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MELTING CURVES OF GAMMAIRRADIATED DNA

HEINZ HOFER
HANS ALTMANN
MANFRED KEHRER

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Heinz Hofer
Hans Altmann
Manfred Kehrer

Arbeitsbericht

Österreichische
Studiengesellschaft für Atomenergie
Ges.m.b.H.
Lenaugasse 10 A-1082 Wien

INSTITUT FÜR BIOLOGIE
Forschungszentrum Seibersdorf

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ABSTRACT

Melting curves of gammairradiated DNA and data derived of them, are reported. The diminished stability is explained by base-destruction. DNA denatures completely at room temperature, if at least every fifth basepair is broken or weakened by irradiation.

KEY WORDS

DNA, gammairradiation, melting curves.

SCHMELZKURVEN VON GAMMA-BESTRAHLTER DNA

ZUSAMMENFASSUNG

Schmelzkurven von Gamma-bestrahlter DNA und daraus abgeleitete Resultate werden in dem vorliegenden Bericht dargestellt. Die verminderte Stabilität wird durch Basenzerstörung erklärt. Die DNA denaturiert vollständig bei Zimmertemperatur, wenn wenigstens jedes 5. Basenpaar zerstört oder durch die Bestrahlung geschwächt wird.

SCHLÜSSELWORTE

DNA, Gamma-Bestrahlung, Schmelzkurven.

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1. INTRODUCTION

Structural changes of DNA after irradiation have been of great interest in the last years. A lowered thermal stability of DNA is one of the effects produced by radiation. We have measured melting curves of gammairradiated DNA solutions to obtain information about the mechanism of radiation induced decreased stability.

2. MATERIALS AND METHODS

2.1. Materials

The DNA (calf thymus) was the Sigma type I material. The other chemicals used were of p.a. grade obtained from Merck, Darmstadt. All solutions were prepared in deionized, double distilled water. DNA was dissolved in sodiumphosphate buffer (pH 6,5; 0,01 m in Na^+) in a concentration of $0,5 \times 10^{-4}$m (P). Concentration was calculated from extinction measurement, using $\varepsilon_{m,258} = 6650$.

2.2. Irradiation

Irradiation was carried out with a 400 Ci Co^{60} source of Atomic Energy of Canada (dose rate 34,5 krad/h) at room temperature. Aerobic conditions were chosen, because we could not detect significant differences for the Tm-values when DNA solution was irradiated either in O_2 atmosphere or under N_2 or in vacuum. The reported measurements were made immediately after irradiation.

2.3. Melting curves

Melting curves were measured on a Zeiss PMQ II spectralphotometer using the continuous method. A linear rise of the temperature was obtained by a similar arrangement as described by Szybalski and Mennigmann (1). No correction for thermal expansion of water was set.

The following abbreviations are used:

$E_{\gamma,T}$: Extinction of irradiated DNA at temperature T at 258 nm.

Tm : Melting point in degree Celsius.

H : Hyperchromicity

$$H = \frac{E_{\gamma,\,max} - E_{\gamma,\,20}}{E_{\gamma,\,20}} \cdot 100$$

S : Steepness of the melting curve. It is expressed by the percent of the maximum rise in extinction per degree Celsius, in the middle of the transition (Tm-value).

$$S = \left(\frac{dD}{dT}\right)_{D = 0,5} \cdot 100, \quad D = \frac{E_{\gamma,T} - E_{\gamma,\,20}}{E_{\gamma,\,max} - E_{\gamma,\,20}}$$

3. RESULTS

Melting curves of DNA, irradiated at different doses, are shown in fig. 1. The drop of the thermal stability of the DNA can be seen by the decrease of the Tm-value (fig. 2). A similar curve has been reported by Hagen and Wild (2), the only difference being is that they had a smaller effect at the same dose, a fact which could perhaps be explained by different irradiation conditions.

It can be shown by the competition method of Blok et al. (3), using iodid ions as OH radical scavengers, that OH radicals are mainly responsible for the diminished stability (fig. 3).

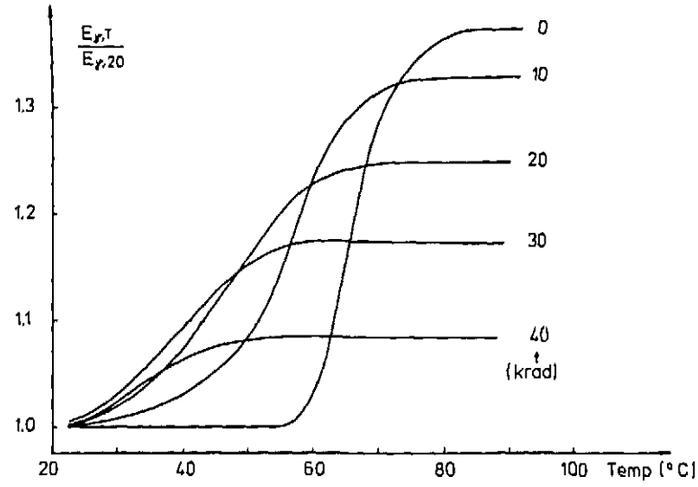
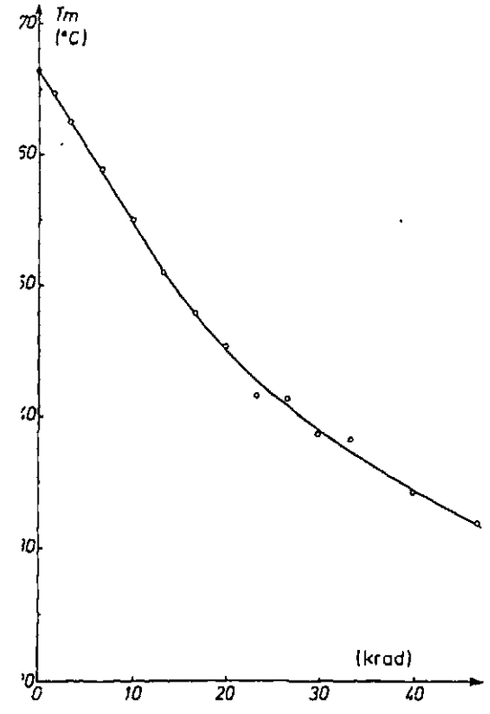


Figure 1: Melting curves of irradiated DNA. Extinction was measured at 258 nm. Concentration: 5×10^{-5} m (P) DNA in sodiumphosphatebuffer (0,01 m in Na^+, pH 6,5). Concentration is the same for all the following figures.

Figure 2: Tm-values of irradiated DNA-solution are plotted as a function of dose.



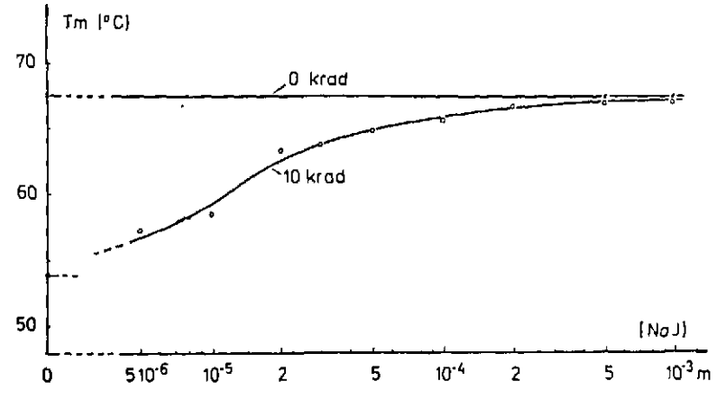


Figure 3: Tm-values of irradiated (10 krad) and control DNA are plotted as a function of NaJ concentration. $\angle Na^+ \_/$ was kept constant in the system by variation of concentration of the Na^+-phosphatebuffer.

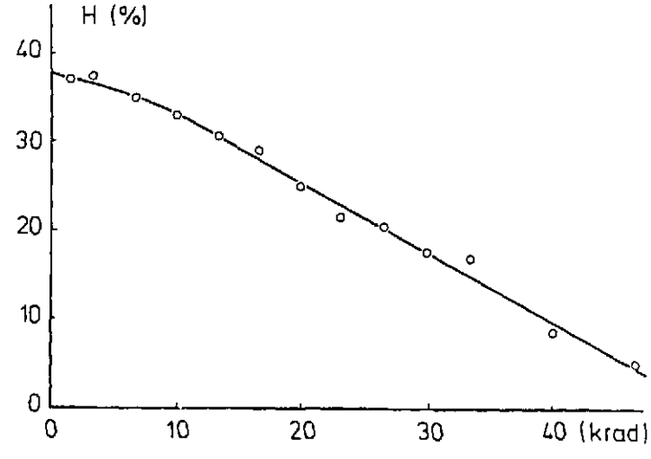


Figure 4: Dependence of hyperchromicity on dose.

Further data derived from the melting curves as hyperchromicity H and steepness S are summarized in fig. 4 and 5. The smaller H at higher doses indicates that parts of the DNA have been denatured.

The alteration of extinction of the DNA at room temperature with dose is shown in fig. 6. There are two different types of mechanism necessary for explaining this curve, as Collyns et al. (4) have pointed out. Radiation induced denaturation produces an increase in extinction that predominates at lower doses over the decrease in absorption by basedestruction. Only at higher doses, when denaturation is almost complete, the drop of the extinction due to basedestruction becomes apparent.

If one is interested in base damage only, extinction of irradiated and then denatured DNA has to be measured (4). We took the values of the maximum extinction of the melting curves - in contrast to Collyns who denatured with acid - and with the aid of the molar extinction coefficient for denatured DNA we obtained the concentration of destructed bases (fig. 7). From the initial part of this plot one can derive the G-value. We obtained G = 0,12 $\pm$ 0,02. The greater yield of damaged bases at higher doses could be explained by the fact that base destruction is more effective in denatured DNA than in native one (5).

The G-value of 0,12 is substantially lower than that reported by Collyns et al. (4) of 1,07. The difference might be explained by taking into account that Collyns did not use stabilising salts for the DNA solution. Furthermore it is possible, as Summers and Szybalski (6) pointed out, that the concentration of DNA is of great influence. We used a 60 times more diluted solution than Collyns. Also contaminations can play an important role in competing for water radicals, especially in very diluted DNA solutions.

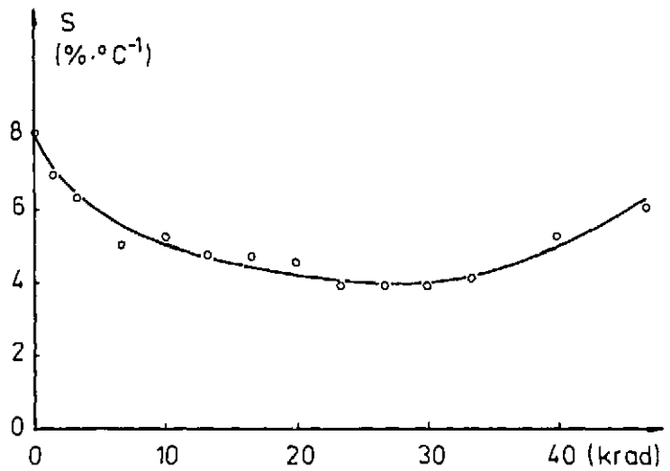


Figure 5: Steepness (definition is given in the text) is plotted against dose.

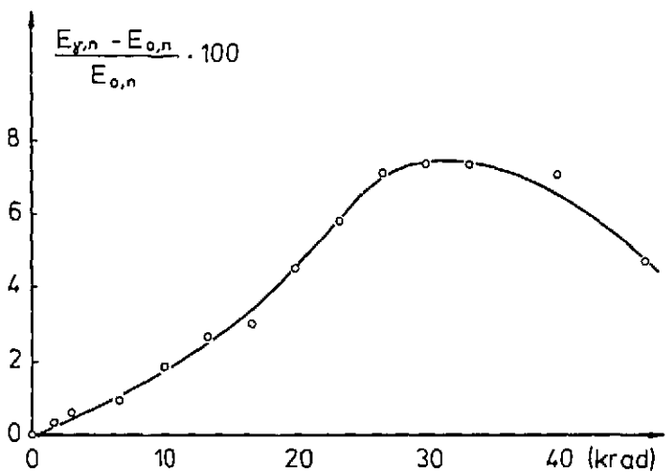


Figure 6: Alteration of extinction at 258 nm of the DNA at room temperature with dose.

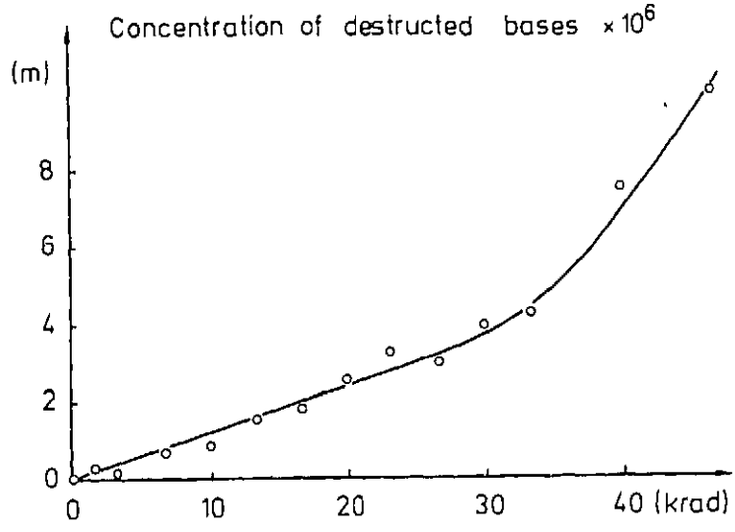


Figure 7: Concentration of destructed bases, that do not contribute to absorption at 258 nm is shown as a function of dose.

4. DISCUSSION

The only explanation for the decrease of the Tm-value after irradiation was given by Hagen and Wild (2). They proposed that single strand breaks (SSB) would yield a greater number of starting points for the unwinding process. The high molecular DNA is thus divided in low molecular parts which melt independent of each other. One can expect a drop of the Tm-value with decreasing chain length L due to the equation of Crothers et al. (7).

$$\frac{1}{Tm(L)} = \frac{K}{L} + \frac{1}{Tm\ (\infty)}, \qquad K = \text{positive factor.}$$

It seems to us that this explanation is not quite satisfactory, because significant deviations from initial Tm-value are produced only by lowest chain lengths, compared to the original length of about 10^4 to 10^5 basepairs. For example, the degradation of a DNA with mean molecular weight of 8.10^6 to a 1/13 of the length showed no deviation of the melting point (8). A steep decrease of the Tm-value of

oligoadenylic acids was produced only when the chain length was not more than about 15 basepairs (9). One can conclude from this, that low doses would have only a small effect on stability and that at higher doses a progressive decrease would appear, which is in contradiction to the measured curve (fig. 2).

We want to show that base destruction is a possible reason for the diminished stability. The G-value of base destruction is about four times as high as that of SSB (4). OH radicals are responsible for both events. Normally UV radiation damages only the bases of DNA leading not to SSB (10). Nevertheless Marmur et al. (8) could observe after UV irradiation of DNA a similar decrease of the stability as is shown in fig. 2.

AT-pairs are less stable than GC-pairs. This produce the linear dependence between GC- (or AT-) content and Tm-value (11). The weakened or broken basepairs, created by destruction of one or both of the two bases in the pair, could lead in a similar manner to a linear decrease of the melting point. The linearity does not have to be fullfilled at higher doses, s there is a competition for OH radicals between original bases contained in a native part of the DNA and bases that are already altered or that are deposited in denatured parts of DNA and therefore do not contribute to more base pair weakening.

When extrapolating the initial decrease of the Tm-value to $20^{o}C$ (fig. 2) one obtaines about 40 krads. Taking this value and the G-value for basedestruction one can say that for complete radiation induced denaturation at least every tenth base must be damaged. In other words, if at least every fifth basepair does not contribute to stability, the ordered structure breaks down. As there might be base alterations leading to basepair weakening but which are not

detected by loss of absorption at 258 nm, the value of 5 should be an upper limit.

This statement is in good agreement with experiments carried out by Eigen (12), who found that the smallest stable oligomer is the basepair triplett. Brahms et al (9) obtained for doublestranded polyadenylic acid built up by 6 - 8 pairs a Tm-value almost equal to room temperature. Results of Hagen (13) are also comparable. He found that there is a doublestrand break if not more than three nucleotide pairs lie between two SSB in opposite position.

Irradiation brings about a lowering of S (fig. 5) which could be due to diminished extent of cooperative melting or increased inhomogenity of the DNA material. The formation of independently melting parts of the DNA molecule separated by SSB or some broken basepairs would reduce the effective chain length for melting and therefore lead to reduced steepness (14). It can be expected that the parts formed by this mechanism are more inhomogenious in GC content and also in the content of damaged bases than the whole molecular which is a second reason for lower S.

The rising steepness of melting curves at higher doses (fig. 5) when Tm-value is rather low, is due to measuring conditions. We started measuring absorption at 20^{o}C, so that especially at higher doses parts of the irradiated DNA were denatured before heating began. One can see from the definition used for S that such a process will rise S artificially.

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