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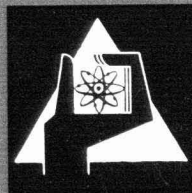
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Electron-spin-resonance study of radiation-induced radicals
in aromatic and aliphatic amino acids and peptides

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Electron-spin-resonance study of radiation-induced radicals in aromatic and aliphatic amino acids and peptides

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Electron-spin-resonance spectrometry at room and at low temperature (100°K) has been used to determine the free-radical yield in the x-irradiated dipeptide glycylglycine, and the aromatic amino acids L-tyrosine and L-tryptophane. Molecular mixtures of L-tyrosine and glycylglycine, as well as L-tryptophane and glycylglycine prepared by freeze-drying of aqueous solutions, have also been studied by this method. The quantitative results obtained with the latter do not support the idea of intermolecular transfer of radicals or excitation energy between the different constituents of the mixtures. As opposed to this, when the aromatic amino acids are bound to glycine by a peptide bond to form glycyl-L-tyrosine and glycyl-L-tryptophane, a marked protection of the aliphatic chain by the aromatic ring system is observed.

1. INTRODUCTION

Although much work has been devoted to the study of electron-spin resonance in irradiated amino acids, the quantitative aspect of the problem has not yet been given the attention it deserves. Only recently, large discrepancies between the yields of radiation-induced radicals found in early determinations could be reduced to values below one order of magnitude (Köhnlein and Müller 1962). On the other hand, qualitative evidence for the occurrence of interactions between the constituents of molecular mixtures of amino acids in the solid state was published more than five years ago (Norman and Ginoza 1958). These conclusions were not based on quantitative determinations, but were drawn from qualitative changes in the patterns of E.S.R.-spectra. It should be noted that one of the compounds of each pair studied by Norman and Ginoza was a sulphur-containing molecule, since it has recently been shown that sulphur-containing molecules in the dry state act as radical acceptors by a chemical mechanism (Henriksen 1962). Thus, it appeared all the more interesting to attempt to replace these sulphurated molecules by aromatic compounds which are known to be radioresistant, to see whether direct evidence could be found in this case for an immediate energy-transfer through the electronic system or by another mechanism as discussed by Zimmer and Müller (1964). In view of the importance of understanding the radiation resistance of

amino acids and the mechanisms of energy-transfer in molecules of biological interest, we decided to resume in a detailed quantitative way the study of radiation resistance in three typical amino acids, in their mechanical and molecular mixtures, and finally in the peptides corresponding to the binary mixtures.

2. MATERIALS AND METHODS

We chose as a typical aliphatic peptide glycylglycine, since its radiation-induced E.S.R.-spectrum can be ascribed to a simple aliphatic carbon radical (Box, Freund and Lilga 1961, Katayama and Gordy 1961, Lin and McDowell 1961) and since, because of the doublet structure of the glycylglycine spectrum, any large overlap is avoided with the single line characterizing the aromatic amino acids which were to be mixed with glycylglycine later on. The typical aromatic molecules were tyrosine, with its benzene ring, and tryptophane, because of its indole structure. Whereas tryptophane was investigated as L-tryptophane, D-tryptophane and the racemic mixture, only the L-form of tyrosine was studied. These compounds were used as crystalline powders sealed in quartz tubes after 15 hours evacuation at 10^{-5} mm Hg. We then prepared mechanical mixtures of glycylglycine with each of the aromatic molecules and, by freeze-drying of an aqueous solution, the corresponding molecular mixtures. In the case of tryptophane, molecular mixtures of L-tryptophane containing 10, 40, 60 and 90 per cent of glycylglycine were prepared. With L-tyrosine, which is less soluble in water, only a mixture containing 50 per cent of glycylglycine was used. Finally we extended our study to the simple peptides, glycyl-L-tyrosine and glycyl-L-tryptophane, the analogy of which to the binary mixtures investigated is obvious. These compounds, too, were obtained commercially of the highest purity available and used as crystalline powders as were all amino acids and peptides studied here.

First derivatives of electron-spin-resonance spectra in irradiated samples were recorded with a commercial X-band spectrometer (Microspin) having a 100 kc/s field modulation, but fitted with a 'double' cavity resonating in the TE_{104} mode. Irradiations and measurements were performed both at room and liquid-nitrogen temperature. Irradiations were carried out using the total radiation emitted by an x-ray tube fitted with a beryllium window of 1.5 mm thickness and operated at 100 kv, 25 ma and the dosimetric procedures reported previously (Köhnlein and Müller 1962). Free-radical concentrations were determined by measuring, with a moment planimeter, the first moment of the derivative spectra recorded at microwave power-levels low enough to avoid saturation, the onset of which was determined for each type of sample.

3. RESULTS AND DISCUSSION

The E.S.R. patterns of the irradiated substances are given in figures 1-3, both for room and low (about 100°K) temperature. In general they correspond to those already reported. As may be seen from figures 4 and 5, similar dose-effect curves are observed in all cases: i.e. an initial linear growth gradually turning at higher doses into a saturation plateau, the change in slope occurring in the Mrad region. Fully-drawn curves in figures 4 and 5 are exponential functions obeying the eqn. $C/C_{\infty} = 1 - \exp(-D/D_{37})$, where C and C_{∞} denote radical-concentrations at dose of radiation D , respectively the maximum reached at saturation, while D_{37}

is the dose at which C is 37 per cent smaller than C_{∞} . D_{37} -values are indicated by arrows in figures 4 and 5. The energy expenditures necessary to create stable free radicals have been deduced from the initial slopes both for room and low temperature and are summarized in the table in units of electron volts per radical. Although other functions may give a better fit to the experimental points in some cases the exponential function is a much better description of the results on *all* substances than any other function containing two parameters only. As is to be expected on the basis of previous results, radiation resistance increases as the degree of delocalization of π -electrons according to the views developed by Duchesne (1957) and Pullman (1961) and recently verified by

Compound	Energy expenditure per radical (ev)		D_{37} -values (Mrad)	
	At 100°K	At 300°K	At 100°K	At 300°K
Glycylglycine	18	18	20	20
L-tyrosine	330	210	4	3
Glycyl-L-tyrosine	50	33	4	3
L-tryptophane	1800	560	> 10	10
Glycyl-L-tryptophane	220	30	7	8

Comparison of energy expenditures and D_{37} -values.

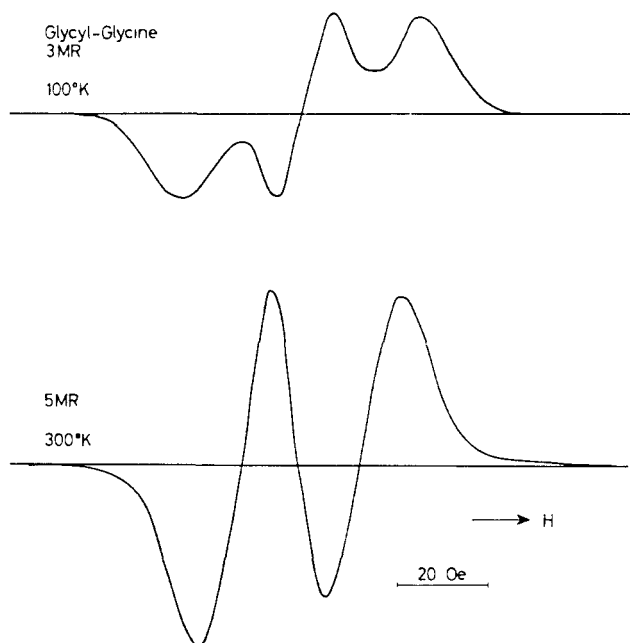


Figure 1. Electron-spin-resonance spectra (first derivative) of glycylglycine at 100°K and 300°K.

Duchesne, Williams-Dorlet and Lacroix (1963). Although in the case of tryptophane both the L-, D- and racemic forms were studied, no significant variation in the behaviour of any of the three compounds was observed.

In the mechanical mixtures, where concentrations of glycylglycine ranged from 10 to 90 mole per cent, no special feature was detected, the E.S.R.-spectrum after irradiation always being the doublet characterizing the aliphatic amino acid. This appears to be quite normal since, owing to the large differences in their radiation resistances, doses necessary to bring about the minimum detectable signals in each of the aromatic acids fall in the range where dose saturation sets in for the aliphatic acid. Provided one takes into account the dilution of the aliphatic amino acid in the mixtures, energy expenditures per stable free radical in the mixtures are always in the vicinity of 15 ev, just as for pure glycylglycine, a finding supporting the above conclusion in a quantitative way. A similar situation has been found for the molecular mixtures prepared in the aforementioned way. As a check for the reality of their existence, we have examined with a hot-stage polarizing microscope the melting behaviour of mixtures containing 40 and 60 mole per cent of glycylglycine in tryptophane. In these cases, the well-known decomposition and sublimation without melting of glycylglycine is hardly observed, and moreover a clear-cut melting of practically the whole of the homogeneous crystalline mass occurs around 254 and 243°C, respectively, instead of 293–4°C for the pure DL-tryptophane used for preparing the mixtures. After irradiation, the E.S.R. spectra gave results identical, both from the qualitative and quantitative points of view, with those obtained for the mechanical mixtures. This does not fit with the variations in spectral patterns reported by Norman and Ginoza (1958), but it should be remembered that one of the components of their mixtures was always a sulphur-containing molecule and that it is now well established that sulphur itself is liable to give rise even in its elementary form to interactions leading to an

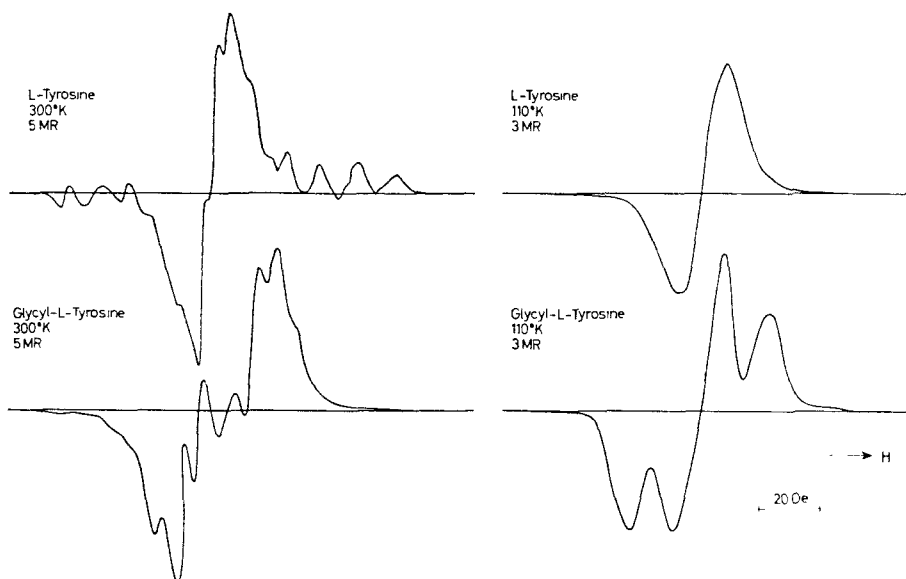


Figure 2. Electron-spin-resonance spectra (first derivative) of tyrosine and glycyl-L-tyrosine at 100°K and 300°K.

efficient radiation protection (Van de Vorst and Duchesne 1963, Charlesby, Garatt and Kopp 1962). The possibility cannot therefore be excluded that some particular mechanism, such as reported previously (Henriksen 1962), might have played some role in the experiments of Norman and Ginoza. In the present case, however, the identity of radiation resistances obtained for glycylglycine, when studied either alone or in mixtures, completely rules out the occurrence of any interaction between the components of our molecular mixtures when irradiated in the solid state.

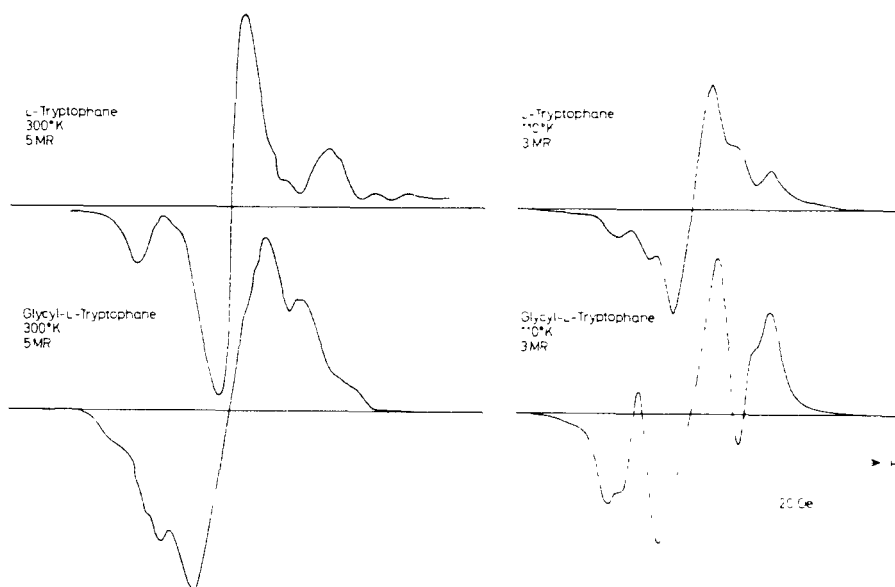


Figure 3. Electron-spin-resonance spectra (first derivative) of tryptophane and glycyl-L-tryptophane at 100°C and 300°C.

It was therefore all the more interesting to study the radiation behaviour of the correlated peptides, glycyl-L-tyrosine and glycyl-L-tryptophane, to see whether the presence of the peptide bond could, through its electronic bridge, give rise to some interactions; this is indeed the case. Figures 2 and 3 show that neither for glycyltyrosine nor for glycyltryptophane is any trace found of the typical doublet or triplet normally given by the aliphatic glycine or glycylglycine of these molecules. Furthermore the single line characterizing each of the aromatic residues is replaced by a multi-line structure, the observation of which seems to have escaped the attention of previous authors (McCormick and Gordy 1958) due to overmodulation or to microwave power saturation.

From the quantitative point of view a marked radiation protection of the aliphatic chain by the aromatic system is observed, as is apparent from the dose-effect curves of figures 4 and 5 and from the values given in the table. Both peptides need at least twice as much energy for the induction of a free radical as their most sensitive component. Another feature emerging from the table is that, although the yields at room temperature are nearly equal for both peptides,

the energy required at low temperature (where secondary reactions are avoided) makes it clear that the protective power of the aromatic member is proportional to its own radiation resistance, the indole ring being more effective than the benzene ring. A point well worth mentioning and partly explaining the mechanism of radiation protection in these compounds is that, in peptides

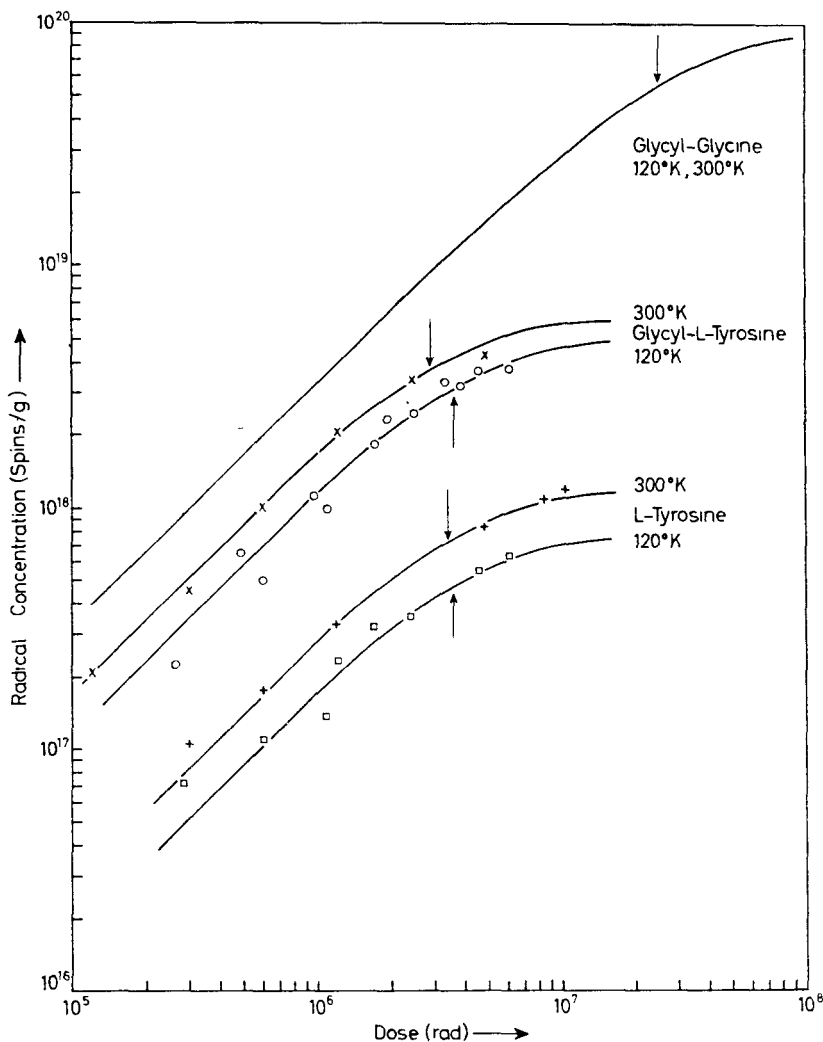


Figure 4. Dose-effect curve of radical induction in glycyglycine, L-tyrosine and glycyl-L-tyrosine at 100°K and 300°K.

irradiated at low temperature then heated up to room temperature, the spectrum changes, accompanied by some loss of radicals, into the spectrum observed for the aromatic component alone and for which no variations in dependence on temperature have been found. These results support the view that the radicals induced by radiation are stabilized in the aromatic part of the peptide molecule.

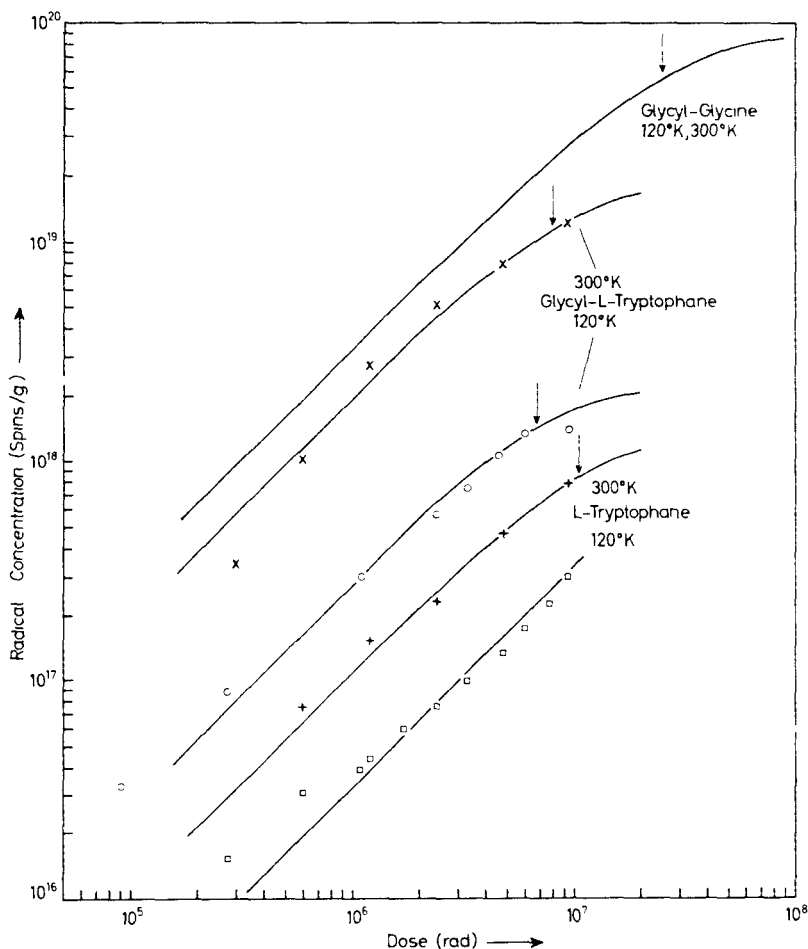


Figure 5. Dose-effect curve of radical induction in glycylglycine, L-tryptophane and glycyl-L-tryptophane at 100 K and 300 K.

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On étudie, par résonance de spin électronique, le rendement en radicaux libres produits par l'irradiation x à température ordinaire et à basse température (100 K) dans un dipeptide aliphatique, la glycyl-glycine, dans les acides aminés aromatiques L-tyrosine et L-tryptophane, ainsi que dans les mélanges moléculaires, préparés par lyophilisation des solutions aqueuses communes, de L-tyrosine et glycyl-glycine d'une part, et de L-tryptophane et glycyl-glycine d'autre part. Les résultats quantitatifs obtenus dans ce cas permettent de conclure à l'absence de transfert des radicaux ou d'excitation entre les constituants des mélanges. Lorsqu'au contraire, les deux molécules sont réunies par une liaison peptidique pour former les peptides simples, glycyl-L-tyrosine et glycyl-L-tryptophane, on observe une protection très marquée de la chaîne aliphatique par le résidu aromatique.

Elektron-Spin-Resonanz Spektrometrie bei tiefer (100°K) und bei Zimmertemperatur wurde zur Bestimmung der Ausbeuten freier Radikale in dem röntgenbestrahlten aliphatischen Dipeptid Glycylglycin und den aromatischen Aminosäuren L-Tyrosin und L-Tryptophan benutzt. Molekulare Mischungen von L-Tyrosin und Glycylglycin sowie L-Tryptophan und Glycylglycin, hergestellt durch Gefriertrocknung wässriger Lösungen wurden ebenfalls mit dieser Methode untersucht. Die mit den letzteren erhaltenen quantitativen Ergebnisse unterstützen nicht die Annahme intermolekularer Übertragung von Radikalen oder Anregungsenergie zwischen den verschiedenen Bestandteilen der Mischungen. Im Gegensatz dazu wird eine deutliche Schutzwirkung beobachtet, wenn die aromatischen Aminosäuren durch eine Peptidbindung an Glycin gebunden sind und Glycyl-L-Tyrosin und Glycyl-L-Tryptophan bilden.

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