DNA-MALONALDEHYDE REACTION: FORMATION OF FLUORESCENT PRODUCTS

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SUMMARY

DNA was reacted with malonaldehyde to form fluorescent products with maximum emission at 460 nm when excited at 390 nm. A new absorption peak at 325 nm was also formed in the course of the reaction between DNA and malonaldehyde. Formation of fluorescent products correlates linearly with loss of DNA template activity.

INTRODUCTION

Malonaldehyde has been shown to react with amino acids and proteins (1,2). The product formed is attributed to crosslinking of amino acids by malonaldehyde with the formation of the amino-iminopropene structure, R-N=C-C=C-N-R. These products have a characteristic maximum fluorescence peak at 465 nm when excited at 390 nm. When the enzyme ribonuclease is reacted with malonaldehyde it loses activity rapidly and the loss of activity is correlated with the formation of the fluorescent product.

Glyoxal and malonaldehyde interact with DNA in vitro and in vivo, as demonstrated by Brooks and Klamerth (3). They found changes in the thermal denaturation profiles and incomplete degradation by deoxyribonuclease of DNA that had been reacted with glyoxal and malonaldehyde. The bifunctional aldehyde reacted, probably by crosslinking, with guanine, cytidine and to a lesser degree with adenine, all of which carry amino groups. The present report describes the formation of fluorescent products resulting from the reaction of DNA with malonaldehyde and the correlation between the formation of these products and the loss of DNA template activity.

MATERIALS AND METHODS

Malonaldehyde was prepared from its ethyl acetal, 1,1,3,3-tetraethoxypropane (J. T. Baker Chemical Co.) by shaking with an excess of an aqueous

suspension of Dowex 50 (X4) as described by Brooks and Klamerth (3). Highly polymerized calf thymus DNA was obtained from Mann Research Laboratories. RNA polymerase was isolated from rat liver as described below. Labeled ^{14}C-ATP was obtained from Amersham/Searle Corporation. Malonaldehyde concentration was determined by the 2-thiobarbituric acid assay (4). Fluorescence measurements were made with an Aminco-Bowman spectrophotofluorometer (American Instruments Company, Inc.) and recorded on an X-Y recorder. Absorption spectra were determined with a Beckman DB-G spectrophotometer.

DNA-malonaldehyde reaction. DNA was dissolved in 0.1 X SSC solution (15 mM NaCl, 1.5 mM trisodium citrate), pH 4.6, at a concentration of 0.5 mg/ml. One drop of toluene was added to prevent bacterial growth. The DNA solution was dialyzed at 37°C against 0.5% (70 mM) malonaldehyde in 0.1 X SSC solution, pH 4.6. Samples were withdrawn at varying time intervals. Excess malonaldehyde was removed by precipitating the DNA with 2 volumes of 95% ethanol containing 2% potassium acetate. The precipitate was washed twice with ethanol, dissolved in 0.1 X SSC solution, pH 6.8, and dialyzed extensively against the same solution until no malonaldehyde was detected in the dialysate. DNA control samples were treated under the same conditions, omitting the malonaldehyde.

Isolation of rat liver nuclei. Rat liver nuclei were prepared as described by Roeder and Rutter (5), with a few modifications. Five week-old male Sprague-Dawley rats (140-150 g) were decapitated and their livers were removed, washed and homogenized in 2 volumes of cold 0.32 M sucrose that contained 1 mM $MgCl_2$. A portion of homogenate (25 ml) was layered on 5 ml of 2.2 M sucrose in centrifuge tubes and centrifuged at 22,000 rev./min (Spinco SW 25.1 rotor) for 1 h. After centrifugation, the supernatant portion was discarded and the nuclear pellet was washed once with 0.22 M sucrose.

RNA polymerase solubilization. The nuclear pellet was treated as described by Roeder and Rutter (5); the final enzyme preparation consisted of fraction 4 as described by these investigators.

Assay for RNA polymerase activity. The procedure used was a modification of the assay described by Roeder and Rutter (6). The 0.275 ml reaction mixture contained 5.0 µmoles of Tris-HCl, pH 8.1, 3.2 µmoles KCl, 3 µmoles NaF, 0.5 µmole 2-mercaptoethanol, 0.5 µmole $MnCl_2$, 0.3 µmole each of UTP, GTP and CTP (Sigma), 0.02 µmole ^{14}C-ATP (specific activity 8 mc/mmole), 70 mM $(NH_4)_2SO_4$, 2.5 µg pyruvate kinase (Sigma), 0.5 µmole phosphoenol pyruvate (Sigma), 40 µg enzyme protein and 60 µg DNA. After incubation for 10 min at 37°C, the reaction was stopped by addition of 0.2 ml of ice-cold 0.1 M sodium pyrophosphate (adjusted to pH 7.0 with HCl), containing 5 mM ATP and 2 mg/ml each of RNA and bovine serum albumin. The reaction mixtures were quickly cooled and 0.2 ml of 5% sodium dodecylsulfate was added followed by addition of 2 ml of 10% trichloroacetic acid containing 40 mM $Na_4P_2O_7$. The acid insoluble material was collected on nitrocellulose filters and washed four times with 10 ml aliquots of the trichloroacetic acid-$Na_4P_2O_7$ solution. The filters were dried and the radioactivity determined in a liquid scintillation counter.

RESULTS

The spectra in Figure 1A show the formation of fluorescent products from the reaction of malonaldehyde with DNA. The fluorescence spectra are characteristic of the amino-iminopropene complexes that have a fluorescence peak at 460 nm when excited at 390 nm. There is an additional excitation peak at 325 nm; this peak disappeared after extending the reaction time to 120 h. Figure 1B shows that the reaction of malonaldehyde with adenine and guanine resulted in similar amino-iminopropene fluorescence spectra.

Figure 2 shows the absorption spectrum of the DNA-malonaldehyde product. The 120 h reaction time curve shows a 325 nm peak. Absorption at 258 nm also increased over the absorption at zero time. As was observed earlier by Brooks and Klamerth (3), absorption-temperature profiles showed shifts in the melting point of DNA-malonaldehyde with increased reaction time. The melting point decreased from 71°C for the control sample to 69°C by 10 h and to 65°C by 50 h.

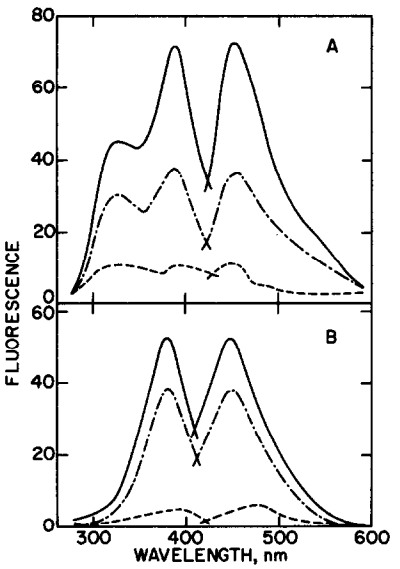

Fig. 1.

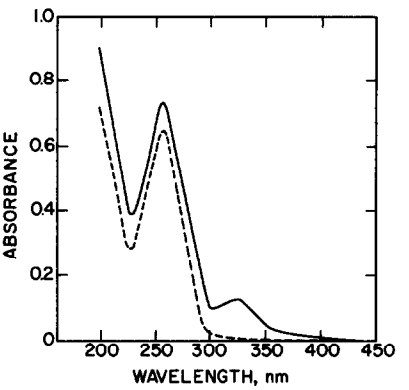

Fig. 2.

Fig. 1. (A) Fluorescence spectra of native DNA (---) and DNA-malonaldehyde products (500 µg/ml 0.1 X SSC, pH 6.8) at 15 h (—•—•) and 50 h (——). Reaction conditions and sample preparations are described in MATERIALS AND METHODS. (B) Fluorescence spectra of adenine and guanine bases reacted with malonaldehyde. 4 mM adenine (—•—•) or guanine (——) was reacted with 8 mM malonaldehyde for 18 h at 37°C. Malonaldehyde was used as a control under the same conditions (---).

Fig. 2. Absorption spectra of control DNA (---) and DNA-malonaldehyde (——) after 120 h. DNA concentration was 40 µg/ml 0.1 X SSC, pH 6.8.

The kinetics of formation of the fluorescent product and the changes in absorption are shown in Figure 3A. The relative increase in absorption at 258 nm between 0 h and 48 h was small as compared to the absorption at 325 nm for the same time interval. The effect of malonaldehyde on the template activity of DNA is shown in Figure 3B. About 64% of the activity was lost during the first 15 h of DNA reaction with malonaldehyde, after which no further loss of activity occurred. There was also a decrease in the template activity of the control samples after 15 h of reaction, probably due to hydrolysis of the purine bases at pH 4.6.

There is a linear correlation (r = 0.95) between the formation of

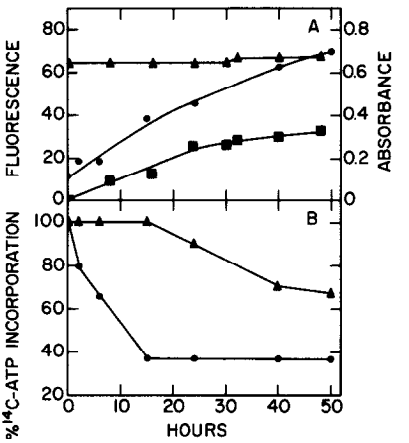

Fig. 3. (A) Fluorescence and UV absorption changes of DNA-malonaldehyde as a function of reaction time. Fluorescence with excitation at 390 nm, emission at 460 nm (●—●); absorbance at 325 nm (■—■); absorbance at 258 nm (▲—▲). (B) The effect of malonaldehyde on the template activity of DNA (●—●) as a function of reaction time compared with that of control DNA (▲—▲). The preparation of DNA samples and assay conditions are described in MATERIALS AND METHODS.

fluorescent product (Figure 3A) and the loss of template activity (Figure 3B) during the first 15 h of reaction which is significant ($p < 0.05$). The very low level fluorescence of control DNA remained constant throughout the experiment.

DISCUSSION

The formation of a fluorescent product having a maximum fluorescence peak at 460 nm when excited at 390 nm can be accounted for by the reaction of the amino groups of the bases of DNA with malonaldehyde to give the conjugated amino-iminopropene structure, R-N=C-C=C-N-R', where R and R' are bases of DNA. Adenine and guanine reacted with malonaldehyde had similar fluorescence spectra. The appearance of the absorption peak at 325 nm probably resulted from the formation of the conjugated amino-iminopropene structure. The kinetic curve for its formation is similar to that for the formation of the fluorescent product. All the results shown here, especially the correlation between the formation of fluorescent products and the loss of template activity, provides evidence that malonaldehyde alters the structure of DNA.

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