CHANGES OF STABILITY AND CONFORMATION OF DNA FOLLOWING THE COVALENT BINDING OF A CARCINOGEN

Robert FUCHS and Michel DAUNE
Centre de Recherches sur les Macromolécules, CNRS, Strasbourg, France

Received 27 March 1971

1. Introduction

Recently Miller [1] has thrown some light on the effects of many carcinogenic chemicals. He has shown that the activity of these compounds or their metabolic derivatives is related to their strong electrophilic reactivity. The active form of those carcinogens is covalently bound to nucleic acids and proteins in induced tumors [1].

In the case of acetylaminofluorene (AAF) Miller et al. [2] have shown that the last metabolic derivative was an ester of *N*-hydroxy-AAF. We have therefore studied the effects of *N*-acetoxy-AAF (*N*-AcO-AAF) on native DNA. The analysis of melting profiles of modified DNA gives evidence for a destabilizing effect which is interpreted as the opening of G–C base pairs. Furthermore it is likely that the carcinogen reacts preferentially with G–C rich regions.

2. Material and methods

Native calf-thymus DNA (V 371) with the following characteristics was used: protein content $< 0.1\%$; hyperchromicity at 260 nm 42%; $S_{20,w} = 21$ S; $[\eta] = 6200$ ml/g in 0.3 M ionic strength.

N-AcO-AAF was synthesized as previously described [3]. The reaction of the carcinogen with DNA was performed according to Miller [4].

When the reaction was completed, the excess of fluorene derivative was extracted twice with ethylic ether. The free residues of fluorene and ethanol were removed by extensive dialysis against 2×10^{-3} M citrate buffer, pH 7.0. This solvent was used for all the experiments. In some cases Sephadex G25 was used with the same results.

The covalent fixation of *N*-AcO-AAF on guanine [4] caused a shift of the U.V. spectrum of DNA from 257 to 262 nm and the appearance of a shoulder at 305 nm. The magnitude of these effects was related to the quantity of bound carcinogen.

We have studied the progressive fixation of *N*-AcO-AFF with an initial ratio C/P (carcinogen/phosphate) varying from 0 to 1.2.

The system described by Wilhelm et al. [5] was used to follow the melting curves at 260 and 305 nm. This apparatus records directly absorbance versus temperature.

3. Results and discussion

The cooperative melting curves obtained at 305 nm (fig. 1) can only be explained if we assume that the

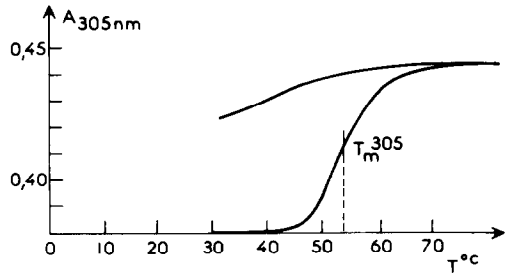

Fig. 1. Melting curve at 305 nm of a modified DNA in which 31% of total guanine have reacted with *N*-AcO-AAF (2×10^{-3} M citrate buffer pH 7). Temperature is increasing linearly at a rate of 1°C/min.

North-Holland Publishing Company – Amsterdam

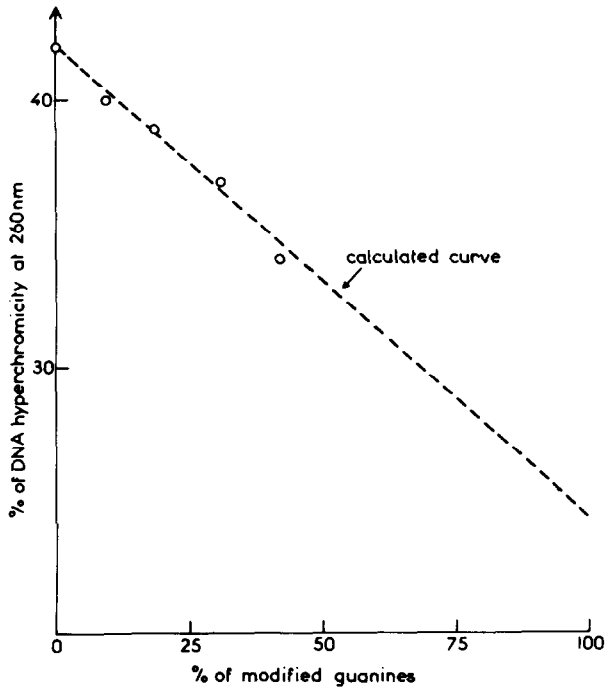

Fig. 2. Correlation between experimental points and curve calculated as described in the text.

carcinogen chromophore is imbedded in the internal field of the double helix. We found that the optical properties of bound carcinogen are independent of C/P, provided that the amount of carcinogen is kept constant [6].

The hyperchromicity at 305 nm enables direct determination of the percentage of modified guanines. If we assume that the specific extinction coefficient of the carcinogen is the same when bound to guanosine [7] or to denatured DNA, we can calculate the amount of modified guanines [6].

If the carcinogen hyperchromicity is independent of wavelength in the range 260–305 nm, the carcinogen contribution can be subtracted from the experimental melting curve in order to calculate DNA hyperchromicity (fig. 2).

A simple way to interpret the data is to assume that the decrease of hyperchromicity is caused by a rupture of hydrogen bonds between G and C. One would expect a linear variation of hyperchromicity with the amount of bound carcinogen; as shown in fig. 2, experimental points fit well with this predicted relationship.

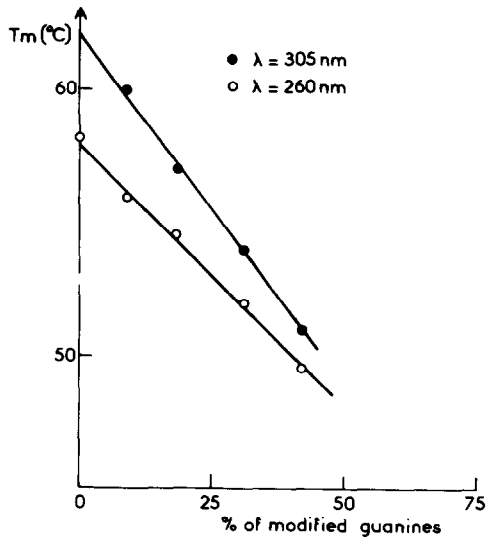

Fig. 3. Variation of T_m at two wavelengths (305 nm and 260 nm) versus amount of modified guanines.

Fig. 3 shows that T_m for both wavelengths (260 nm and 305 nm) decreases linearly with the amount of modified guanine. T_m at 260 nm (T_m^{260}) was always lower than T_m at 305 nm (T_m^{305}). The first observation indicates a strong destabilisation of modified DNA. The extrapolation of the curves T_m^{260} to 100% of reacted guanines gives T_m of DNA consisting only of A–T pairs. The value found (38°) is comparable to the melting temperature of poly dA–dT in the same conditions [8].

The systematic higher value of T_m^{305} compared to T_m^{260} may be explained in two ways:

a) The intercalation of carcinogen aromatic ring between base plates and the strong interaction with those bases would increase the local stability in DNA. Therefore the melting temperature of this region should be higher. But this hypothesis is hardly compatible with the fact that T_m^{260} decreases as the amount of modified guanines increases.

b) The carcinogen reacts preferentially with regions of high G–C content. Even if the modification of guanine destabilizes the base pair involved, the carcinogen environment may have a higher melting temperature than the other parts of the DNA molecule.

As the amount of bound carcinogen increases, the difference between T_m^{305} and T_m^{260} must decrease since any heterogeneity in the DNA tends to disappear.

We are now developing new analytical methods to test this last assumption, which so far represents a more convenient explanation of the experimental facts. It offers, moreover, a new insight into the biological mechanism of carcinogenesis.

Acknowledgements

This research was supported by a grant of the Ligue Nationale Française contre le Cancer. It is a pleasure to thank Dr. Biellmann for providing laboratory facilities to synthesize the carcinogen.

References

[1] J.A. Miller, Cancer Res. 30 (1970) 559.
[2] J.A. Miller and E.C. Miller, Prog. Exptl. Tumor Res. 11 (1969) 273.
[3] P.D. Lotlikar, J.D. Scribner, J.A. Miller and E.C. Miller, Life Sci. 5 (1966) 1263.
[4] E.C. Miller, U. Juhl and J.A. Miller, Science 153 (1966) 1125.
[5] F.X. Wilhelm, M.H. Champagne and M.P. Daune, European J. Biochem. 15 (1970) 321.
[6] R. Fuchs and M. Daune, manuscript in preparation.
[7] E. Kriek, J.A. Miller, U. Juhl and E.C. Miller, Biochemistry 6 (1967) 177.
[8] M. Riley, B. Maling and M.J. Chamberlin, J. Mol. Biol. 20 (1966) 359.