rates are proportioned to the concentration of hydroxide ion, and reflect the attack of this species on neutral cytidine.

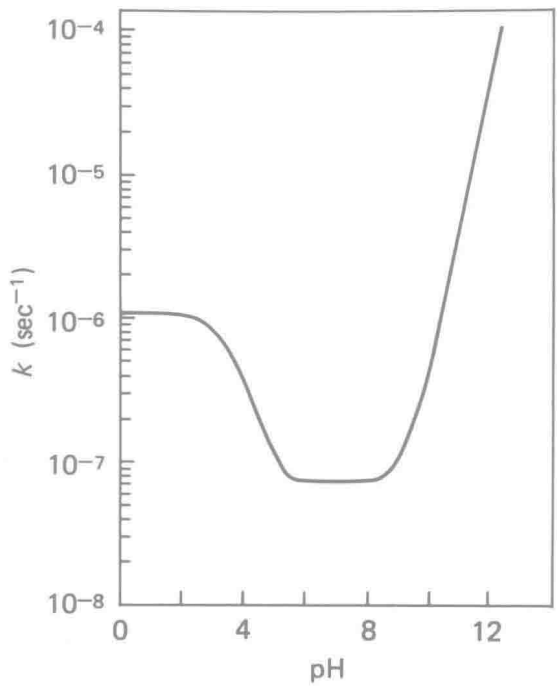

Fig. 1. The pH-rate profile for hydrolytic deamination of cytidine at 80° (2).

Two possible mechanisms have been suggested for the mechanism of deamination (1) (Figure 2), The scheme shown describes the reaction in acid, but other equivalent ones would exist in