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PRODUCTION OF FREE RADICALS IN SOLID BIOLOGICAL COMPOUNDS
EXPOSED TO VARIOUS IONIZING RADIATIONS

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July 20, 1965

SUMMARY

The effect of the irradiation temperature on the production of secondary radicals in glycine, trypsin, and lysozyme has been studied. The solid compounds were irradiated in vacuum, with fast stripped helium, carbon, and argon ions at different temperatures in the range 77° to 320°K. The number of secondary radicals was measured with an ESR spectrometer after the samples had been kept at room temperature for 20 minutes.

It was found that the radical yield was independent of the irradiation temperature up to 100° to 120°K. Above this level the yield increased and in the temperature range 77° to 295°K the total variation averaged a factor of 2.2. This variation is assumed to be due to a temperature effect on the radical reactions that take place during irradiation. It was found that the yield-versus-temperature curves could be adequately described by a sum of exponential functions.

The experiments on trypsin showed that there is a good correlation between the production of secondary radicals and the loss of enzymatic activity when the irradiation temperature varies. Similarly, a good correlation was found in experiments in which the stopping power of the radiation varied. Based on these results, a model for the inactivation of

solid enzymes has been proposed. It is assumed that the loss of enzymatic activity is the result of a sequence of events in which free radicals are important intermediates.

INTRODUCTION

When solid biological compounds are irradiated at low temperatures the types and yields of radicals formed are usually different from those observed at room temperature (1-4). However, the room-temperature types are formed when the low-temperature-irradiated samples are annealed for a few minutes at room temperature. It appears that the yields of these radicals, which will here be called the "secondary radicals," are influenced by the irradiation temperature. In a series of experiments, including more than 40 different substances, it was found that the yield of secondary radicals after irradiation at 295°K was larger than that observed after irradiation at 77°K followed by annealing at 295°K, by a factor of approximately 2 to 3 (3, 5). It is of interest to note that the inactivation of solid enzymes (6) shows a temperature dependence similar to that for the production of secondary radicals. Recently Brustad (7) studied the effect of the irradiation temperature on the inactivation of trypsin in the temperature range 10° to 420°K. The results show that above 100°K the apparent inactivation cross section increased with increasing temperature. The results were interpreted to indicate that three different processes are involved in the inactivation mechanism--one temperature-independent, and two that depend upon the irradiation temperature but can be distinguished by different activation energies.

In the experiment reported here the enzymes trypsin and lysozyme as well as the amino acid glycine have been irradiated with heavy ions at different temperatures ranging from 77° to 320°K. The yield of secondary radicals has been measured by ESR spectroscopy, and is given as a function of the irradiation temperature. The purpose of these experiments is first to study the effect of the temperature on the reactions leading to the secondary radicals, and secondly to correlate the yield of secondary radicals with the loss of enzymatic activity. It is hoped that such correlations may shed some light on the mechanism for radiation inactivation of solid enzymes.

EXPERIMENTAL PROCEDURE

The ESR spectrometer and the procedure used to obtain quantitative data have been described elsewhere (8).

Glycine and trypsin were obtained from Nutritional Biochemical Corporation, and lysozyme from Worthington Biochemical Corporation. The samples were irradiated in thin layers (20 to 50 mg/cm²), in brass holders, in vacuum. During irradiation the samples were mounted on a metal surface where the temperature could be controlled by circulating liquid nitrogen, nitrogen gas, or water inside the metal plate. During each run, 10 samples were irradiated. The sample temperature was lowered in steps, between exposure of successive samples, in order to slow down or stop those secondary reactions that may begin during exposure of a sample to the radiation. When the samples subsequently were brought to room temperature these reactions continued (an example is shown in Fig. 1) under conditions that were alike for all samples.

After exposure the samples were first brought to liquid nitrogen temperature, then transferred to glass sample tubes, evacuated, and sealed off. During this procedure the samples reached room temperature for approximately 5 minutes. They were also exposed to air for 1 min during the transfer process. Before the ESR measurements the samples were kept at 295°K for an additional 15 min. As mentioned above, this procedure allowed the secondary radical reactions to take place (Fig. 1), and ensured that all samples of the same substance exhibited the same

resonance spectrum characteristic of the secondary radicals.

Radiation Source and Dosimetry

The samples were irradiated with fast stripped helium, carbon, and argon ions from the Berkeley heavy-ion linear accelerator. The radiation dose was obtained by measuring the charge collected by a Faraday cup. In these experiments the same dose was given to all samples irradiated with one particular type of ions. The dose was in the range 2 to 5×10^6 rads, and was given in the course of a 2-min irradiation. The numbers of ESR centers produced per gram irradiated were obtained, and are given in relative units. A more detailed description of the quantitative procedure is given elsewhere (8).

RESULTS

When glycine is irradiated at room temperature, or at low temperatures followed by heat treatment at room temperature, the ESR spectrum of a polycrystalline sample is the well-known triplet with a weak line on each side (8). Experiments on single crystals (9—12) indicate that the two radicals $^+\text{NH}_3\dot{\text{C}}\text{HCOO}^-$ and $\cdot\text{CH}_2\text{COO}^-$ may be responsible for this spectrum. In these experiments the same resonance pattern was found for all three types of radiation used (8). The yields of the secondary radicals at different irradiation temperatures are shown in Fig. 2. It appears that the yield is constant up to a temperature of about 100°K. Above this level the yield seems to increase almost linearly with the irradiation temperature, and the total variation in the temperature range here studied is by almost a factor of 3. The results in Fig. 2 show that the temperature effect is little influenced by the type of radiation, although it seems to be more pronounced

for radiation with small stopping power.

In Fig. 3 the results for glycine are replotted in a semilogarithmic plot, where the reciprocal of the irradiation temperature is given along the abscissa. The purpose of this plot is to see if the yield versus the irradiation temperature can be expressed by a sum of exponential functions,

$$Y_T = \sum_i Y_{i0} \exp(-E_i/kT),$$

where Y_T is the radical yield at the irradiation temperature T . The parameter E_i describes the slope of the straight line in this Arrhenius plot. This parameter may also be interpreted as the apparent activation energy for the underlying process (see below). A similar procedure has previously been used by Brustad (7) to describe the effect of the irradiation temperature on the inactivation of solid trypsin.

It appears that the curves in Fig. 2 can be adequately fitted by a sum of two straight lines for which the values of E_i are $E_1 = 0$, and $E_2 = 800$ to 1200 cal/mole (see also Table I).

The two enzymes trypsin and lysozyme both exhibit a composite resonance pattern when irradiated at room temperature (Fig. 4). The spectrum consists of a doublet with approximately 17-oersted (or gauss) splitting and a g factor of $g = 2.0028$, and a broad resonance with principal g values $g_1 = 2.003$ (masked by the doublet), $g_2 = 2.022$, and $g_3 = 2.055$. Good evidence is now available (13) for the interpretation that the broad resonance is due to a sulfur radical in which the unpaired electron is localized mainly on the sulfur atom in the cysteine residue, and that the doublet is due to a radical in which the unpaired electron is localized in a π orbital on an α -carbon atom in the protein backbone:

The group R is primarily a hydrogen atom.

When the proteins are irradiated at low temperatures the ESR spectra are completely different from those at room temperature (the results for lysozyme are shown in Fig. 1) (2,4). When the samples are annealed at higher temperatures radical reactions take place, as indicated in Fig. 1, and the two types of secondary radicals are formed. The effect of the irradiation temperature on the yield of these secondary radicals is shown in Fig. 5. The curves for both trypsin and lysozyme have the same shape as that found for glycine. The total variation in the yield, within the temperature range studied, is by a factor 2 to 2.5. The results for trypsin and lysozyme can also be fitted by a sum of either two or three straight lines, and the values for the slopes of these lines (E_1) are given in Table I.

Correlation Between the Production of Secondary Radicals and the Loss of Enzymatic Activity

In Fig. 6 the production of secondary radicals in trypsin, irradiated with fast stripped carbon ions, is correlated with the loss of enzymatic activity. Within the temperature range studied there appears to be a surprisingly good correlation between these two types of measurements. Thus, the yields seem to be constant up to about 120°K, and the yields at 77°K is approximately half that at 295°K for both types of measurement. The radical measurements have been carried out only up to 320°K, because the secondary radicals become unstable at elevated temperatures. We may

therefore expect that the radical curve will drop below the inactivation curve at temperatures above 300°K.

A quantitative measure for the correlation between the production of secondary radicals and the biological end effect can be obtained from Arrhenius plots like those in Figs. 3 and 5. If the procedure of using a sum of exponential functions is applied, the values for the slopes of these lines can be correlated. In Table I are assembled the results so far obtained for the two types of measurements. The inactivation data for trypsin are taken from Brustad's experiments (7), whereas the results for lysozyme and ribonuclease are taken from Fluke's experiments (14). The following observations can be made from this table:

(a) The results for all four compounds can be described by a sum of two or three exponential functions. The apparent activation energies are zero, about 1 kcal/mole, and approximately 3 to 5 kcal/mole.

(b) In some experiments, in which the radical measurements were carried out after the samples had been kept at room temperature for 20 min and 2 days respectively, it was found that the curves changed slightly with time. However, the changes in the apparent activation energies are small.

(c) The results indicate that there is a good correlation between the production of secondary radicals and the loss of enzymatic activity. It is therefore reasonable to assume that the same processes are responsible for both the observed effects.

The data presented in Table I are, to the author's knowledge, the only experiments so far reported in which the effect of the irradiation temperature on the radiation yield has been described in detail. On the

other hand, a number of experiments have been reported in which a few specific temperatures, like 77°K and 295°K, have been used (3—6, 15, 16). Although the yield ratio $Y_{295^{\circ}\text{K}}/Y_{77^{\circ}\text{K}}$ cannot give a detailed description of the temperature effect, it gives an indication of how large the effect is within this range. In Table II are assembled the results of this work together with a number of other experiments. Again there appears to be a good correlation between the production of secondary radicals and the loss of enzymatic activity. Thus, the average value for all the radical data is 2.34, whereas the corresponding value for the inactivation data is 2.23. The radical data include several small-molecule compounds, and if we take only the ESR results for the different proteins the ratio drops to 2.24.

DISCUSSION

The experiments described here have demonstrated that the production of secondary radicals in solid biological substances is influenced by the irradiation temperature. The observed radical yield is usually independent of the irradiation temperature up to 100° or 120°K. At higher temperatures the yield increases with the temperature and the total variation in the range 77° to 295°K is of order 2 to 3.

When radiation energy is absorbed in a compound a variety of initial ESR centers are formed which subsequently take part in several secondary reactions (1—4). These reactions lead both to nonradical species and to the secondary radicals here observed. Since these reactions certainly have different activation energies, their relative importance can be varied by changing the temperature at which they proceed. In these experiments only the irradiation temperature was varied, and the subsequent procedure was the same for all samples. Since previous experiments (3, 4) have

shown that radical reactions may proceed in solids above 100°K, it can be concluded that radical reactions are already taking place, to a greater or lesser extent, during exposure. It seems reasonable therefore to ascribe the results of this work to a temperature effect on the secondary radical reactions.

The yield-versus-temperature curves can be described mathematically by a sum of exponential functions. One interpretation of this is that several processes are involved in the formation of the secondary radicals. The results in Table I may be interpreted to mean that we have one temperature-independent process ($E_1 = 0$) and one or two processes that depend upon the irradiation temperature. However, whether this is a correct interpretation is questionable. Thus, the observation that the yield of secondary radicals and the loss of enzymatic activity are independent of the irradiation temperature below approximately 100°K may be due to another cause. Absorption of radiation energy may lead to a localized heating of small regions of the sample. This may allow radical reactions or energy transfer to take place in a small volume near the site of the absorption at a rate characteristic of a much higher temperature than the actual average temperature of the sample. Approximately 100°K may then be an "effective temperature" describing the rate at which the secondary reactions take place in the "hot spot" produced by a localized absorption of radiation energy. This means that the observed invariance of the radiation yield below 100°K is due to an "effective localized irradiation temperature" independent of the actual sample temperature. When the yield-versus-temperature curves are analyzed we must therefore neglect the part below 100°K. It then appears that the curves can be described mathematically by

a sum of only two straight lines for which the $E_1^{\ddagger}$ values are much smaller than those obtained by the first interpretation (see Table I). According to the latter interpretation we have only two temperature-dependent processes, with activation energies $E_1^{\ddagger} = 0.1$ kcal/mole and $E_2^{\ddagger} = 1.5$ to 3 kcal/mole. It is not possible, at present, to decide which interpretation is the more correct, but it should be pointed out that in either case there is a good correlation between the production of secondary radicals and the loss of enzymatic activity.

A topic of general interest is the relation between the radicals produced and the observable biological damage. It is generally accepted that in aqueous solutions the chemical damage is produced largely by the radiation-induced free radicals. This has certainly prompted the assumption that the radiation damage on solid biological systems is also caused fully or partly by the free radicals (17—22). However, so far we have relatively little experimental evidence for this assumption.

In a recent paper Braams (17) has outlined in more detail a free-radical mechanism for the radiation damage in the solid state. Based on our ESR experiments (3, 4), which demonstrate that a series of reactions takes place before the secondary radicals are formed, as well as on the experiments demonstrating the intermolecular transfer of unpaired spins (20), an extended free-radical mechanism has been proposed. The main steps in this mechanism, which has certain features in common with that of Braams, is visualized in Fig. 7. It is assumed that the radiation inactivation of solid enzymes is the end result of a sequence of events in which free radicals are transient intermediates. Although there are large gaps and unknown reactions in this model the main idea is that the initial processes in the inactivation

mechanism are the same as those leading to the secondary protein radicals (steps I and II in Fig. 7). These radicals (step III) will subsequently be involved in new reactions either when the enzymes are still in the solid state or when they are dissolved in an aqueous solution in order to allow activity measurements. According to the present model it is assumed that the secondary radicals are precursors to the enzyme inactivation, but we have so far no information about the reactions that take place between step III (Fig. 7) and step V. The assumption that the secondary radicals are intermediates in the inactivation mechanism can be tested in experiments in which the production of radicals is correlated with the loss of enzymatic activity.

The first attempts to correlate the production of free radicals with the enzyme inactivation were reported by Hunt, Till, and Williams (21, 22). Since they found that the yield of radicals was of the same order of magnitude as the inactivation yield, they concluded that a large fraction of the biological damage can be accounted for by the radicals. However, the most interesting part of their work, with regard to the free-radical mechanism, is their experiments on the oxygen effect. With ribonuclease they found that oxygen changed the ESR spectrum as well as the inactivation yield. They assumed that the sulfur groups lead to the formation of SH groups, and, since the yield of SH groups was found to be almost independent of oxygen, they concluded that the sulfur radicals are unlikely to take part in the oxygen effect. The assumption was made that the doublet-type radicals lead to inactivation when they react with oxygen. On the basis of this assumption, they calculated an expected inactivation curve for posttreatment in oxygen by using the observed dose-effect curve for the radical concentration

in vacuum. The good correlation between the expected and observed inactivation curve, in a certain dose range, seems to support the free-radical mechanism. However, as pointed out in a previous paper (13), a direct correlation of this kind where one secondary radical is correlated with the inactivation of one macromolecule is uncertain, and may lead to considerable errors because of the great problems involved in determining the concentration and the stability of the radicals. In the experiments reported here efforts have therefore been made to overcome the difficulties involved in direct correlations. The physical conditions for the reactions that take place prior to the formation of the secondary radicals (that is, steps I and II in Fig. 7) have been varied. These variations resulted in changes in the yield of secondary radicals, and according to the model in Fig. 7 we would expect similar changes in the inactivation yield.

In these experiments it was found that the production of secondary radicals exhibits the same relation to the irradiation temperature as that reported for the loss of the enzymatic activity (7, 14). Furthermore, a good correlation between production of secondary radicals and inactivation was found for trypsin in experiments in which the stopping power of the radiation varied in the range $2 \text{ MeV g}^{-1} \text{ cm}^2$ to about $1.6 \times 10^4 \text{ MeV g}^{-1} \text{ cm}^2$ (8).

Another source of support for the free-radical mechanism is the experiments on molecular mixtures (20). Thus, it was found that the reactions leading to the secondary radicals (step II in Fig. 7) can be partly intercepted in molecular mixtures by small-molecule substances. A large fraction of the unpaired electrons initially formed in the proteins becomes transferred to sulfur compounds, with the result that the proteins were protected against the formation of the secondary radicals. According to the

model in Fig. 7 this would imply that we also have a protection against the biological end effect (step V). It is well known, from several experiments on molecular mixtures, that the radiosensitivity of solid enzymes can be changed by the presence of small-molecule compounds (23—27).

It would be of interest to discuss the model in Fig. 7 in view of some of the other inactivation mechanisms proposed. In the target theory a hit or an absorption event must take place within the sensitive target (in the case of enzymes it was assumed to be a primary ionization within the macromolecule), and no attempts were made to describe either the primary species formed or the early secondary reactions. The model seems incapable, in its original form, of accounting for the observation that the enzyme inactivation can be influenced both by the presence of other solid substances and by the irradiation temperature. Since we now have methods available for studying secondary radical reactions and intermediate species, it is possible to take another step and either extend the old target theory or introduce some other models. The latter possibility has been adopted by Augenstein, Brustad, and Mason(6). These authors introduce a series of processes in order to account for the temperature dependence of the enzyme inactivation. They cast serious doubts upon the validity of the one-ionization model, and suggest that nonionizing excitations may be more important at room temperature. The inactivation is assumed to be completed when enough radiation energy has been transferred from the place where it is absorbed to what they call "the crucial site" in the molecule. The important step in their model is the many processes they introduce for the transport of radiation energy in solid proteins. They assume that intermediate species, such as charges and excitons, can migrate in proteins, and that some of

these processes take place during irradiation. Since our knowledge about the migration of charges and excitons in proteins is very limited, no attempts are made here to discuss the reliability of these processes. It should, however, be pointed out that Augenstein, Brustad, and Mason seem to give little room for the part played by the free radicals and radical reactions in transfer of radiation energy and in inactivation. The radical model in Fig. 7 represents, therefore, an alternative to the many physical processes proposed.

It was mentioned above that the radical reactions in step II also resulted in the disappearance of some of the initial ESR centers. The possibility that the nonradical species thus formed may in turn lead to the inactivation of the enzyme should also be considered. Furthermore, the secondary radicals produced in the proteins are very sensitive to oxygen, and the radical concentration usually decreases rapidly when oxygen is admitted to the sample. Except for the experiments on ribonuclease by Hunt and Williams (22), we have insufficient information to determine whether the radicals that react with oxygen become unavailable for enzyme inactivation, or whether radical reaction with oxygen is an important step in the inactivation mechanism. It would therefore be of considerable interest to carry out experiments on several enzymes in which the production of radicals is correlated with the loss of enzymatic activity.

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Table I. The apparent activation energies for the processes leading to the production of secondary radicals and the loss of enzymatic activity. ^a

Compound	Type of radiation	Type of measurement	Activation energies (cal/mole)					Reference
			E ₁	E ₂	E ₃	(E' ₁)	(E' ₂)	
Glycine	He ⁴ ions	Radicals	0	800	--	(130)	(1250)	This Work
"	C ¹² "	"	0	1200	--	(100)	(1450)	"
"	Ar ⁴⁰ "	"	0	900	--	(80)	(1550)	"
"	Ar ⁴⁰ " b	"	0	900	3700	(70)	(2200)	"
Lysozyme	He ⁴ "	"	0	1000	5000	(90)	(2600)	"
"	He ⁴ " b	"	0	1000	4000	(90)	(3200)	"
"	C ¹² "	"	0	1000	4000	(100)	(2150)	"
Trypsin	He ⁴ "	"	0	950	3600	(50)	(1650)	"
"	C ¹² "	"	0	950	3300	(70)	(2050)	"
"	C ¹² " b	"	0	1150	--	(70)	(2400)	"
"	Ar ⁴⁰ "	"	0	1300	--	(120)	(1700)	"
"	Ar ⁴⁰ " b	"	0	800	3100	(60)	(2350)	"
<hr/>								
Trypsin	D ² ions	Inactivation	0	1350	4700	(110)	(2200)	Brustad (7)
"	He ⁴ "	"	0	1350	5200	(110)	(2200)	"
"	B ¹¹ "	"	0	1100	3400	(70)	(2300)	"
"	C ¹² "	"	0	1150	4200	(70)	(2300)	"
"	Ne ²⁰ "	"	0	1050	3800	(70)	(2200)	"
"	Ar ⁴⁰ "	"	0	1300	3700	(70)	(2300)	"
Lysozyme	Electrons	"	0	620	2540	--	--	Fluke (14)
Ribonuclease	"	"	0	1060	6100	--	--	"

^a. The dependence of the radiation yields upon the irradiation temperature are given by the curves such as those presented in Figs. 3 and 5. These curves can mathematically be described by a sum of exponential functions. The E_i values represent the slopes of the straight lines in the semilogarithmic plot. The E_i values may also be interpreted as the apparent activation energies of the underlying processes. In this table the results of two alternative interpretations are presented. In the first place the total curve is decomposed into a sum of exponential functions (the values E₁, E₂, and E₃). In the other alternative the results below 100°K have been neglected and the high-temperature parts of the curves have been decomposed into two straight lines (see text).

The apparent activation energies E'₁ and E'₂ are given in parentheses.

^b. These results refer to experiments carried out on samples kept at room temperature for 2 days between the exposure and the measurement.

Table II. The effect of the irradiation temperature on the radiation yield.

Compound	Type of radiation	Type of measurement	$Y_{295^\circ\text{K}}/Y_{77^\circ\text{K}}$	Reference
Thiols (6) ^a	γ rays	Radicals	1.6	Henriksen (5)
Disulfides (9) ^a	"	"	2.1	"
Other sulfur compounds (10) ^a	"	"	3.2	"
Amino acids (8) ^a	"	"	2.7	"
Peptides (3) ^a	"	"	1.3	"
Proteins (10) ^a	"	"	2.3	"
Glycine	He ⁴ ions	"	2.6	This work
Glycine	C ¹² "	"	2.5	"
Glycine	Ar ⁴⁰ "	"	1.8	"
Lysozyme	He ⁴ "	"	1.7	"
Lysozyme	C ¹² "	"	1.8	"
Trypsin	Electrons	"	3.2	"
Trypsin	He ⁴ ions	"	1.9	"
Trypsin	C ¹² "	"	2.1	"
Trypsin	Ar ⁴⁰ "	"	2.2	"

Trypsin	D ² ions	Inactivation	2.8	Brustad (7)
Trypsin	He ⁴ "	"	3.0	"
Trypsin	B ¹¹ "	"	2.0	"
Trypsin	C ¹² "	"	2.1	"
Trypsin	Ne ²⁰ "	"	1.9	"
Catalase	D ² "	"	1.8	Setlow and Doyle (15)
Invertase	D ² "	"	2.0	Pollard et al. (16)

^a. The number in parenthesis represents the number of compounds studied for each group. The value given in this table is the average. The largest ratio (7.5) was found for S-methyl-cysteine.

FIGURE LEGENDS

Fig. 1. The ESR spectrum of lysozyme irradiated and measured at 77°K.

In this particular example the enzyme was irradiated in the solid state, in vacuum, with 6.5-MeV electrons. An X-band spectrometer was used, and the first derivative of the absorption spectrum is given. The microwave frequency was measured by a wavemeter and the magnetic field by a proton resonance fieldmeter. After the first measurement (upper figure) the sample was annealed at room temperature and then measured again at 77°K. The spectral changes reveal, therefore, the secondary radical reactions. The dashed curve represents measurement at high gain. The relative spectrometer sensitivity is otherwise given by the columns to the left.

Fig. 2. The yield of secondary radicals in glycine as a function of the irradiation temperature. Solid glycine was irradiated in vacuum with fast stripped helium, carbon, and argon ions at different temperatures. The samples were kept at room temperature for 20 minutes before the ESR measurements were carried out. The dose was the same for all samples irradiated with one type of ions. The results are given in relative units, and the average value for the temperature region 77° to 400°K has been set equal to 1.0 in all experiments.

Fig. 3. The yield of secondary radicals in solid glycine as a function of the reciprocal of the irradiation temperature. The curves were decomposed into a sum of straight lines and the apparent activation energies obtained from the slopes of these lines are given in Table I.

Fig. 4. The ESR spectrum of trypsin irradiated and measured at room temperature. According to Kneubühl (28) the principal g values for a

polycrystalline sample correspond to maxima and zero point in the derivative curve. The principal g values for the broad resonance in the protein spectra are in excellent agreement with those found for organic sulfur radicals (29).

Fig. 5. The yield of secondary radicals in solid trypsin as a function of the reciprocal of the irradiation temperature. Otherwise as in Figs. 2 and 3.

Fig. 6. Effect of the irradiation temperature on both the production of secondary radicals and the loss of enzymatic activity for trypsin. The enzyme was irradiated in the solid state, in vacuum, with fast stripped carbon ions. The inactivation curve is exponential with respect to the radiation dose. The parameter used for the inactivation is therefore either the reciprocal of the D_{37} dose or, as in this figure, the apparent inactivation cross section. All inactivation data, which were taken from Brustad's experiments (7), have been recalculated and are given in relative units in order to be plotted on the same figure. The average value of the data below 100°K has been set equal to 1.0.

Fig. 7. An attempt to visualize a free-radical model for the radiation inactivation of solid enzymes. The biological end effect is assumed to be the result of a sequence of events in which free radicals are important intermediates.

MUB-5438

Fig. 1.

MUB-5010

Fig. 2.

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Fig. 3.

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Fig. 4.

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Fig. 5.

MUB-5015

Fig. 6.

MUB-4211

Fig. 7.

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