The Deamination of Glycine by α -Radiation from the Disintegration of Boron in a Nuclear Reactor

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A correct assessment of the effects of α -radiation as an example of densely ionizing radiations, in comparison with a less densely ionizing radiation (e.g. X-rays), on a variety of solutes in aqueous solutions, is of importance for an understanding of the mode of action. There are a few such comparative investigations reported in the literature (Dale, Gray & Meredith, 1949; this paper contains references to relevant literature). In general α -radiation is less effective than X-radiation. In some of these investigations it is doubtful whether there was a sufficient consideration of the part played by simultaneous β - and γ -radiation and, therefore, the small effect observed may have been entirely due to radiation other than α -rays. Such effects were fully taken into account in a recent investigation on a comparison between α - and X-radiation effects on carboxypeptidase (Dale, Gray & Meredith, 1949). It was also shown that an increase of the concentration of the solute led to an increase of the α -ray effect, and the authors inclined to the view that the effects observed were due to the δ -rays issuing from the α -ray track. Radiation from a polonium source would entirely avoid interference by β - or γ -radiation, but it is experimentally difficult to make sufficiently strong sources of radiation. This paper reports an investigation of the effects of α -radiation using the disintegration particles from the action of slow neutrons on a boron salt present in the solution of the solute to be investigated. The advantage of this procedure is that not only a large radiation dose can easily be obtained using the neutron flux available in nuclear reactors, but also that the yield from the interfering β - and γ -radiation can be directly measured.

The reaction investigated was the deamination of glycine by radiation, and this reaction is of interest for several reasons. Glycine has a low molecular weight (75) in contrast to the high molecular weight of carboxypeptidase (35,000); furthermore, solutions of higher concentrations than are possible with carboxypeptidase can be made, and finally the deamination has been shown (Dale & Davies, 1949; Dale, Davies & Gilbert, 1949) to have an ionic yield for X-rays rising to nearly 3 on increasing the concentration of glycine to 20%, whereas the X-ray ionic yield with carboxypeptidase remains constant at 0.19 over a very wide range of concentrations.

EXPERIMENTAL

Dosimetry

The exposure to slow neutrons of a solution containing a B salt produces an irradiation of the solution by α -particles and slow Li particles resulting from the nuclear reaction $^{10}\text{B} (n\alpha) ^7\text{Li}$. With a neutron flux of $10^{10}/\text{cm}^2/\text{sec}$. or more, available in nuclear reactors, one can obtain in a few hours sufficient radiation from the heavy particles to give good yields of NH_3 from the deamination of glycine. However, in a nuclear reactor there is also a considerable flux of fast neutrons and γ -radiation and these will produce further deamination via their corresponding secondary radiations, protons and electrons. There is also the slow neutron reaction $^{14}\text{N} (np) ^{14}\text{C}$ originating from the nitrogen in the glycine. These effects can be eliminated by exposing two solutions of the same glycine concentration side by side in the neutron flux, one containing a B salt and one without, the difference in the yields being due to the heavy particle radiation only. We, in fact, irradiated a third solution containing double the amount of B salt, to check that the deamination by the α -particles was proportional to the B concentration. For reliable results the deamination in presence of B should be as large as possible compared with the background effect, and this is achieved by using the highest possible concentration of B. It was found by preliminary experiments that up to 2.25M-KBO₂ (potassium metaborate) could be added to the glycine solution without affecting the deamination yields by X-radiation. The resulting solution was, however, strongly alkaline, and accordingly special experimental procedure had to be adopted, as discussed later. Little prior information about the intensity of the γ -radiation in the nuclear reactor was available, but rough estimates indicated that this amount of B would be sufficient, as was finally shown by the experiment.

The rate of liberation of energy/unit volume of solution by the B disintegration can be calculated from the B concentration, the disintegration cross-section for thermal neutrons and the average neutron flux in the solution. The mean energy release in the slow neutron disintegration of B can be calculated from the masses (Mattauch & Flügge, 1942), remembering that only about 8% of the disintegrations liberate the full energy, the remainder leaving the ^7Li in an excited level of 474 keV. (Bøggild, 1945; Gilbert, 1948; Rosenfeld, 1948). These calculations give a mean energy/disintegration of 2.49 MeV. The effect of the 474 keV γ -radiation resulting from the de-excitation of the ^7Li will not be eliminated by comparison with the B-free solution, but the solution was in a layer of only 2 mm. thickness and absorbs a negligible amount of the γ -radiation. The

capture cross-section for the B disintegration is taken as 703×10^{-24} cm.², and therefore the rate of energy liberation/ml. of molar solution of B for a neutron flux n /cm.²/sec. is

$$6.06 \times 10^{20} \times 703 \times 10^{-24} \times 2.49 \times 10^6 n = 1.06 \times 10^6 n \text{ eV./sec.}$$

For the purpose of calculating the ionic yield we can define an ion pair as the absorption of 32.5 eV. and hence the rate of production of ion pairs/ml. of m-borate solution due to the α and Li particles is 3.26×10^4 n/sec.

For the determination of n , the average neutron flux, account must be taken of the considerable local disturbance of the neutron flux produced by the presence of so much B, and also of the fact that the flux will vary markedly throughout the solution. These effects can be allowed for by using the presence of K as a monitor, through the activity of the ⁴²K produced by the slow neutron reaction ⁴¹K ($n\gamma$) ⁴²K, for the number of ⁴²K atoms produced is proportional to the number of B disintegrations independent of the spatial variations of the neutron flux. All solutions were therefore made to have nominally the same K concentration, the appropriate amount of K₂CO₃ being added to the solutions with less or no KBO₂.

Since the half life of ⁴²K is 12.4 hr. and the irradiation of the B solution was 20.9 hr. allowance was made for the decay of the ⁴²K during irradiation in calculating the integrated dose. A similar correction for decay between the time of irradiation and measurement was also made. The activity of a K sample was followed for 72 hr. to check the half life and a value of 12.8 hr. was obtained. This is higher than the generally accepted value of 12.44 (Seaborg & Perlman, 1948) by more than the statistical errors. The measured value was used in the corrections. If this higher value were due to an impurity, its adoption gives the smaller error.

The K monitor was calibrated by two methods. In the first, some samples of 1.48 M-K₂CO₃ solution were irradiated for several days in the nuclear reactor in positions where the neutron flux had been calibrated. The second method involved calibrating the efficiency of a Geiger counter for ⁴²K and using the activation cross-section for ⁴²K (Seren, Friedlander & Turkel, 1947). The counter was of the Veall type (Veall, 1948) and was calibrated with a ⁴²K solution which had been measured at the Atomic Energy Research Establishment, Harwell, in a counter of known efficiency. The two methods give results which differ by about 14%, the first method giving the lower value of the neutron flux. This difference probably indicates the order of magnitude of the error in determining the absolute value of the neutron flux. The mean of the two methods was adopted in the calculations. The neutron flux determined by this method for the B-free solutions compared well with the estimated value for their positions in the nuclear reactor.

Chemical method

The general chemical procedure for estimating the NH₃ liberated in the deamination by radiation was the same as in previous work (Dale, Davies & Gilbert, 1949), but since the highly concentrated solution of KBO₂ required could not be neutralized without precipitation it was necessary to carry out the irradiation in the Conway units themselves to prevent loss of NH₃. The standard pyrex or soda glass

Conway units are not suitable for irradiation inside a nuclear reactor because of the boron and sodium content of the glasses, which either absorb neutrons too strongly or are too radioactive after irradiation. Perspex was therefore used, and sets of three units were hollowed out of solid blocks 6 × 2 × 1 in. with perspex sheets ground to form covering lids, which were held in place by clips. Each block was packed into an aluminium can with absorbent cotton wool for insertion into the nuclear reactor. The three Conway units in each block had inner compartments and outer rings which were filled with 1 ml. of fluid to a depth of about 2 mm. An 0.25 N-H₂SO₄ solution was put in all inner compartments. The outer rings contained the glycine solution with varying amounts of KBO₂ and K₂CO₃, the glycine content being the same in the three units of any one block. The same pattern of KBO₂ and K₂CO₃ concentration was repeated in all blocks and is shown in Table 1.

Two glycine concentrations were investigated, 200,000 and 10,000 μ g./ml. made up by weight, the higher concentration being duplicated. In all, three perspex blocks were irradiated for 20.9 hr. in a neutron flux of about 10^{10} /cm.²/sec. in the nuclear reactor GLEEP at the Atomic Energy Research Establishment, and after removal from the reactor the units were rocked for 0.5 hr. to ensure the completion of the diffusion of NH₃ into the acid solution. The solutions from all compartments in the Conway dishes were then transferred quantitatively to test tubes and the subsequent measurements made in our laboratory in Manchester.

Blank experiments to determine the yield of NH₃ in the absence of radiation were made at the same temperature (31°) experienced during the irradiation, and for the same duration, and also control experiments under the same conditions but with added H₂O₂. According to Bonet-Maury & Lefort (1948) H₂O₂ is formed in pure water proportionately to the α -ray dose. If a solute is present which is sensitive to H₂O₂ part of the radiation effect might be due to H₂O₂ formed during irradiation. On extrapolating the curves published by these authors to the two doses of α -radiation used in the present experiment, one arrives at H₂O₂ concentrations of 7.35×10^{-3} and 3.68×10^{-3} M, which are the average concentrations of H₂O₂ built up during the experiment in the nuclear reactor. These concentrations are maximum concentrations, since one can assume that the presence of a solute reacting with OH radicals and/or H atoms will reduce the formation of H₂O₂.

RESULTS

The results of the α -radiation are summarized in Table 1. The ionic yields of the heavy particle radiation are 0.56 and 0.17 for the 200,000 and the 10,000 μ g./ml. solutions respectively. Comparing these with the corresponding yields for X-radiation (Dale, Davies & Gilbert, 1949) the relative efficiencies for deamination by α -radiation and X-radiation were 19.5 % for the 20 % solution, and 15.2 % for 1 % solution.

Table 2 shows the ammonia yield due to hydrogen peroxide. The hydrogen peroxide effect, therefore, is at most 12.5 % of the α -ray effect for the 200,000 μ g./ml. glycine solution and 7.5 % for the 10,000 μ g./ml. solution.

Table 1. *Deamination of glycine by α -particles*

Conway unit ...	Block 1			Block 2			Block 3		
	A ₁	B ₁	C ₁	A ₂	B ₂	C ₂	α	β	γ
Glycine concn. (μ g./ml.)		200,000			200,000			10,000	
KBO ₃ concn. (m)	2.25	0	1.13	2.25	0	1.13	2.25	0	1.13
Total K concn. (m)	2.25	2.96	2.60	2.25	2.96	2.60	2.25	2.96	2.60
NH ₃ yield (μ g./ml.)	800	73	539	968	65	462	312	26	180
Blank (μ g./ml.)	12	3	12	12	3	12	1	1	2
Net NH ₃ yield (μ g./ml.)	788	70	527	956	62	450	311	25	178
NH ₃ yield due to α -rays (μ g./ml.)	718	—	457	894	—	388	286	—	153
Mol. NH ₃ /ml. $\times 10^{-10}$	2.57	—	1.64	3.20	—	1.39	1.02	—	0.55
Potassium activity* $\times 10^{-5}$	1.36	1.64	1.31	1.20	1.35	1.17	1.48	1.74	1.48
Neutron flux $\times 10^{-10}$	1.02	1.24	0.99	0.90	1.02	0.89	1.12	1.31	1.12
Ion pairs/ml. $\times 10^{-10}$	5.64	—	2.76	4.99	—	2.47	6.20	—	3.10
Ionic yield for α -radiation	0.46	—	0.59	0.64	—	0.56	0.16 _s	—	0.17 ₇
Mean ionic yield				0.56				0.17 ₇	
Ionic yield for X-radiation†				2.89				1.13	
Ratio (' α yield' as % of 'X yield')				19.5%				15.2%	

* Potassium activity is the counts/min./ml./'molar' K, 58 hr. after the end of the irradiation.

† Dale, Davies & Gilbert (1949).

Table 2. *Deamination of glycine by hydrogen peroxide at 31° during 21 hr.*

Glycine concn. (μ g./ml.) ...	200,000		10,000	
H ₂ O ₂ concn. (mm)	7.35	3.68	7.35	3.68
NH ₃ yield (μ g./ml.)	104	67	25	11
Blank (μ g./ml.)	12	12	1	1
NH ₃ due to H ₂ O ₂ (μ g./ml.)	92	55	24	10

DISCUSSION

In Table 3 are set out the ammonia yields from the boron-free solutions, which will be due to radiation by electrons and protons, reduced to a standard neutron flux of 10^{10} /cm.²/sec. On the assumption that the ionic yield for these radiations is the same as for X-radiation, the total dose and the dose rate in r./min. has been calculated. This estimation, however, only gives a lower limit to the radiation dose, because the proton part, having a higher ion density, is probably not so efficient in deamination as the electron part, but there is reasonable agreement between the values calculated from solutions of different concentrations.

Table 3. *Effect of background radiation*

Glycine concn. (μ g./ml.)	Block 1	Block 2	Block 3
	200,000	200,000	10,000
NH ₃ yield/ml. for neutron flux of 10^{10} /cm. ² /sec.	57	61	19
Mol. NH ₃ /ml. $\times 10^{-18}$	0.70	0.75	0.61
Total dose $\times 10^{-5}$ (r.)	4.35	4.66	3.78
Dose rate in flux 10^{10} r./min.	347	372	302

In any comparison between radiation effects produced by the boron disintegration and those by α -

particles from radon or polonium account must be taken of the different densities of the ions along the tracks of the particles. The boron disintegration produces two ionizing particles, an α -particle with range in standard air of 0.77 cm. or 7.7 μ . in water and a Li particle of range 0.48 cm. in air or 4.8 μ . in water (Gilbert, 1948). The maximum ion density of the latter is only just greater than that of the α -particle (Bower, Bretscher & Gilbert, 1938) because its range is shorter than the Bragg maximum in the ionization curve for lithium particles. The mean linear density of ionization along the disintegration track is 5900 ion pairs/ μ . of track in water, the mean density of the lithium particle being about 13% less than in the α -track. For comparison the corresponding figure for the mean of the three α -particles emitted by radon and its short-lived daughter products is 3500 ion pairs/ μ . and for the electrons produced by a 200 kV. X-ray tube 80 ion pairs/ μ . (Gray, 1947).

The relative yield for α -particles compared with X-radiation (15–19%) for deamination of glycine is considerably higher than the values (3–10%) for a range of concentrations for the inactivation of carboxypeptidase by α -particles from radon and its short-lived products (Dale, Gray & Meredith, 1949). The increased efficiency in the present experiment is not likely to be due to the fact that the linear ion density was greater than in the radon experiment, for this should rather result in a decreased efficiency. Both experiments show an increase of the ionic yields for α -radiation with concentration of solute, though not proportional to concentration. The high value of 0.56 obtained for the highest concentration of glycine is of particular interest showing that the whole effect cannot be attributed to the δ -rays but

that the main column of the α -track must play an important part.

Bonet-Maury & Lefort (1948) have shown that hydrogen peroxide is formed in pure water by α -radiation, and therefore part of the α -radiation effect could be due to the presence of hydrogen peroxide if the solute is sensitive to it. The presence of a solute however may reduce the formation of hydrogen peroxide by reacting with the primary products of radiations (OH radicals and H atoms). Carboxypeptidase was shown to be insensitive to high concentrations of hydrogen peroxide (Dale, Gray & Meredith, 1949) and though the hydrogen peroxide can deaminate glycine, the effect is not more than 12% of the total radiation effect as shown by the present control experiments.

Since neither the quality of the radiation nor the effect of hydrogen peroxide can wholly account for the observed differences, one should assume that these are linked in some way with the fact that the X-radiation effects on amino-acids were very different from those on macromolecules, as was fully discussed in an earlier paper (Dale, Davies & Gilbert, 1949). There a chain reaction or the action of radicals by excitation was invoked for explaining the kinetics of

the deamination of amino-acids by X-rays, and it was also pointed out that these theoretical considerations are not yet fully in agreement with current concepts of the indirect action of radiation.

SUMMARY

1. The deamination of glycine by the densely ionizing particles from the disintegration of boron by slow neutrons in a nuclear reactor has been studied. An account of the necessary technique is given and the dosimetry fully discussed.

2. The ionic yields for the liberation of ammonia were 0.17 and 0.56 for 1 and 20% solutions of glycine, respectively, being 15 and 19% of that due to X-radiation. The higher efficiency of deamination by this radiation compared with the inactivation of an enzyme by α -rays from radon and its products is discussed.

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Observations on Antisera Against Complexes of Di-2-chloroethyl sulphide and Protein

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Human beings and animals, after receiving repeated skin exposures to di-2-chloroethyl sulphide (mustard gas, 'H'), often develop an increased sensitivity to this substance, and it was felt that the factors concerned might be investigated by methods developed in the study of immunity. Berenblum & Wornall (1939) previously published an account of an investigation undertaken with this end in view, and the

present communication provides additional information about the system first described by these authors. It was also hoped that by studying different derivatives of diethyl sulphide as inhibitors in a specifically precipitating immunity system, some evidence might be obtained for the nature of the linkage formed when H reacts with proteins.