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GESELLSCHAFT FÜR KERNFORSCHUNG M. B. H.

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G. HOTZ, R. MAUSER and R. WALSER

Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, Germany

(Dedicated to K. G. Zimmer on the occasion of his sixtieth birthday)

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Infectious T1-DNA stored for about 20 days at 4°C in an appropriate buffer contains on average 5 single-strand breaks per phage genome without detectable loss of infectivity.

T1-DNA in 0·2 M NCE-buffer thermally treated for 6 min at 100°C and rapidly cooled forms an infectious molecule of mismatched configuration probably containing short-range folded single-stranded regions, 3 single-strand breaks per phage genome, a low intrinsic viscosity of $[\eta] = 34{\cdot}5$ [dl./g], and an increased sedimentation coefficient indicating a change in molecular conformation.

No double-strand breaks and a negligible number of single-strand breaks are observed in unsubstituted infectious T1-DNA after inactivation with U.V.-light of 2537 Å. After incorporation of 5-bromouracil, however, single-strand breaks are produced in infectious T1-DNA at a yield of 0·9 breaks per lethal hit requiring an exposure to 45 ergs/mm^2 per break. The relation of double to single-strand breaks is 1/17. The relevance of the breaks to the biological activity of infectious T1-DNA is discussed.

1. Introduction

The question of the relevance of DNA strand breaks for the infectious process of phage DNA is a major problem in molecular radiobiology. There is increasing evidence for the hypothesis that single-strand breaks in DNA of bacteriophage are not lethal (Summers and Szybalski 1967). In a previous paper on the infectious DNA from coliphage T1 (Hotz and Mauser 1969), it was noted that this DNA retained its biological activity over a long period of storage, and was infectious even when the molecules are artificially mismatched containing single-strand breaks.

Both sedimentation (S) and viscosity (η) measurements provide information on size and conformation of the DNA molecules. The narrow coiling of mismatched T1-DNA molecules observed after heat treatment for 6 min at 100°C in a buffer of high molarity, and subsequent rapid cooling (Hotz and Mauser 1969), should produce an increase in S and a decrease in η. From sedimentation data of such mismatched molecules in a CsCl-density gradient, one could expect an answer to the question whether single-stranded regions exist.

DNA breaks were observed recently (Stephan, Miltenburger and Hotz 1970) which had been induced by U.V.-light (2537 Å) after irradiation of T1-phage particles substituted by the base analogue 5-bromouracil. The effect of U.V.-light (2537 Å) on the infectious activity of irradiated T1-DNA molecules

(Davison, Freifelder and Holloway 1964) were studied. The average molecular weight of the preparations is derived from the $S^0_{20,w}$ (Eigner and Doty 1965).

Storing infectious T1-DNA up to 4 months in NCE-buffer at 4°c changes the mean sedimentation coefficient of native DNA but slightly (table). However, if the DNA is denatured before sedimentation, a significant decrease of the $S^0_{20,w}$

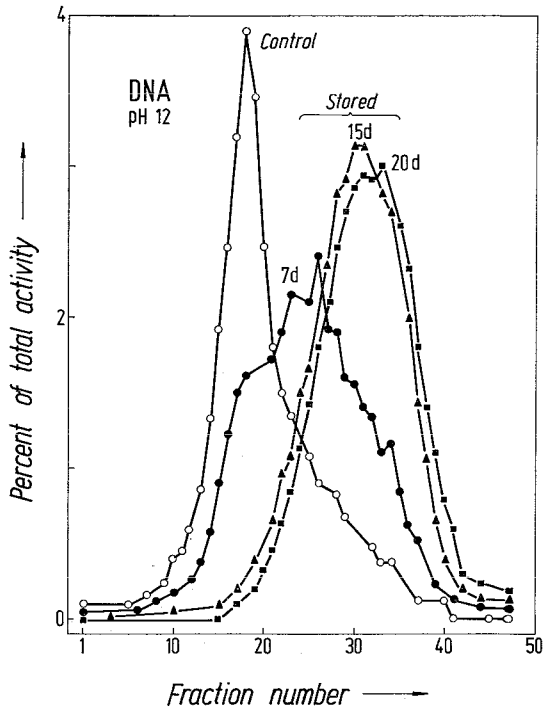

Figure 2. The effect of storage (7, 15, and 20 days under conditions described in the legend of figure 1 on the sedimentation pattern of alkali-denatured infectious T1-DNA. All preparations of denatured DNA were sedimented in an alkaline sucrose gradient of pH 12 for 210 min at 35 000 r.p.m. under the same experimental conditions as are described in the legend of figure 1 for native DNA.

Duration of storage at 4°c in NCE-buffer	DNA preparation and type	$S^0_{20,w}$	MW × 10^{-6}
6 days	IV. 70; native	35·5	30
3 weeks	,,	34·0	27
4 months	,,	34·2	27·5
1 day	XII. 69; denatured	35·2	16
6 months	,,	21·7	6·6
3 weeks	II. 70; denatured	25·7	9·4
4 weeks	,,	23·8	8
2 months	,,	20·4	5·8

The dependence of $S^0_{20,w}$ on storage time of infectious T1-DNA.

was observed. We conclude that single-strand breaks occur in the native state of DNA and/or bonds of the double helix are weakened during prolonged storage of the DNA and broken thereafter during the procedure of denaturation. The extent of decrease in molecular weight of denatured DNA after storage depends on the batch of DNA studied, as can be seen from the table.

Furthermore, these physicochemical alterations in stored DNA do not alter the infective activity of T1-DNA significantly (figure 3). This stability of infectious T1-DNA is independent of the host and is observed when speroplasts, made from both HCR-positive and HCR-negative cells of *E. coli* K12, are infected.

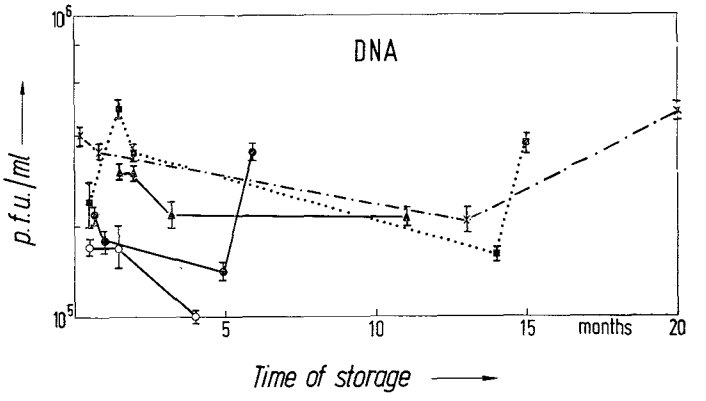

Figure 3. The effect of time of DNA-storage on the infectivity (plaque-forming units per ml) of T1-DNA. Five stock solutions of different DNA-preparations were kept at 4°c in NCE-buffer for the time indicated. Standard errors marked.

3.2. *Hydrodynamic properties of thermally-treated infectious T1-DNA (sedimentation, viscosity, and density)*

It was shown recently that T1-DNA, thermally-treated in 0·2 M NCES for 6 min at 100°c and then quickly cooled in ice-water, has an unusual structure (Hotz and Mauser 1969) but an almost normal infectious activity. The pictures obtained with the electron microscope are consistent with a DNA structure mismatched with loop-like formation. It was the aim of the experiments to be described to give further information on the physicochemical structure of thermally-treated infectious T1-DNA.

Figures 4 (*a*)–4 (*c*) give the results of three runs performed at different speeds and durations of centrifugation in neutral sucrose gradients containing ' heat-treated ' infectious T1-DNA (HT-DNA) and an untreated preparation serving as a reference of sedimentation behaviour. The molecules of the control are homogeneous as regards their molecular weight and configuration. The untreated DNA is travelling in a narrow band, and the distribution pattern is not influenced by time or speed of centrifugation.

However, from the sedimentation pattern of thermally-treated infectious T1-DNA, shown in figures 4 (*a*)–4 (*c*), we conclude that the molecules are heterogeneous to a high degree in their configurations and sedimentation properties. After 180 min at 27 000 r.p.m., a pattern with a still defined peak can be observed (figure 4 (*a*)). After 210 min at 30 000 r.p.m., however, a broad

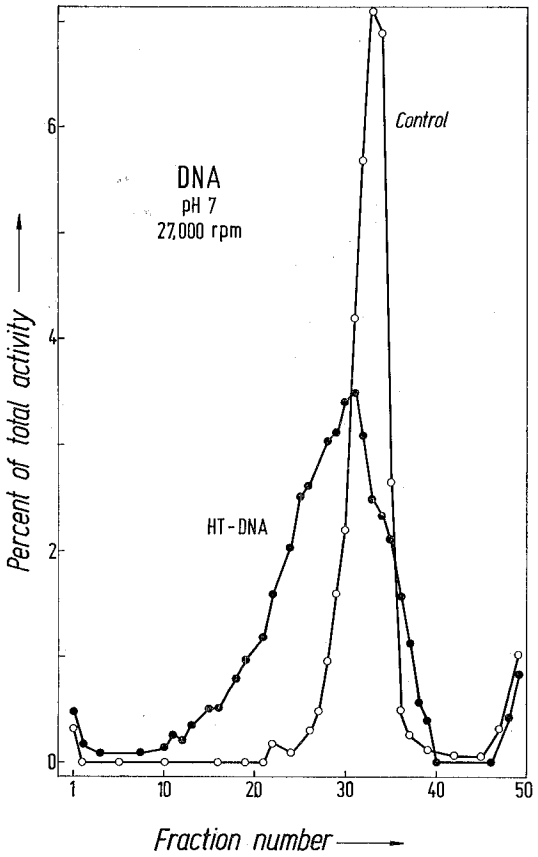

Figure 4 (*a*).

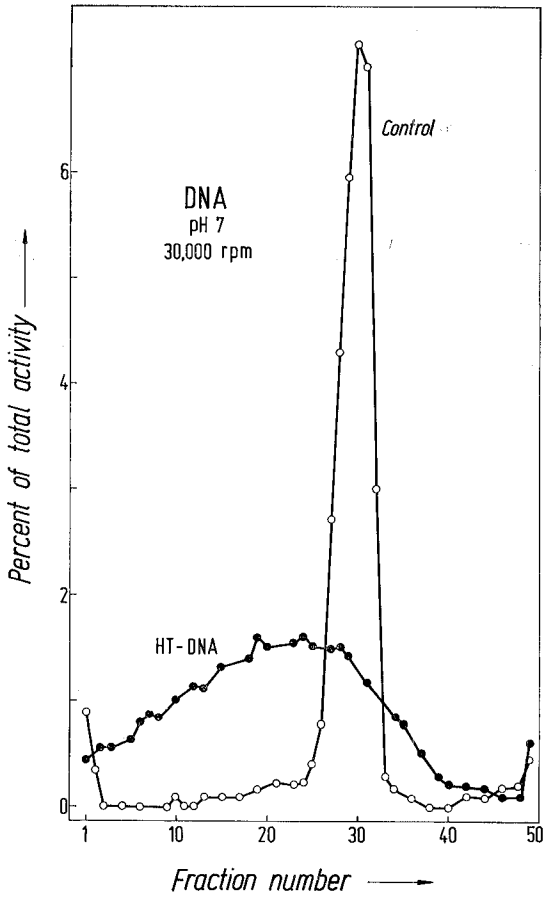

Figure 4 (*b*).

Figure 4 *continued on next page*

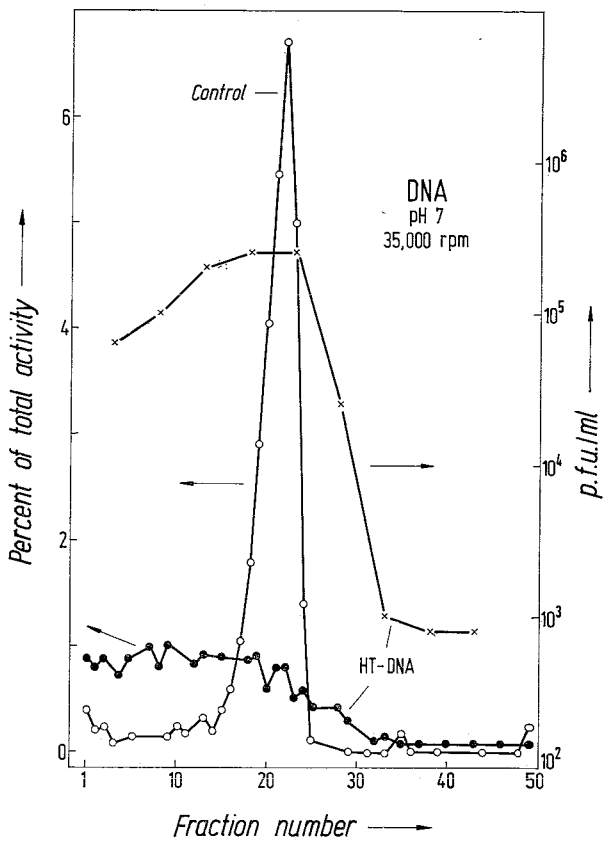

Figure 4 (*c*)

Figure 4. The effect of heat treatment (6 min at 100°C in 0·2 M NCES-buffer and then quickly cooled in ice water) on the sedimentation properties of infectious T1-DNA (HT-DNA) at three different speeds (*a*, *b*, *c*). (*c*) gives in addition the infectivity (p.f.u./ml) of the fractions of HT-DNA throughout the gradient.

distribution of the sedimenting molecules is observed, which is even more pronounced at 35 000 r.p.m. Under these conditions, a defined peak is not visible at all.

In addition, figure 4 (*c*) gives information about the infectivity of thermally-treated molecules in different fractions of a sucrose gradient. It is obvious from the data that the portion of HT-DNA molecules sedimenting faster than the control differ slightly in their infectious activity compared with the portion of HT-DNA molecules travelling at the speed of the reference molecules. There is only a small amount of material detectable in preparations of HT-DNA which sediments at a slower rate than the mass of untreated reference DNA and gives a low titer of plaque-forming units (p.f.u.) in the assay (fractions No. 25–32). We conclude from the sedimentation behaviour shown in figures 4 (*a*)–4 (*c*) that no detectable double-strand breaks are produced in HT-DNA, but that aggregation and unusual folding of the molecules occur as a result of heating and rapid cooling of T1-DNA in a buffer of high molarity.

To get further information on the physical configuration of thermally-treated infectious T1-DNA, we measured the intrinsic viscosity $[\eta]$ of such preparations.

Figure 5 shows a plot of η_{sp}/c against the concentration of HT-DNA. The extrapolation to zero concentration gives an intrinsic viscosity of $[\eta]=34{\cdot}5$ [dl./g]. Comparing this value with the intrinsic viscosities of native T1-DNA $[\eta]=145$ [dl./g] and denatured T1-DNA $[\eta]=26$ [dl./g] (Bohne, Coquerelle and Hagen 1970), we conclude that thermally-treated infectious T1-DNA has a collapsed, narrow-coiled structure similar to the compact coils of denatured DNA with low viscosity.

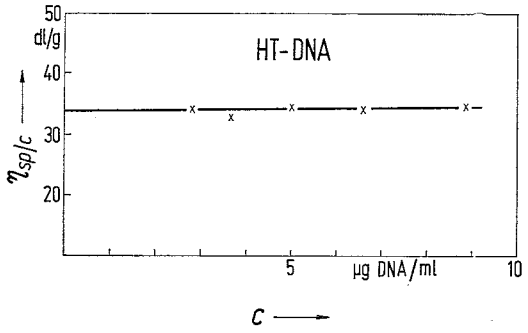

Figure 5. Dependence of the relative viscosity (η_{sp}/c) on concentration of infectious T1-DNA (HT-DNA) after heat treatment as is described in the legend of figure 4. The extrapolation to zero concentration gives the intrinsic viscosity $[\eta]=34{\cdot}5$ [dl./g].

Figure 6 gives the results of experiments performed to demonstrate single-strand breaks in thermally-treated infectious T1-DNA. It is evident from the data that the sedimentation behaviour of T1-DNA, denatured with alkali after heat-treatment of the molecules and sedimented in an alkaline sucrose gradient at pH 12, shows a reduced sedimentation rate compared with untreated T1-DNA. The shape of the curves giving the molecular distribution in the gradients is similar for control DNA and HT-DNA. From the position of the band of sedimenting denatured HT-DNA, we calculated the reduction of molecular weight of the single-stranded molecules according to the relationship proposed by Burgi and Hershey (1963). The average molecular weight is $6{\cdot}5\times10^6$. Using the relation mentioned under (§ 4.1) in the discussion, the mean number of single-strand breaks responsible for the reduction of the molecular weight is about 3 per T1 genome.

A partially-denatured structure of the HT-DNA molecules could be expected (Hotz and Mauser 1969) from the small increase in relative absorbance up to about 1·15 at 260 nm. To prove this, the buoyant density of HT-DNA was measured by CsCl density-gradient centrifugation (Meselson, Stahl and Vinograd 1957) as shown in figure 7. Here the sedimentation properties of a mixture of native and denatured T1-DNA were compared with those of thermally-treated infectious T1-DNA. Evidently, thermally-treated infectious T1-DNA bonds at a buoyant density corresponding to the density of 1·705 for native T1-DNA (Creaser and Taussig 1957). From these data we conclude that only a small portion of partially-denatured molecular regions might be produced by heat treatment (6 min at 100°c in 0·2 M NCES and then quickly cooled in ice-water) not detectable in the density-gradient. Otherwise we would expect the band of HT-DNA being broadened, with a second peak shifted towards the position of denatured T1-DNA.

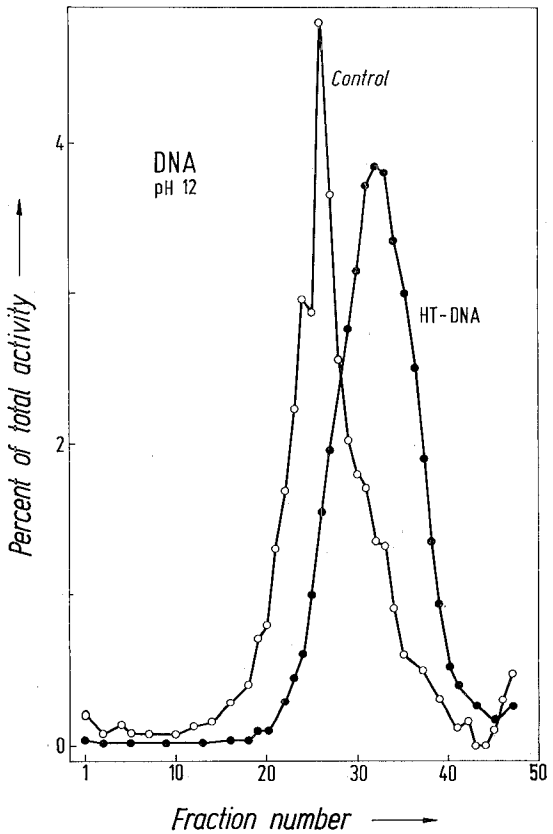

Figure 6. The effect of heat treatment (6 min at 100°c in 0·2 M NCES-buffer and then quickly cooled in ice-water) on the sedimentation pattern of infectious T1-DNA (HT-DNA) after alkali denaturation.

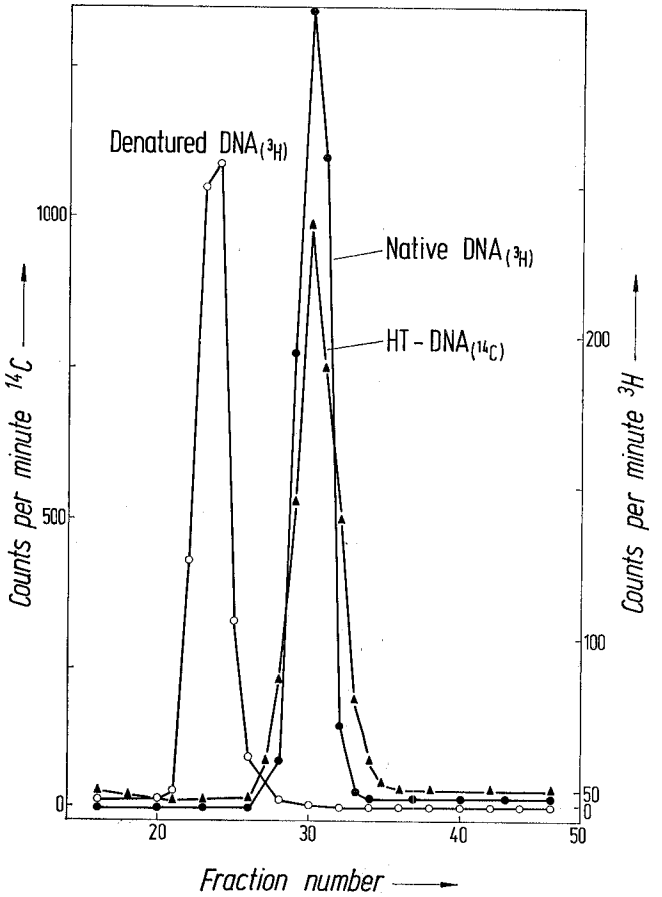

Figure 7. Density distribution of native, denatured and thermally treated (HT-DNA) infectious T1-DNA banded in CsCl-solution at 30 000 r.p.m. for 72 hrs in a rotor SW65K (Spinco L2-65) at 20°c.

3.3. *Sedimentation behaviour of UV-irradiated infectious T1-DNA*

Figure 8 shows the distribution of native infectious T1-DNA before and after U.V.-irradiation with a dose of 40 LH ($\sim 6 \times 10^3$ ergs/mm^2) sedimenting in a neutral sucrose gradient. 1 LH (lethal hit) inactivates infectious T1-DNA to a surviving fraction of 37 per cent. The number of hits and the corresponding radiation dose in ergs/mm^2 are taken from complete dose-effect curves of U.V.-irradiated infectious DNA unsubstituted or substituted by 5-bromouracil (BU). After application of the high dose mentioned above, very little change is noted in the sedimentation behaviour of the DNA. However, the molecular distribution of the sedimenting DNA is not as homogeneous as in the control, and a small portion of slower sedimenting material is observed at the base of the profile in figure 8.

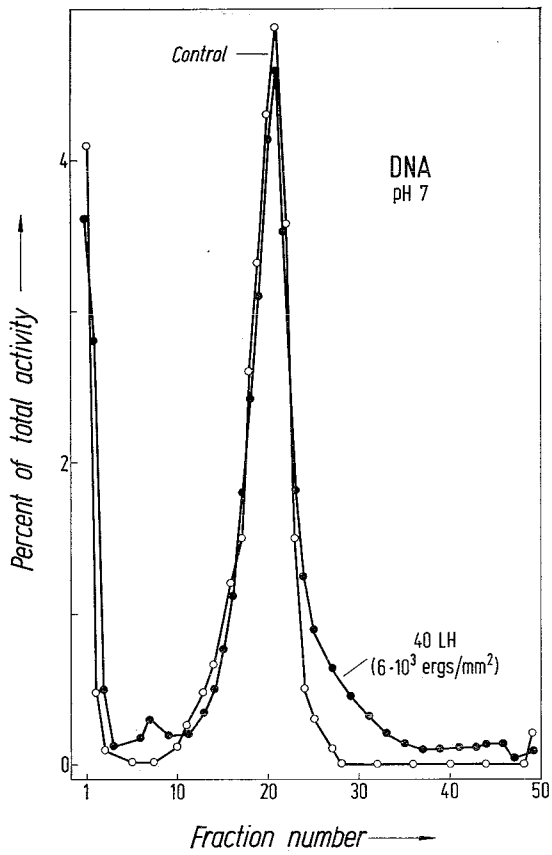

Figure 8. Sedimentation patterns of native infectious T1-DNA before and after U.V.-irradiation (2537 Å) with 6×10^3 ergs/mm^2 (40 LH).

A sedimentation pattern of T1-DNA denatured after U.V.-irradiation with 40 LH and analysed in an alkaline sucrose gradient is shown in figure 9. Compared with the unirradiated control DNA, whose sedimentation profile is already described in figure 2, the shape of the curve shows a more

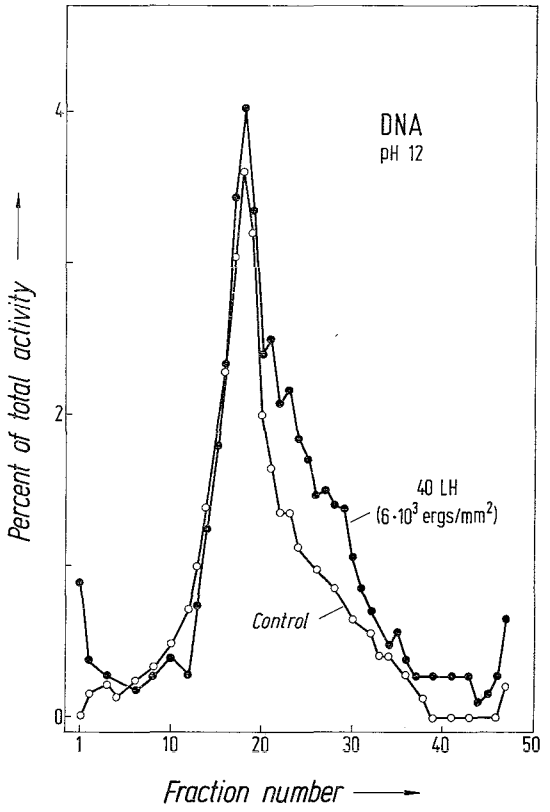

Figure 9. The effect of U.V.-light (6×10^3 ergs/mm^2; 40 LH) on the sedimentation pattern of alkali-denatured infectious T1-DNA (irradiation on native DNA).

heterogeneous distribution of the DNA molecules, with a small portion of molecules sedimenting at a slower rate. This fraction of DNA can be attributed to molecules with reduced molecular weight owing to single-strand breaks.

Figure 10 gives the results of a study of the sedimentation behaviour of U.V.-irradiated native infectious T1-DNA substituted by 5-bromouracil. The BU-DNA was irradiated with increasing doses of U.V.-light. The unirradiated control DNA shows a sedimentation profile similar to that of unsubstituted DNA (figure 8). As to their molecular weight, the distribution indicates homogeneity of the molecules. After U.V.-irradiation with 20 LH (corresponding to about $1{\cdot}2 \times 10^3$ ergs/mm^2), the material sedimenting at the position of the control decreases and the molecular distribution is more heterogeneous with a small fraction of molecules sedimenting at a slower rate than the control. Obviously this fraction represents broken DNA molecules and increases with increasing U.V.-dose. The new peak increases in size, and the band is shifted towards the meniscus indicating BU-DNA with smaller molecular weight. We conclude from the sedimentation behaviour of BU-DNA in a neutral sucrose gradient that double-strand breaks are produced by irradiation of infectious T1-DNA with high doses of U.V.-light when the molecules have been substituted by 5-bromouracil.

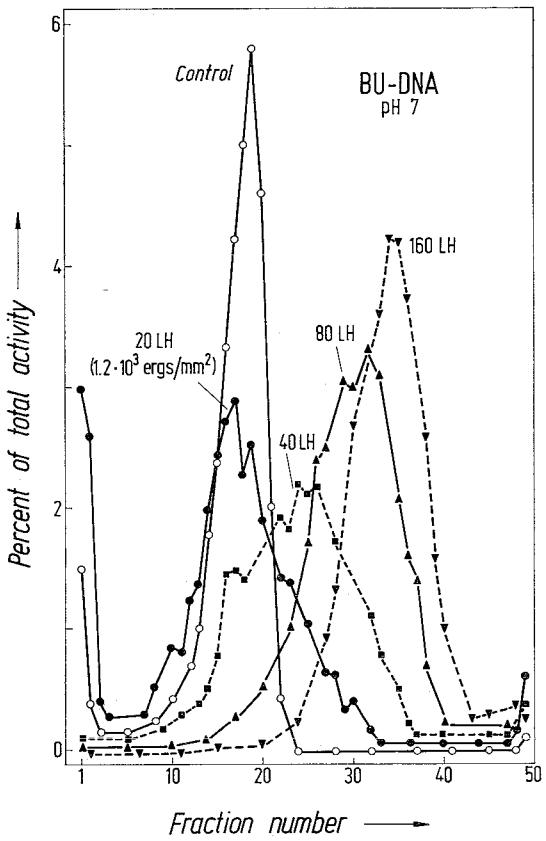

Figure 10. Effect of increasing U.V.-dose on the sedimentation pattern of native infectious BU-DNA of phage T1 (65 per cent of thymine substituted).

Figure 11 gives the results of experiments with infectious BU-DNA denatured after U.V.-irradiation with various doses and sedimented in an alkaline sucrose gradient at pH 12: the unirradiated control BU-DNA is comparable to unsubstituted DNA as far as the shape and position of the sedimentation band are concerned (figure 9). A U.V.-dose as small as 2 LH (corresponding to a surviving fraction of 13·5 per cent infectious molecules and to about $1{\cdot}2 \times 10^2$ ergs/mm^2) applied to native BU-DNA shifts the sedimentation band of denatured DNA significantly towards the meniscus. From the altered sedimentation behaviour of U.V.-irradiated BU-DNA in an alkaline sucrose gradient we conclude the occurrence of single-strand breaks to be proportional to the radiation dose.

Figures 12 and 13 demonstrate the effect exerted by 0·01 M cysteamine · HCl (Calbiochem, U.S.A.) on U.V.-induced strand breaks in infectious BU-DNA. It is obvious from figure 12 that the sedimentation behaviour of native BU-DNA, irradiated in the presence and absence of cysteamine is different, though an identical dose of 40 LH was applied in both experiments. Since cysteamine has a pronounced protective effect on the biological activity of infectious DNA of phage BU-T1 (Hotz and Mauser 1970) 40 LH applied to BU-DNA in the

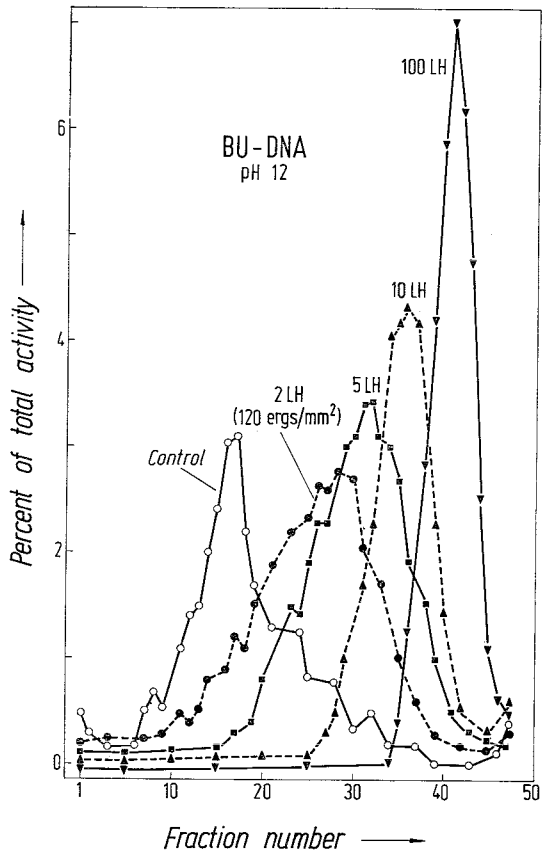

Figure 11. Effect of increasing U.V.-dose on the sedimentation pattern of alkali-denatured infectious BU-DNA of phage T1 (irradiation on native DNA).

presence of cysteamine correspond to an absolute U.V.-dose that is four times higher than the dose required for inactivation of BU-DNA in the absence of the protective substance. We conclude from the data shown in figure 12 that the radical scavenger present during irradiation reduces effectively double-strand breaks. The sedimentation profile of BU-DNA irradiated with 40 LH in the presence of cysteamine is comparable to the profile of BU-DNA irradiated with 20 LH in the absence of the protective substance (figure 10) as far as the shape and position of the band are concerned.

However, a different effect of cysteamine is observed when the BU-DNA is sedimented after denaturation in an alkaline sucrose gradient (figure 13). It is evident from these profiles that roughly the same number of single-strand breaks are produced by radiation in the presence and absence of cysteamine when the same dose (expressed in terms of lethal hits) is applied to the infectious BU-DNA. The question about the relevance of single-strand breaks to the radiosensitizing effect of BU-incorporation will be discussed in the following section.

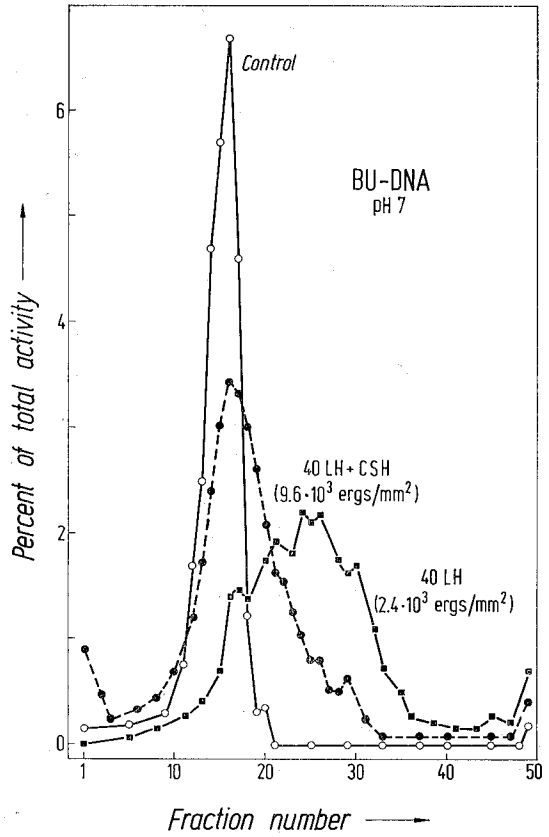

Figure 12. Effect of 0·01 M cysteamine · HCl (CSH) (Calbiochem, U.S.A.) on the sedimentation pattern of native BU-DNA of phage T1. The infectious T1-DNA received an u.v.-dose of 40 LH in the presence ($9{\cdot}6 \times 10^3$ ergs/mm²) and in the absence ($2{\cdot}4 \times 10^3$ ergs/mm²) of cysteamine.

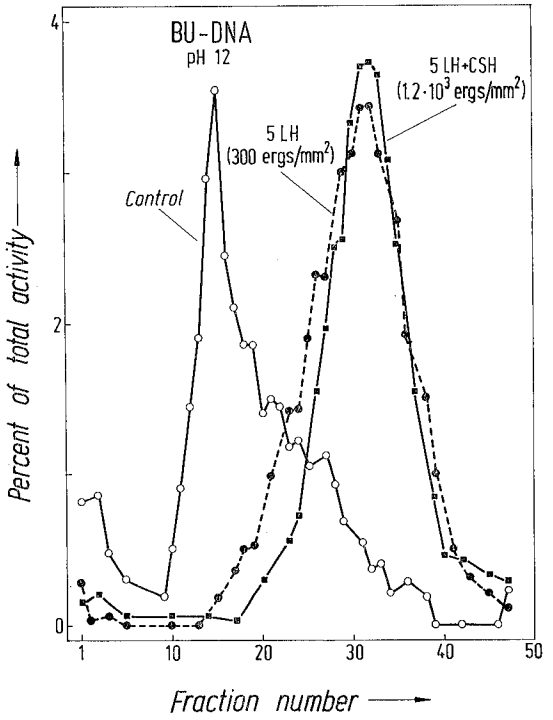

Figure 13. Effect of 0·01 M cysteamine · HCl (CSH) on the sedimentation pattern of alkali-denatured BU-DNA of phage T1. The infectious T1-DNA (native) received an u.v.-dose of 5 LH in the presence ($1{\cdot}2 \times 10^3$ ergs/mm²) and in the absence (300 ergs/mm²) of cysteamine.

4. Discussion

Evidence has been presented that storage, heat treatment and U.V.-light produce single-strand breaks in the infectious DNA of phage T1 under certain experimental conditions. Since the relevance of single-strand breaks to the loss of biological activity (infectivity) in phage DNA is still an open question, we concentrated our study on such breaks produced by different physicochemical events. As outlined in the introduction there is some evidence for the hypothesis that single-strand breaks without concomitant damage to DNA subunits are not lethal for phage-DNA.

4.1. *Alterations in stored T1-DNA*

Single-strand breaks are observed after storage of infectious T1-DNA (labelled and unlabelled with ^{14}C, respectively) in an appropriate buffer at 4°C. The relative molecular weight of the DNA species can be calculated from the distances (D) between meniscus and peak of the sedimentation profiles applying the formula proposed by Burgi and Hershey (1963): $(D_2/D_1)=(M_2/M_1)^{0\cdot4}$. The average number of single-strand breaks per DNA strand is given by the relation $(M_n/M_{n(stored)})-1$, with M_n being the number average molecular weight of an intact strand and $M_{n(stored)}$ the value of a broken strand. Since the mean molecular weight of denatured DNA is decreased to 1/6 after 20 days of storage (figure 2) this value corresponds to a mean number of 5 single-strand breaks per T1 genome. However, it is not known yet if single-strand breaks are formed during storage of the DNA or because of an alkaline lability of unknown primary lesions. Nevertheless, it is interesting to note that physicochemical changes do not alter the infectivity of T1-DNA molecules to any extent measurable by the assay system. From this observation we conclude that the phage host is able to repair certain molecular lesions occurring during prolonged storage of T1-DNA and that this repair system must obviously be different from those responsible for host-cell repair of U.V.-damage. However, other cellular mechanisms are known which repair damaged DNA especially under circumstances of high multiplicity of infection as is the case in all assay systems for infectious DNA. For a detailed discussion of the subject of DNA repair reference should be made to the publication by Strauss (1968). It is interesting to note that even single-strand breaks produced by ionizing radiation in bacteria, in phage particles or their infectious DNA are probably not lethal (Freifelder 1968, Ginsberg and Webster 1969). The repair of single-strand breaks caused by different physicochemical events must be a general phenomenon that is also associated with vital biological functions of DNA replication and recombination (Summers and Szybalski 1967).

4.2. *Alterations in thermally-treated T1-DNA*

From electron microscope pictures of ' heat-treated ' infectious T1-DNA (HT-DNA), a hypothetic model of an imperfect Watson–Crick helix was proposed (Hotz and Mauser 1969). A helical but unusual narrow-coiled DNA, partly mismatched, with loop-like formation, low viscosity and sedimentation behaviour differing from the $S^0_{20,w}$ measured for native, untreated T1-DNA, could be expected. The experimental data given in § 2 of this publication are consistent with this model, i.e., an intrinsic viscosity of $[\eta]=34\cdot5$ [dl./g] and an increased sedimentation coefficient. After denaturation of the HT-DNA three

single-strand breaks per T1 genome were measured on the average. Denatured molecular regions in HT-DNA could not be detected in our experiments based on CsCl-density gradient centrifugation. As to the absorbance at 260 nm of HT-DNA, it was noted earlier (Hotz and Mauser 1969) that this value was not equal to the level measured with native T1-DNA but was increased to about 1·15. This is probably due to the fact that single strands in the starting material were broken during heating and rapid cooling, and additional degradation also occurred during the experimental procedure, which prevented renaturation of a certain percentage of the strands.

Summing up, we conclude that heat treatment of T1-DNA under the experimental conditions used (high ionic strength and rapid cooling) does not favour unfolding of the single strands but leads to an improper renaturation with non-specific molecular associations. However, an infectious molecule is formed, though it probably contains many short-range folded single stranded regions similar to those proposed by the model of Doty, Boedtker, Fresco, Haselkorn and Litt (1959) and studied extensively by Studier (1969 a, b) with respect to their effects on renaturation of DNA. He concluded from his results on intra-strand interactions in T7-DNA that, if single strands are folded, nucleation and zippering of the DNA are affected, both of which are necessary for proper renaturation of a native molecule. From these studies of T7-DNA (Studier 1969 b) we can expect that in our preparations of infectious HT-DNA also complexes of more than two single strands are formed. It would be interesting to know whether such complexes are still infectious for spheroplasts.

4.3. *Alterations in U.V.-irradiated T1-DNA*

The results obtained in our experiments for U.V.-irradiated infectious unsubstituted and BU-substituted T1-DNA are in good agreement with data recently published on the same subject:

(*a*) No double-strand breaks and a negligible number of single-strand breaks are observed in unsubstituted infectious T1-DNA after U.V.-doses of biological interest. After U.V.-irradiation of coliphage T1 (Stephan, Miltenburger and Hotz 1970), phage T3 (Lion 1970) and phage T4 (Hotz and Walser 1970) similar results were reported, when the DNA was extracted after irradiation of the particles and analysed by sucrose gradient centrifugation.

(*b*) At small U.V.-doses corresponding to a few inactivation doses for infectious BU-substituted T1-DNA, no double-strand breaks are visible in a sucrose gradient, which is consistent with results obtained after irradiation of other T-phage mentioned above. At higher doses, e.g. 80 LH (corresponding to about 5×10^3 ergs/mm^2), all native molecules contain double-strand breaks, the yield, however, being very low.

(*c*) It is evident from our data that the yield of single-strand breaks is high in U.V.-irradiated BU-DNA, i.e. about 0·9 breaks per lethal hit, when calculated from the reduction of the molecular weight after 2 LH (figure 11) according to the formula of Burgi and Hershey (1963). This value is in good agreement with 1 single-strand break per lethal hit produced in BU-substituted T3 phage (Lion 1970). According to the formula outlined in § 4.1 we calculated from the sedimentation pattern (figures 10 and 11) the relation of double-strand breaks to single-strand breaks with 1/17 for infectious BU-DNA, which is different from the results obtained after U.V.-irradiation of BU-substituted T1-phage particles

(1/160) (Stephan *et al.* 1970). We conclude from this relation that double-strand breaks are not relevant to inactivation of the biological activity due to BU-incorporation. For the production of one single-strand break in infectious BU-DNA, an exposure to 45 ergs/mm^2 is needed. It is tempting to infer from these data that a single-strand break in BU-DNA is a lethal event and responsible for the increased U.V.-sensitivity of the plaque-forming ability of phage DNA, as was proposed recently for BU-substituted transforming DNA of *Bacillus subtilis* (Hutchinson and Hales 1970). Some results, however, make it questionable whether this hypothesis can be applied to phage DNA: Single-strand breaks are probably lethal events in the transformation of unsubstituted bacterial DNA, but they are repaired in phage DNA by the host (Summers and Szybalski 1967) as well as in bacterial DNA irradiated *in vivo* even in radiosensitive strains (Freifelder 1968, Ginsberg and Webster 1969). When the U.V.-dose is expressed in terms of phage lethal hits, the same number of single-strand breaks is observed in BU-phage T1 (Stephan *et al.* 1970) and in BU-T4 (Hotz and Walser 1970) in the absence and presence of 0·01 M cysteamine, respectively; however, unsubstituted phages which are inactivated to the same survival rate do not contain breaks. The observation that BU-phage and unsubstituted phage, U.V.-irradiated in the presence of cysteamine have the same D_{37} calls for further elucidation of the relevance of single-strand breaks to the specific damage to plaque-forming ability in BU-phage. Lesions to the deoxyribose and/or the bases were proposed recently to be the lethal event in BU-phage which can be influenced by the presence of cysteamine (Hotz and Reuschl 1967, Stephan *et al.* 1970).

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Au cours du stockage de l'ADN infectieux du phage T1 dans des conditions optimales de tampon (NCE) et de temperature (4°c) on a observé la formation de cassures simples dans la chaîne polynucléotidique. Après environ 20 jours le génome de T1 renferme 5 ruptures sans que l'infectiosité ne soit affectée.

Lorsque l'ADN-T1 infectieux est chauffé 6 min à 100°c en tampon NCE 0,2 M puis refroidi brusquement à 0°c, il y a renaturation désordonnée de l'ADN; l'activité infectieuse est alors quasi normale, bien qu'on ait à faire à une molécule fortement agglomérée de faible viscosité ([η] = 34,5 [dl/g]), à coefficient de sédimentation élevé et renfermant 3 cassures simples par génome.

Après inactivation ultraviolette (2537 Å) il n'apparaît qu'un nombre négligeable de cassures simples et aucune cassure double. Lorsque le 5-bromouracile est incorporé dans l'ADN infectieux on observe 0,9 cassure simple par évènement inactivant et une exposition de 45 ergs/mm^2 est nécessaire pour provoquer une rupture. On discute le rôle joué par les ruptures de chaîne dans l'activité biologique de l'ADN infectieux de T1.

Bei der Lagerung infectiöser T1-DNS unter optimalen Bedingungen, d.h. in einem geeigneten Puffer (NCE) und bei 4°c, wurden Einzelstrangbrüche gemessen. Nach etwa 20 Tagen enthält das T1-Genom im Mittel 5 Brüche, ohne dass die Infektiosität messbar abnimmt.

Wenn infektiöse T1 - DNS in 0,2 M NCE-Puffer während 6 min bei 100°c thermisch behandelt und anschliessend rasch auf 0°c abgekühlt wird, erfolgt eine

ungeordnete Renaturierung. Das Molekül besitzt fast eine normale infektiöse Aktivität, obwohl es sich um ein hochgradig geknäueltes Molekül mit niederer Viskositat ($[\eta]$ = 34,5 [dl/g]), einem erhöhten Sedimentationskoeffizienten und 3 Einzelstrangbrüchen pro Genom handelt.

Nach Inaktivierung infektiöser T1-DNS mit U.V.-Licht der Wellenlänge 2537 Å treten keine Doppel- und nur vernachlässigbar wenige Einzelstrangbrüche auf. Andererseits werden nach Einbau von 5-Bromuracil in die infektiöse DNS 0,9 Einzelstrangbrüche pro inaktivierendem Treffer gemessen, wobei zur Erzeugung eines Bruches eine Exposition von 45 erg/mm² benötigt wird. Die Bedeutung der DNS-Brüche für die biologische Aktivität der infektiösen T1-DNA wird diskutiert.

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