RADIOLYSIS OF THE AQUEOUS SOLUTIONS OF SELENOMETHIONINE

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$5 . 10^{-3} - 5 . 10^{-2}$ M solutions of selenomethionine were irradiated by gradually increased doses of gamma radiation in the oxygen, nitrogen and nitrous oxide atmospheres. The values, calculated for rate constants of radiolysis (K) and for initial yields (G_i) have shown that degradation of selenomethionine proceeds as the result of the indirect effect of radiation, predominantly due to OH radicals. The hydrated electrons are scavenged by the selenium atoms without any degradation of the original substance. The main radiation-degradation reactions of selenomethionine include, in addition to deamination reactions, also reactions leading to the cleavage of —SeCH$_3$ and —CH$_3$ groups, as well as recombination reactions, in contrast to radiation-degradation reactions of methionine irradiated under identical conditions.

In recent years an enhanced attention has been paid to selenium which, as an essential trace element, plays an important role in life processes of organism. An interest has been focused also to the biologically important organic compounds of selenium, namely to the selenium analogue of methionine. This compound has a high biological activity, manifested namely by its ability to reduce peroxides[1]. The radiation-protective effectiveness of selenomethionine has been proved to be much higher than that of methionine[2,3].

It seemed therefore interesting to study both these compounds from the point of view of radiation chemistry and to compare the mechanisms of their radiolyses. A number of papers[4-7] deals with the detail studies of aqueous solutions of methionine. The nature and yields of the main radiolytic products have been described and a radiation degradation scheme of this compound has been proposed. Certain kinetic data have been obtained[8] characterizing the decrease of methionine by radiolysis and the course of de-amination reactions as a function of the radiation dose and of the solution concentration. Up to the present time analogous data on the radiolysis of selenomethionine were not available.

The aim of this study was to obtain more detailed information on the radiation reactions of selenomethionine under the same irradiation conditions as those that had been used in the study of methionine and to work out an idea on the radiation-chemical behaviour of the two analogues from the comparison of the results obtained.

EXPERIMENTAL

DL-Selenomethionine, prepared in our laboratory, has been used for this study. In several cases selenomethionine-^{75}Se (also prepared in this laboratory) has been used for the determination

of quantitative data. Aqueous solutions of selenomethionine of the concentrations of 10^{-3}, $5 . 10^{-3}$, $8 . 10^{-3}$, 10^{-2}, $2 . 10^{-2}$ and $5 . 10^{-2}$M, respectively, have been prepared. Activity of the labelled selenomethionine was about $3 — 5$ mCi/ml.

Previous to irradiation the samples were bubbled either with nitrogen or with nitrous oxide (a bubbler with pyrogallol was used to remove oxygen that could be eventually present). The ampoules were then sealed and irradiated. Samples for the studies of the effect of oxygen on the course of the radiolysis were bubbled with oxygen during the irradiation.

5 kCi of ^{60}Co was used as the source of radiation. The dose rate of this source was $1 \cdot 03 . 10^{19}$ eV/ml h. Samples were irradiated by doses ranging from 0·25 to $5 \cdot 5 . 10^{20}$ eV/ml, in certain cases from 0·66 to $9 \cdot 26 . 10^{20}$ eV/ml.

The decrease of selenomethionine and the formation of decomposition products were followed by paper chromatography and electrophoresis and quantitatively evaluated by the radiometric and colorimetric analyses. The amount of ammonia was determined by the microdiffusion method of Conway. The formation of radiation-chemical products containing four-valent selenium was followed on the paper chromatograms and electrophoreograms using the iodine-starch spray according to Scaly and Williams[9]. Gas chromatography was used for the detection of $(CH_3)_2Se$ and $(CH_3)_2Se_2$ in the fraction of volatile decomposition products[10]. Selenohomo-lanthionine, isolated from the solution of selenomethionine irradiated in the nitrogen atmosphere, was identified by its chromatographic and polarographic behaviour as well as by the products of its deselenization with Raney nickel.

RESULTS AND DISCUSSION

The effect of the absorbed dose of radiation on the decrease of selenomethionine in solutions of various concentrations in the nitrogen, oxygen or nitrous oxide atmospheres is illustrated in Figs 1 and 2. Denoting the initial concentration of seleno-methionine by S_0 and the concentration of the irradiated samples by S, the values of the relative decrease of selenomethionine, S/S_0, plotted in a semilogarithmic scale as a function of the absorbed dose D, lie on a straight line. Slopes of these straight lines determine the rate constants of the decrease of selenomethionine at the given initial concentration. It turns out from this graph that the values of rate constants K are inversely proportional to the initial concentration S_0,

$$K = A/S_0 , \qquad (1)$$

where A is a constant characteristic for the medium of the irradiated solution. From Figs 1 and 2 we can conclude that the decrease of selenomethionine follows the reaction of the first order

$$S = S_0 \, e^{-KD} , \qquad (2)$$

or, eventually,

$$S = S_0 \, e^{-AD/S_0} . \qquad (3)$$

The results were evaluated similarly as the experimental data obtained for the irradiated solutions of methionine[8]. Eq. (4) for the G-value (*i.e.* for the number of mole-

cules of the irradiated substance transformed per 100 eV of the absorbed energy),

$$G = \frac{6{\cdot}023 \cdot 10^{22}(S_0 - S)}{D} = \frac{6{\cdot}023 \cdot 10^{22}S_0(1 - e^{-AD/S_0})}{D} , \qquad (4)$$

can be simplified for D approaching zero, so that we obtain

$$G_i = 6{\cdot}023 \cdot 10^{22}KS_0 = 6{\cdot}023 \cdot 10^{22}A . \qquad (5)$$

The experimental results are given in Table I. Values listed in this table show that the initial yields G_i are practically independent of the initial concentration of seleno-methionine. This can be considered as a proof of the indirect action of radiation on the degradation of selenomethionine. Values obtained for solutions of higher concentrations of selenomethionine in the oxygen atmosphere are an exception. Namely,
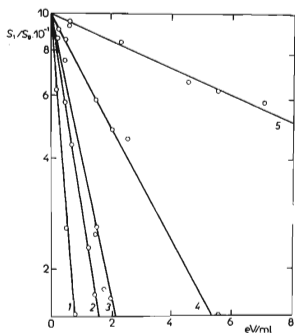

FIG. 1

The Dependence of the Decrease of Seleno-methionine S/S_0 (in the logarithmic scale) on the Dose D (in 10^{20} eV/ml)

The straight lines $1-5$ correspond to the initial concentrations of the solution of sele-nomethionine 0·005, 0·008, 0·01, 0·02 and 0·05M, respectively. Samples were irradiated in the oxygen atmosphere.
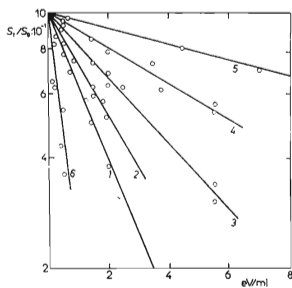
FIG. 2

The Dependence of the Decrease of Seleno-methionine S/S_0 (in the logarithmic scale) on the Dose D (in 10^{20} eV/ml)

The straight lines $1-5$ correspond to the initial concentrations of the solution of sele-nomethionine 0·005, 0·008, 0·01, 0·02 and 0·05M, respectively. Samples were irradiated in the nitrogen atmosphere. The straight line 6 represents the dependence for the 0·005M solution, irradiated in the nitrous oxide atmosphere.

the G_i values obtained for the 0·02M and 0·05M solutions are substantially lower than the overall average of yields that were found in this atmosphere for all other solutions.

Similar results have been also obtained when evaluating the experimental data characterizing the course of de-amination reactions. The amount of selenomethionine decomposed due to the de-amination is denoted by $(S_0 - S_1)$. The dependence of S_1/S_0 on the dose is again given by a set of straight lines (Figs 3 and 4). The slopes correspond to the rate constants K_1 of the de-amination reactions, for which we get

$$S_0 - S_1 = S_0(1 - e^{-K_1 D}) . \qquad (6)$$

The mean value of $G_i(NH_3)$ in the oxygen-free atmosphere is 0·52 ± 0·01 which is practically constant for all concentrations studied. Larger scattering of the results has been observed for experiments carried out in the oxygen atmosphere. The value of $G_i(NH_3) = 1·5 ± 0·2$ has been adopted as the mean value. In this case, similarly as for $G_i(-M)$, the values of $G_i(NH_3)$ for the irradiation of more concentrated solutions of selenomethionine in oxygen atmosphere tend to decrease.
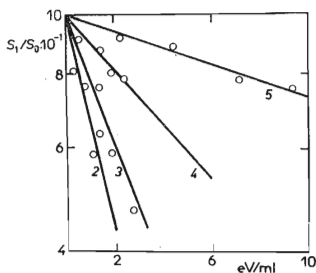

Fig. 3

The Dependence of the De-amination of Selenomethionine, S_1/S_0 (in the logarithmic scale) on the Dose D (in 10^{20} eV/ml)

The straight lines 2—5 correspond to the initial concentrations of the solution of selenomethionine 0·008, 0·01, 0·02 and 0·05M, respectively. The samples were irradiated in the oxygen atmosphere.
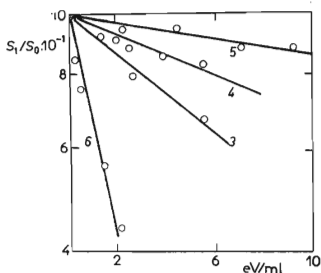

Fig. 4

The Dependence of the De-amination of Selenomethionine S_1/S_0 (in the logarithmic scale) on the Dose D (in 10^{20} eV/ml)

The straight lines 3—5 correspond to the initial concentrations of the solution of selenomethionine 0·01, 0·02 and 0·05M, respectively. The samples were irradiated in the nitrogen atmosphere. The straight line 6 corresponds to the 0·005M solution irradiated in the nitrous oxide atmosphere.

Using the radical scavengers and comparing the obtained yields we have tried to explain certain details in the mechanism of the radiation degradation of seleno-methionine. The yields $G_i(-M)$ and $G_i(NH_3)$ determined in the solution of seleno-methionine irradiated in the presence of nitrous oxide are given in Table II, together with yields obtained in the oxygen-free atmosphere and in the oxygen atmosphere at the same concentration of the solution and at the same radiation dose.

In the atmosphere of nitrous oxide, in which all the hydrated electrons are substituted by OH radicals because of the reaction
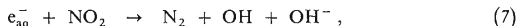
$$e_{aq}^- + NO_2 \rightarrow N_2 + OH + OH^-, \tag{7}$$

TABLE I

Kinetic Data for the Radiolysis of the Aqueous Solution of Selenomethionine Irradiated in the Oxygen- or Nitrogen Atmospheres

$S/S_0 = 0.5$.

	$S_0 \cdot 10^{-2}$ mol/l	$D \cdot 10^{20}$ eV/ml	$A \cdot 10^{22}$ mmol/eV	$\overline{A} \cdot 10^{22}$ mmol/eV	$G_i(-M)$	$G_i(-M)$
	5	8·3	(0·42)		(2·5)	
	2	1·9	(0·73)		(4·4)	
O_2	1	0·75	0·92		5·6	
	0·8	0·6	0·93	0·95	5·6	5·7
	0·5	0·35	0·99		6·0	
	5	13·9	0·25		1·5	
	2	6·25	0·22		1·3	
N_2	1	3·3	0·21	0·23	1·3	1·4
	0·8	2·15	0·26		1·5	
	0·5	1·5	0·23		1·4	

TABLE II

The Average Initial Yields G_i for the Decrease of Selenomethionine and for the Formation of Ammonia on 0·005M Solutions of Selenomethionine, Irradiated in the Atmosphere of Nitrogen, Nitrous Oxide or Oxygen

Yield	N_2	N_2O	O_2
$G_i(-M)$	1·4	5·2	5·7
$G_i(NH_3)$	0·52	1·2	1·5

the yield of OH radicals increases practically twice ($G_{OH} = 5.5$). If compared with yields in the oxygen-free atmosphere, the decrease of selenomethionine in nitrous oxide is substantially higher ($G_i(-M) = 5.2$). It is therefore probable that the OH radicals play a decisive role in the degradation of selenomethionine.

Higher values of $G_i(-M)$ have been found even in the atmosphere saturated with oxygen. In this case the irradiated substance is decomposed by OH and HO_2 radicals as well as by oxygen anions formed by the reaction

$$e_{aq}^- + O_2 \rightarrow O_2^- . \qquad (8)$$

In this atmosphere the decrease of the irradiated substance, given by $G_i(-M) = 5.7$, means that practically all active species of water participate in the degradation of selenomethionine.

Shimazu and Tappel[2,3,6] studied the radiation stability of selenomethionine in comparison with methionine. They proved a higher radiation stability of seleno-methionine and they found that the radiation-protective effectiveness of this compound is in certain cases three to five times higher than that of methionine. They ascribe the enhanced radiation-protective effectiveness of selenomethionine to the ability of se-lenium to scavenge free radicals and to the reparation mechanism that proceed in the molecule of selenomethionine during the irradiation.

We have compared the overall kinetics of the radiation degradation of seleno-methionine with the kinetic data found in the study of methionine irradiated under the same conditions[8]. It turns out that the rate constants of radiation degradation of methionine are substantially higher. This can be illustrated by the yields of $G_i(-M)$ and $G_i(NH_3)$ for the irradiation of methionine (*i.e.* 4.7 and 2.1, respectively, in the nitrogen atmosphere and 9.7 and 4.7, respectively, in oxygen) which are about two or three times higher than those for selenomethionine. In contrast to methionine, where the initial yields $G_i(-M)$ and $G_i(NH_3)$ are independent through the whole range of studied concentrations, in the 0.02M solution and namely in the 0.05M solution of selenomethionine, irradiated in the oxygen atmosphere, the initial yields are lower. These results indicate that at higher concentrations of selenomethionine — along with reactions between oxygen and hydrated electrons — competitive reactions between hydrated electrons and molecules of selenomethionine take place without any degradation of selenomethionine. Pulse radiolysis could substantially contribute to the verification of this assumption and to the elucidation of certain interesting properties of selenomethionine but, unfortunately, this method has not yet been applied to this system.

We have tried to outline the mechanism of the radiation-degradation processes taking place in the irradiated solution of selenomethionine based on the detailed analysis of the main decomposition products. For this purpose the results of the radiolysis of 0.05M solution of selenomethionine irradiated by doses ranging from

0·66 to 9·26 . 10^{20} eV/ml[11] in the nitrogen- and oxygen atmospheres have been used. From the preceding studies on the radiolysis of methionine[7,8] it follows that, along with the de-amination, the most important radiation-chemical reaction in methionine is the oxidation of sulphur from the lowest to the highest valence state, preserving, however, the integrity of the original molecule. Methionine sulfoxide, methionine sulfone and the homocysteic acid were found to be the degradation products in the irradiated solution. The cleavage of —SCH₃ and —CH₃ groups is a minor process and the corresponding radical intermediates are evidently so unstable that they cannot form, *e.g.*, the recombination products. Actually, no such products were detected in the irradiated solution of methionine.

On the other hand, in the case of the selenium analogue, the cleavage of —SeCH₃ and —CH₃ groups belongs, together with the subsequent independent reactions of these fragments, to the most important reactions. In the nitrogen atmosphere the recombination reactions prevail. The formation of recombination products of the type of selenohomolanthionine (HOOC.CHNH₂CH₂.CH₂SeCH₂CH₂.CHNH₂.COOH) and selenohomocysteine (HOOC.CHNH₂.CH₂CH₂.SeSe.CH₂CH₂.CHNH₂.COOH) has been observed. This proves the relatively stable nature of the radical intermediates formed by the cleavage of —SeCH₃ and —CH₃ groups. Even the recombination of the remaining fragments takes place, as proved by the presence of $(CH_3)_2Se$ and $(CH_3)_2Se_2$ in the irradiated solutions. In the oxygen atmosphere in the diluted solutions of selenomethionine the oxidation of selenium takes place, forming partly selenomethionine oxide, partly leading to the cleavage of the molecule of selenomethionine. In more concentrated solutions (0·05M) reactions leading to the cleavage of —SeCH₃ and —CH₃ groups prevail and the oxidation reactions take place only on the carbon skeleton and on the cleaved groups. Homoserine and aspartic acid are the final products in the former case, in the latter one the selenium acids of various oxidation degrees, mainly the methylselenious and selenious acids, are formed.

From the observed radiation products it can be concluded that the degradation of selenomethionine is mainly due to the reactions of OH radicals, in oxygen atmosphere also to the reactions of HO₂ radicals and oxygen anions O_2^-. They preferably attack the α-position of the irradiated amino acid, giving rise to the de-amination reactions, or the —SeCH₃ group, where the C—Se bond is being cleaved and the oxidation of selenium takes place. The hydrated electrons are evidently scavenged by the selenium atom without any degradation of the original amino acid.

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