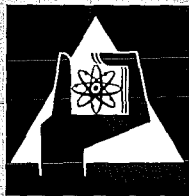# KERNFORSCHUNGSZENTRUM

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Temperature-Dependence of Radiation Sensitivity in the Dry State: A Modell Derived from Experiments Using Atomic Hydrogen. II

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Temperature-dependence of radiation sensitivity in the dry state: A model derived from experiments using atomic hydrogen.   II

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In a previous series of experiments it was shown that activation energies of roughly 1 kcal/mole, quite frequently found for the dependence on temperature of the sensitivity of biological material towards ionizing radiation in the dry state, are most probably due to the action of atomic hydrogen.   In all our experiments, diffusible and reactive species were liberated by proton bombardment of thin foils of organic material and allowed to react with the sample material. Whereas in the earlier experiments the temperature of either the samples alone (method A), or both foil and samples (method B), were varied, a third mode of exposure has now been used (method C) allowing the temperature of the bombarded foil alone to be varied (down to 88°K), while the phage samples were kept at room temperature.   The rate of phage-inactivation by radical attack in dependence on temperature leads to activation energies of $0 \cdot 65 \pm 0 \cdot 1$ kcal/mole for method A, $0 \cdot 95 \pm 0 \cdot 1$ kcal/mole for method B, and $0 \cdot 34 \pm 0 \cdot 05$ kcal/mole for method C.   This result lends strong support to the somewhat speculative interpretation given earlier.   According to this interpretation, H radicals separated from macromolecules by ionizing radiation need an activation energy to leave the site of their production and be liberated for good.   There is now good experimental evidence that this activation energy for liberation accounts for the difference of roughly $0 \cdot 3$ kcal/mole between the activation energies found in methods A and B.

1.   Introduction

Inactivation of biomolecules by the action of atomic hydrogen (e.g. from a gas discharge) is a well-known phenomenon.   Furthermore, there is good reason to assume that radiation-induced hydrogen atoms contribute to the total damage caused by ionizing irradiation of biological material in the dry state (cf. the model suggested by Braams, 1963).   Experimental evidence for this hypothesis was furnished by a series of experiments (Jung and Kürzinger 1968). These initial results allowed reconsideration of some vague hypotheses about the correlation between the temperature dependence of radiation sensitivity and the contribution of mobile radicals to the total damage.   To check the validity of these hypotheses, the influence of temperature on the inactivation rate of phage T1 exposed to radiation-induced atomic hydrogen was investigated (Kürzinger 1969).   The reactive species were produced by 2 MeV protons impinging on thin foils of mylar (polyethyleneterephthalate) or formvar (polyvinylformal) at a small angle of incidence and could reach the samples arranged *in vacuo* at a short distance.   The experimental set-up allowed two modes of exposure: In method A, only the temperature of the test material was varied, whereas the irradiated foil was kept at room temperature.   Thus,

the yield of atomic hydrogen remained constant, and the influence of temperature on the reaction and/or diffusion rate within the sample material could be studied. In method B, with foil and samples at the same temperature, conditions prevailing at direct inactivation by irradiation were simulated: the entities giving off the diffusible radicals and the molecules reacting with these were at the same temperature. The plaque-forming ability of phage was destroyed exponentially with exposure time in both cases. From a kinetic point of view, the reciprocal of the 37 per cent survival exposure time $(1/t_{37})$ is a measure of the velocity or the reaction constant of the inactivating process. Plotted in a semi-log scale versus reciprocal of temperature (Arrhenius plot), the $1/t_{37}$ values for the various inactivation curves fell on straight lines for both methods. From the slopes of these lines, activation energies of 0.65 ± 0.1 kcal/mole and 0.95 ± 0.1 kcal/mole could be derived for methods A and B, respectively. The difference between these two values could only be ascribed to the temperature dependence of the yield of reactive species liberated from the foil. Therefore, an activation energy E_a^L (L = liberation) of roughly 0.3 kcal/mole was postulated. This amount of thermal energy should help the split-off small radical to leave the site of its production, i.e. to overcome steric restrictions or ' cage-effects at the parent molecule '. Therefore, an investigation was started aiming at the temperature dependence of the radical yield alone. To this end, a third mode of exposure (method C) was devised, in which the temperature of the irradiated foil (the radical source) was the only variable parameter.

2. Materials and methods

2.1. *Microbiological assay*

Purification and concentration of phage T1 batches as well as sample preparation and plating of phage was performed in exactly the same way as in the previous studies (Jung and Kürzinger 1968, Kürzinger 1969). In spite of the identical procedure, the survival rate of phage after freeze-drying was lower by nearly one order of magnitude in the present experiment. For that reason, two or three exposure runs at the same temperature were combined in any survival curve in order to compensate for the poorer statistics of the single experiment.

2.2. *Exposure chamber*

Again the principle of the ' condenser experiment ' (Jung and Kürzinger 1968) was used. A schematic representation of the arrangement chosen for the present investigation (method C) is given in figure 1. This arrangement is the reverse of method A: The temperature-adjustable magazine is coated with the foil, and the sample glass discs are fastened to the polygonal metal plate kept at room temperature. Geometrical conditions, i.e. angle of incidence of the protons and foil-to-sample distance, were identical to those in methods A and B (Kürzinger 1969). Again a grid was put in proper position and at an adequate negative potential to keep off secondary electrons. The sample holder is a 24-cornered polygonal steel plate in a horizontal position, providing for 48 sample discs. It can be revolved, so that the samples are brought successively to a position opposite to the foil and exposed in pairs. Through its supporting shaft, water can be circulated into a pipe system within the sample-holder to
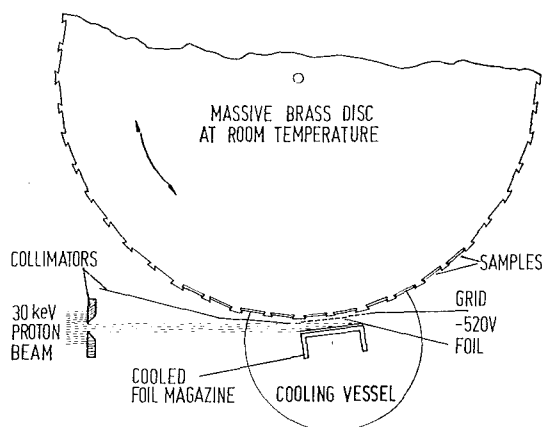
Figure 1.   Schematic representation of the arrangement for exposing specimens to atomic hydrogen generated by bombarding a thin foil of organic material with 30 keV protons according to method C (see text).

avoid heating or cooling by photon radiation.   The magazine bearing the organic foil is inserted into a vertical thermostat vessel.   During the irradiation, it is moved up and down automatically across the exposure slot by means of a motor-driven screw rod.   Temperature adjustment and measurement is performed as in the earlier study (Kürzinger 1969).   The lowest temperature obtained for the foil was 88°K, whereas the value for the samples (room temperature) could be stabilized within 2 degrees.   During operation, the pressure within the chamber was lower than 3×10^{-5} Torr.

2.3.   *Irradiation and exposure procedure*

As the 2 MeV Van de Graaff machine used previously was no longer at our disposal, the exposure chamber was installed at a home-made low-energy accelerator (Jung 1965) producing protons of 30 keV.   Because of this low energy, the thickness of the formvar foil was reduced to 1200 Å to avoid charge-up effects.   The procedure of coating the foil magazine is already described (Kürzinger 1969).   During exposure, the magazine was kept moving up and down with a velocity of 3·5 mm/sec; thus no perceptible warming of the irradiated section of the foil could occur.   The proton beam could be defocused, so that the beam intensity was sufficiently homogeneous across the aperture of the irradiation diaphragm.   In each run 48 samples were exposed, and an additional dozen served as control samples.

3.   Results and discussion

As in our earlier experiments using methods A and B, the plaque-forming ability of phage samples decreased with increasing exposure time, reaching an asymptotic value (5 to 30 per cent) for the longer exposures (cf. figure 3 in Kürzinger 1969) which is due to lack of penetration into parts of the sample. After subtracting this constant fraction, exponential curves are obtained in all cases.   As the rate of inactivation depends on the intensity of the proton beam (Kürzinger 1969, equation (6)), the proton flux was varied to obtain the same t_{37} value at room temperature as had been found with methods A and B (at room

temperature all three exposure methods are identical). This could be achieved with a 30 keV proton flux of 5×10^{11} p/cm² sec, or a beam current of 8×10^{-8} amp/cm², compared with $1 \cdot 56 \times 10^{12}$ p/cm² sec and $2 \cdot 5 \times 10^{-7}$ amp/cm², respectively, for the 2 MeV proton irradiation. It is seen that the slow protons are more effective by a factor of 3 in releasing reactive species from the irradiated foil. This is not surprising, as the slow protons are ranging near the Bragg peak
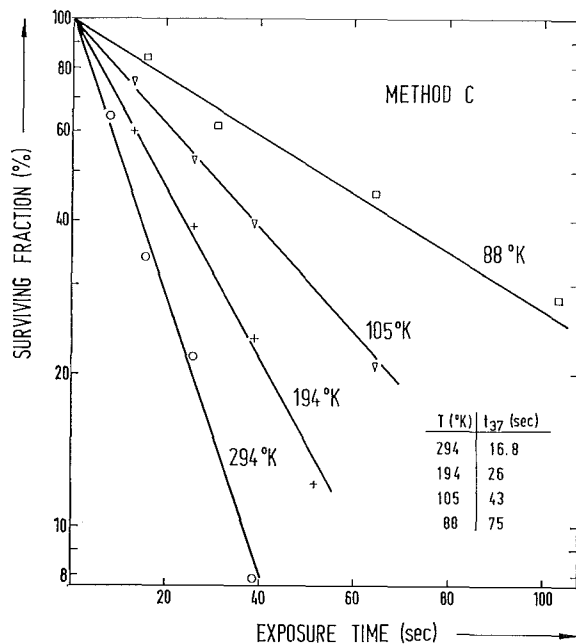

Figure 2. Inactivation of dry phage T1 by exposure to radiation-induced atomic hydrogen (irradiated foil at various temperatures).

(80–90 keV) and, therefore, have a higher LET value. Furthermore, this difference between the beam intensities in both series indicates again that the diffusion length of the split-off radicals is very short (probably a few hundred angstroms at most), as the radicals liberated deeper within the foil by fast protons obviously cannot diffuse out of the foil surface (Kürzinger 1969). Some typical inactivation curves as obtained with method C at various temperatures are shown in figure 2. The reciprocal of the t_{37}-values derived from all inactivation curves are plotted versus the reciprocal of temperature (Arrhenius plot) in figure 3. The data can be represented quite well by a straight line that is described by the equation:

$$1/t_{37} = c_0 \exp\left(-E_a{}^C/RT\right) \quad (\text{sec}^{-1}), \qquad (1)$$

with $c_0 = 0 \cdot 084$ sec^{-1} and $E_a{}^C = 0 \cdot 34 \pm 0 \cdot 05$ kcal/mole. Again, as for methods A and B, there is no indication of a temperature-independent component to be detected. In figure 4, the results obtained with the three exposure methods A, B and C are summarized. Within experimental error the activation energy $E_a{}^C$ is equal to the difference between the values $E_a{}^A$ and $E_a{}^B$ derived from previous results. For this difference the symbol $E_a{}^L$ was chosen, as it was ascribed to some act of liberation after the radiation-induced primary segregation of a small radical from a macromolecule. The present experimental findings demonstrate
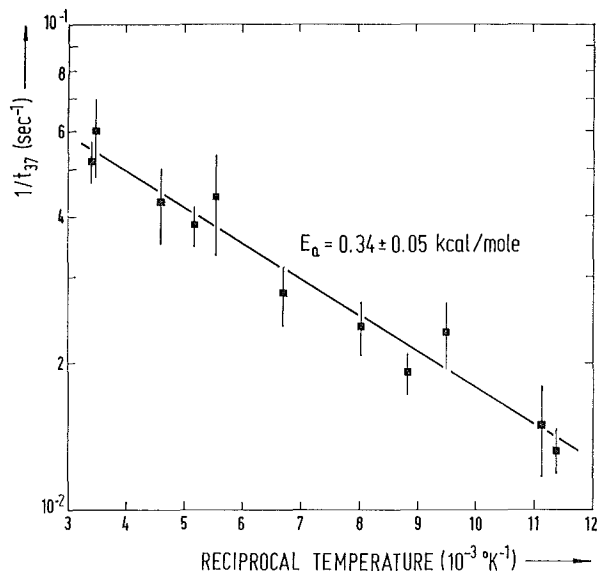
Figure 3. Reciprocal of 37 per cent survival exposure time for the inactivation of dry phage T1 by radiation-induced atomic hydrogen versus reciprocal of temperature (Arrhenius plot—method C).

that this notation and the interpretation on which it is based are reasonable. Corresponding investigations with infectious DNA of phage $\emptyset$X174 (Wintermantel 1971) indicate that the values of activation energy reported here are not restricted to one type of biomolecules. Additional information regarding the meaning of our results for molecular radiobiology may be found in a review article by Zimmer and colleagues (1969) and in a textbook by Dertinger and Jung (1970).
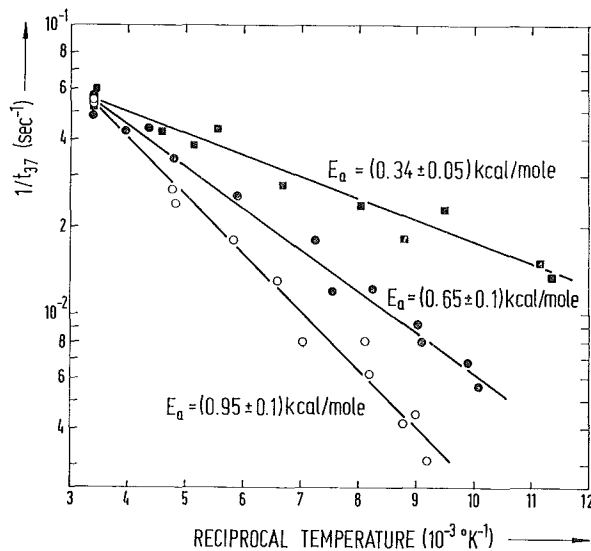

Figure 4. Arrhenius plots for the inactivation of dry phage T1 by radiation-induced atomic hydrogen. Closed circles: method A. Open circles: method B. Squares: method C.

Au cours d'études antérieures relatives à l'influence de la température sur l'inactivation du phage T1 par des radicaux mobiles radio-induits, on a montré que des énergies d'activation de l'ordre de 1 kcal/mol, valeur d'ailleurs souvent déterminée pour la radio-sensibilité de macromolécules biologiques en absence d'humidité, relevaient très vraisemblablement de l'action de l'hydrogène radicalaire. Pour les expériences décrites ici, les agents réactifs diffusibles responsables de l'inactivation ont été produits par bombardement protonique de membranes artificielles. Alors que pour les mesures antérieures, on variait seule la température des échantillons T1 (méthode A) ou bien simultanément celle des échantillons et de la membrane génératrice d'agents réactifs (méthode B), une troisième méthode C permet de varier uniquement la température de la membrane (jusqu'à 88°K) tout en maintenant les échantillons phages à la température ambiante de 293°K environ. L'énergie d'activation déterminée par les différentes méthodes est (en kcal/mol): méthode A: $0,65 \pm 0,1$; méthode B: $0,95 \pm 0,1$; méthode C: $0,34 \pm 0,05$. Cette dernière valeur confirme l'interprétation plus ou moins spéculative des résultats antérieurs: les radicaux hydrogène, arrachés des macromolécules par les radiations ionisantes, nécessitent pour leur libération totale du lieu de génération une énergie d'activation d'environ 0,3 kcal/mol.

In vorausgegangenen Untersuchungen über den Einfluß der Temperatur auf die Inaktivierungsrate von T1-Phagen durch strahleninduzierte mobile Radikale im Vakuum konnte gezeigt werden, daß Aktivierungsenergien von etwa 1 kcal/Mol, wie sie für die Temperaturabhängigkeit der Strahlenempfindlichkeit trockener makromolekularer biologischer Objekte häufig berichtet werden, mit großer Wahrscheinlichkeit auf die Wirkung atomaren Wasserstoffs zurückzuführen sind. In allen hier geschilderten Experimenten wurden durch Protonenbeschuß von dünnen Kunststoff-Folien diffusible reaktionsfähige Agentien freigesetzt, die auf das Probenmaterial einwirken konnten. Während in den früheren Experimenten die Temperatur entweder der Proben allein (Methode A) oder der Proben und der Folie zusammen (Methode B) variiert wurde, ließ sich nun in einer dritten Expositionsanordnung (Methode C) lediglich die Temperatur der bestrahlten Folie ändern (bis 88°K), wobei die Phagenproben auf Raumtemperatur gehalten wurden. Für die Temperaturabhängigkeit der Inaktivierungsrate von T1-Phagen durch Radikalangriff hatten sich Aktivierungsenergien von $0,65 \pm 0,1$ kcal/Mol (Methode A) und $0,95 \pm 0,1$ kcal/Mol (Methode B) ergeben. Nun konnte aus den Inaktivierungsraten in Methode C ein Wert von $0,34 \pm 0,05$ kcal/Mol abgeleitet werden. Dieses Ergebnis untermauert die kürzlich gegebene, bisher noch etwas spekulative Interpretation. Danach benötigen H-Radikale, die durch ionisierende Strahlung von Makromolekülen abgespalten wurden, eine Aktivierungsenergie, um ihren Entstehungsort verlassen zu können und damit endgültig freigesetzt zu sein. Die jetzt gewonnenen Ergebnisse legen die Deutung nahe, daß die Differenz von etwa 0,3 kcal/Mol zwischen den nach Methode A und B gefundenen Energiewerten als Aktivierungsenergie für die Radikalfreisetzung anzusehen ist.

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