A Comparison of the Effects of Ultraviolet and Ionizing Radiations on Trypsin Activity and on its Constituent Amino Acids

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Photons of 254 nm. u.v. light, ^{60}Co γ -rays and 1 Mev electrons produce different patterns of destruction of individual amino acids in dried films of trypsin and in the corresponding amino acid mixture. For example, in the amino acid mixture u.v. light destroys tyrosine, tryptophan and cystine, whereas in trypsin only cystine is disrupted but with 10 times the initial yield. Further, in the amino acid mixture loss of half-cystine is a simple exponential function of dose, but in trypsin there appear to be two exponential components of the loss with yields that differ by a factor of 35. Both the γ -rays and electrons destroy half-cystine, tryptophan, histidine and methionine in the amino acid mixture with remarkably high yields, whereas in trypsin doses that destroy almost all of the enzymic activity produce no detectable destruction of amino acid residues. These marked differences between the two preparations show that the radiation-sensitivity of a given amino acid alone and in a protein is different, and suggests that in trypsin there is fairly extensive migration of energy, charge or both with localization of damage at specific sites determined by this enzyme's internal organization. All three types of radiation produce appreciable amounts of 'damaged' (not completely inactivated) molecules which are prevented from reassuming an active configuration by the addition of 5.5 M-urea; thiol reagents have a similar effect after bombardment with u.v. light or electrons. The patterns of destruction produced by γ -rays and by electrons in both the amino acid mixture and in trypsin are different (some of the yields vary by a factor of 30). This result appears to be inconsistent with the popular belief that most of the energy absorbed from γ -rays is associated with very-high-energy electrons.

There have been a number of conflicting views on the mechanisms by which radiation destroys the biological potency of proteins (for review, see Augenstein, 1962a). The experiments reported here were designed to investigate further three questions dealing with this topic: (i) to what extent does incorporation of an amino acid into a protein change the sensitivity of that residue to various types of radiation? (ii) are there mechanisms of enzyme inactivation that are common to all types of radiation, both ultraviolet and 'ionizing'? and (iii) are the biochemical effects of γ -rays and fast electrons the same?

Specifically, we have studied the effects of u.v. radiation (254 nm.), ^{60}Co γ -rays and 1 Mev electrons on dried films of either trypsin or a mixture of amino acids having the same composition as this protein. Destruction of the various amino acids

was measured in the two conditions and the yields were compared with the loss of enzymic activity. Further, the residual enzymic activity was measured both with and without exposure to urea and thiol reagents to investigate the possible production of trypsin molecules that have been 'damaged' but not irreversibly inactivated by the radiations (Augenstein & Ghiron, 1961). Since it is known that the method of preparing samples can cause large changes in radiation-sensitivity (Butler & Robins, 1960), we adsorbed either the amino acid mixture or trypsin on to dialysis membranes to be able to use the same method of preparing films for all three types of radiation.

EXPERIMENTAL METHODS AND MATERIALS

Preparation of enzyme and amino acid samples. Dialysis membranes were cleaned by the standard pretreatment method described by Hughes & Klotz (1956).

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To make the protein solution, 10 or 40mg. of twice-crystallized salt-free trypsin from Worthington Biochemical Corp. (Freehold, N. J., U.S.A.) was dissolved in 2ml. of dil. acetic acid, pH3.0. A 40 μ l. sample of one of these solutions was applied to a dialysis membrane stretched taut over a circular frame. This droplet was spread over a circular area 2.4cm. in diameter and allowed to dry at room temperature (about 45min.).

Also, 10 or 40mg. of the following amino acid mixture was dissolved in dilute acetic acid, pH3.0, containing the minimum amount of conc. HCl necessary to dissolve the cystine present: DL-aspartic acid, 17.3mg.; L-threonine, 11.9mg.; L-serine, 17.3mg.; L-glutamic acid, 10.3mg.; L-proline, 5.2mg.; glycine, 9.8mg.; DL-alanine, 6.2mg.; DL-valine, 10.5mg.; L-isoleucine, 9.8mg.; L-leucine, 10.2mg.; L-tyrosine, 8.2mg.; L-phenylalanine, 2.5mg.; L-methionine, 1.5mg.; L-lysine hydrochloride, 13.7mg.; L-histidine, 2.3mg.; L-arginine hydrochloride, 2.1mg.; L-cystine, 6.8mg.; L-tryptophan, 4.1mg. This mixture is identical in composition with trypsin except that it contains the same amounts of glutamic acid and aspartic acid as the sums of glutamic acid plus glutamine and aspartic acid plus asparagine normally found in trypsin. Samples of these solutions were applied to the dialysis membranes as described above.

Since it is well known that either adsorption or drying of a protein may lead to denaturation and inactivation, we tested extensively the activity which could be reclaimed from our preparations. Fortunately, the residual enzymic activity in the unirradiated samples redissolved from the membranes was identical with that of trypsin that had not been adsorbed on to a membrane.

Irradiations. (i) Electron irradiation. A flux of 1 Mev electrons that gave 8.3Mrads/min. as measured with an FeSO₄ dosimeter was obtained from a linear accelerator located in the Agricultural Engineering Department of Michigan State University. The dialysis membranes containing either trypsin or the amino acid mixture were attached to Plexiglass sheets, thickness $\frac{1}{8}$ in., which were placed on solid CO₂ during the irradiation. Since irradiation times of 9min. or less were used, no appreciable heating of the samples occurred. These and all other irradiations were conducted with normal laboratory air present.

(ii) γ -Ray irradiation. The samples were placed in 2 dram glass tubes (Kimble) and inserted into the ⁶⁰Co γ -ray facility at the Phoenix Laboratory of the University of Michigan. The dose rate was 0.65Mrad/hr. as measured with an FeSO₄ dosimeter.

(iii) Ultraviolet irradiation. A bank of 8w u.v. lamps placed at a distance of 50cm. from the sample provided a u.v. flux of 4.5×10^{-8} einsteins/cm.²/min., where 1 einstein is defined as 6×10^{23} photons. The 0.2mg. of trypsin applied to the dialysis membrane had E_{254} approx. 0.05 and had 12% reflectance. Since approx. 88% of the light was transmitted through the membrane and film, the intensity of light throughout the sample was nearly constant.

Measurement of enzymic activity. After irradiation the samples were put in 5ml. of 0.01M-sodium phosphate buffer, pH3.0, for 6min. to elute the trypsin from the membrane. Measurements of both enzymic activity and extinction showed that the trypsin was eluted quantitatively under these conditions. The residual esterase activity in irradiated trypsin was determined from the

rate of hydrolysis of the substrate BAEE* in 0.01M-sodium phosphate buffer, pH8. To ascertain the fraction of 'damaged' molecules produced (Augenstein & Ghiron, 1961), the irradiated enzyme solutions were treated with the thiol reagents PCMB (Boyer, 1954; Augenstein & Ghiron, 1961) or DTNB at pH8.0 (Ellman, 1959) before assay in both the absence and presence of 5M-urea.

Determination of amino acid contents. Seventeen of the 18 constituent amino acids of trypsin were determined by the methods of Grundlach, Moore & Stein (1963) with a Beckman amino acid analyser. The results obtained were standardized against valine, leucine and phenylalanine separately (these three amino acids are stable towards acid hydrolysis and also apparently are unaffected by the types of radiation employed; Augenstein & Riley, 1964). The values reported are averages of the three sets of standardized values. Tryptophan was determined separately by the u.v. difference-spectral technique with *N*-bromosuccinimide (Patchonik, Lawson & Witkop, 1958). Since we did not determine glutamine and asparagine separately from glutamic acid and aspartic acid, nothing can be said about the possible radiation deamination of them.

The amino acid mixture was eluted from the dialysis membrane with a 0.2M-sodium citrate buffer, pH2.2, and assayed directly on the Beckman amino acid analyser.

RESULTS

The effects of the three types of radiation on the enzymic activity of trypsin and also on the individual amino acids in trypsin or in the amino acid mixture are shown in Figs. 1, 3 and 4. The values of yields derived from these results are tabulated in Tables 1 and 2.

Ultraviolet irradiation. The half-cystine residues are the only ones that decline in number significantly (Fig. 1) when trypsin is exposed to u.v. radiation. Previously it was reported that tryptophan was destroyed by u.v. radiation at a rate approximately equal to that for inactivation of trypsin (Augenstein & Ghiron, 1961). However, those results were obtained by means of alkaline hydrolysis of the trypsin, whereas in our present analysis of tryptophan we used *N*-bromosuccinimide. This implies that whatever destruction occurs does not affect the indole moiety: consistent with this we observed no change in E_{280} even with exposures that destroyed 90% of the enzymic function.

The decline in number of the half-cystine residues can be resolved into two obvious components (Fig. 2): three of the six constituent cystine residues are destroyed with a quantum efficiency of about 4 times that for the inactivation of trypsin, and the other three are destroyed at a much lower rate (Table 1). Although this complex pattern has been observed previously for trypsin irradiated in

* Abbreviations. BAEE, benzoyl-L-arginine ethyl ester; PCMB, *p*-chloromercuribenzoate; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid).

Fig. 1. Effect of u.v. radiation (254 nm.) on tryptic activity and the integrity of individual amino acids either in trypsin (a) or in the amino acid mixture (b) corresponding to the composition of trypsin. All samples were irradiated as films dried on dialysis membranes. The different reagents used in the assays of trypsin activity are as follows. ×, BAEE; △, BAEE+PCMB; ▲, BAEE+DTNB; ○, BAEE+urea; □, BAEE+PCMB+urea; ■, BAEE+DTNB+urea. Methionine was poorly resolved in all cases.

solution, in the earlier studies the disruption of cystine proceeded at approximately the same rate as the loss of enzymic activity. In accord with previous reports (Augenstein & Ghiron, 1961; Augenstein & Riley, 1964; Risi, Dose, Rathinasamy & Augenstein, 1967) the inactivation of trypsin induced by u.v. light can be correlated with the appearance of groups that can be titrated with either PCMB or DTNB. The two methods, however, give different results: the values respectively are 3.1 and 1.0 reactive groups/mol. of trypsin inacti-

vated. It has been shown that PCMB and other mercurial reagents react with both thiol groups and monoxycystyl residues that may be formed by oxidation or photo-oxidation (MacLaren, Savage & Sweetman, 1965). The value of 1 thiol group/mol. of trypsin inactivated is consistent with the values obtained with *S*-carboxymethylation of u.v.-irradiated trypsin (Risi *et al.* 1967). Since the use of the DTNB reagent is based on a disulphide-interchange reaction, it should be specific for free thiol groups. Thus we believe that the difference

Fig. 2. Comparison of the destruction by u.v. radiation of the half-cystine content (C) and the esterase activity in trypsin (I). The two broken lines represent a resolution of the results for the loss of half-cystine in trypsin into two exponential components.

between the two estimates of numbers of thiol groups reflects the presence of partially oxidized cystine residues formed in trypsin by irradiation. Nevertheless, the specificity of non-mercurial reagents such as DTNB for thiol groups has not been established unequivocally.

In contrast with the situation in trypsin, u.v. irradiation of the amino acid mixture causes degradation of both tyrosine and tryptophan in addition to cystine. Although destruction of cystine proceeds much more rapidly than that of the two aromatic amino acids, it still occurs at a much lower rate than is the case for loss of half-cystine in trypsin. This is particularly evident when calculations are made of the yields based on those photons that are presumably absorbed only in cystine (assuming the extinction of cystine in trypsin is the same as that for the free amino acid). The observation of an estimated yield in excess of 1 is in agreement with previous results (Risi *et al.* 1967; Rathinasamy & Augenstein, 1968) and presumably reflects disruptions resulting from photons absorbed in other residues in the trypsin.

The effects of u.v. irradiation on the esterase

activity of trypsin are shown in Fig. 1 for various assay conditions. The quantum yield of 0.014 measured by the most common assay method (BAEE added immediately after irradiation) agrees reasonably well with those reported previously for u.v. irradiation of trypsin adsorbed on to glass or quartz (Lofrth & Augenstein, 1967) and of this enzyme in solution under different conditions (Augenstein & Ghiron, 1961; McLaren & Luse, 1961). Clearly, treatment of irradiated trypsin with either PCMB or DTNB after irradiation but before the addition of substrate increases the irradiation yield by essentially the same amount. Similarly, addition of urea to the substrate mixture at the time of assay increases even more the amount of inactivation observed after a given irradiation exposure. As recorded in Table 2, the ratios of the yields under these conditions are very nearly the same as those reported for u.v. irradiation of trypsin in solution (Augenstein & Ghiron, 1961).

Irradiation with 1 Mev electrons. The D_{37} dose (i.e. the dose required to decrease the initial enzyme activity to $e^{-1} = 0.37$ of its initial value) of 38 Mrads measured from the results in Fig. 3 is somewhat higher than the values of 11.8–36 Mrads reported by other workers (Table 2). However, a direct comparison with previous results is not possible since practically all of the earlier workers irradiated their samples in either thin-glass or plastic vials: this surrounding material probably changed the spectrum of energies of the electrons striking the samples. As with u.v. light, the addition of either a thiol reagent or urea before exposure to substrate increases the irradiation yield. However, as shown in Table 2, the fractional increase for either of these treatments is less than is the case with u.v. irradiation: the ratio 1.5 for the effect of urea is very close to the value of 1.7 reported by Brustad (1961).

Amino acid analyses of trypsin exposed to these fast electrons revealed no loss of any of the constituent amino acids with doses sufficient to destroy 80% of the enzyme activity. In addition, this radiation produces no decrease in the indole content that can be demonstrated by titration with *N*-bromosuccinimide. Although the half-cystine content remains constant, groups are produced that can react with both PCMB and DTNB. Again, the numbers of reactive groups estimated by these two methods are different; they are respectively an increase of 1.4 and 0.5 reactive groups/mol. of trypsin inactivated. These results would seem to place an upper limit of 1 cystine residue disrupted/mol. inactivated by fast electrons and indicates that the chances that the disruption of a cystine residue will produce a thiol are less than the chances of producing an oxidized by-product.

In contrast with the situation for trypsin, there are losses of cystine, tryptophan, tyrosine, methio-

Table 1. *Parameters for the inactivation of trypsin, the destruction of amino acid residues, or the production of thiol groups by different radiations*A. Ultraviolet irradiation (flux 4.5×10^{-8} einsteins/cm.²/min.)

	D ₃₇ (min.)	Yield	
		(moles/einstein absorbed/mole of trypsin)	(moles/einstein absorbed/mole of the pertinent amino acid)
Trypsin			
Enzyme inactivation	34.5	0.014	
Thiol production (measured by PCMB)		0.023	0.22
Thiol production (measured by DTNB)		0.009	0.09
Destruction of half-cystine (measured by amino acid analysis)	8 300	0.69, 0.02	6.6, 0.2
Amino acid mixture (determined by amino acid analysis)			
Half-cystine destruction	150		0.17
Tryptophan destruction	250		0.01
Tyrosine destruction	650		0.04

B. 1 Mev electrons (dose rate 8.3 Mrads/min.)

	D ₃₇ (min.)	Yield	
		(molecules/ev absorbed in trypsin)	(molecules/ev absorbed in pertinent amino acid)
Trypsin			
Enzyme inactivation	4	0.012	
Thiol production (measured by PCMB)		0.017	
Thiol production (measured by DTNB)		0.006	
Amino acid mixture (determined by amino acid analysis)			
Half-cystine destruction	8.8		0.55
Methionine destruction	(16?)*		(0.5)
Histidine destruction	16		0.5
Tryptophan destruction	4.8		1.2
Tyrosine destruction	47.3		0.14

C. ⁶⁰Co γ-rays (dose rate 650 000 rads/hr.)

	D ₃₇ (hr.)	Yield	
		(molecules/ev absorbed in trypsin)	(molecules/ev absorbed in pertinent amino acid)
Trypsin			
Enzyme inactivation	25	0.025	
Thiol produced (measured by PCMB)		0.025	
Cysteic acid produced		0.016	
Half-cystine destruction	120	0.06	
Amino acid mixture (determined by amino acid analysis)			
Half-cystine destruction	17 260		3.6, 0.2
Tryptophan destruction	17		4.3
Methionine destruction	80		1.4
Arginine destruction	(80?)†		(1?)†
Histidine destruction	250		0.4
Tyrosine destruction	360		0.25

* See Fig. 1.

† See scatter of results in Fig. 1.

nine and histidine on electron irradiation of the amino acid mixture with comparable doses (Fig. 3). Only tryptophan is destroyed in the free state at

a rate comparable with that for trypsin inactivation. However, as shown in the second column in Table 1, the yields are large when they are calculated on the

Table 2. *Comparison of the present results with previous reports for the irradiation of trypsin*

References: * present results; ^aAugenstein & Ghiron (1961); ^bLofrth & Augenstein (1967); ^cMcLaren & Luse (1961); ^dAlexander (1957); ^eHutchinson & Watts (1961); ^fHutchinson (1960); ^gAugenstein (1959); ^hAlexander & Hamilton (1960).

A. Parameters for the inactivation of trypsin

	Ultraviolet (quantum yield)	1 Mev electrons (D ₃₇ in Mrads)	γ -Rays (D ₃₇ in Mrads)
Non-urea assay with BAEE	0.007 ^a , 0.010 ^b 0.014 [*] , 0.015 ^c	11.8 ^d , 28 ^e , 36 ^b , 38 [*]	16 [*] , 32–35 ^e
Assay with 5.5 M-urea present with BAEE	0.017 ^a , 0.023 [*]	26 [*]	10 [*]

B. Effects of exposing irradiated trypsin to thiol reagents or urea before assay^a. All values normalized to those obtained with normal assay procedure, i.e. only substrate present

	Ultraviolet (quantum yield)	1 Mev electrons (D ₃₇ in Mrads)	γ -Rays (D ₃₇ in Mrads)
PCMB or DTNB present	1.4 [*] , 1.4 ^f , 1.7 ^a	1.2 [*]	1 [*]
5.5 M-Urea	1.7 [*] , 2.4 ^a , 3.6 ^b , 4 ^g	1.5 [*] , 1.7 ^h	1.6 [*]

basis of molecules destroyed/ev absorbed directly by that particular amino acid.

Irradiation with ⁶⁰Co γ -rays. The D₃₇ dose of 16 Mrads measured from the results in Fig. 4 for the destruction of trypsin activity as determined by the immediate addition of substrate is appreciably less than that found by other workers, who have reported values of more than 30 Mrads (Hutchinson & Watts, 1961). After γ -irradiation there is a minimal effect of adding PCMB and none of DTNB, but there is a 30% enhancement in the yield when urea is present during the assay (Table 2).

The only amino acid that is significantly affected by γ -irradiation of trypsin is cystine (D₃₇ = 79 Mrads) (Fig. 4). Its disruption yields mainly cysteic acid as measured by the normal amino acid assay procedure. However, neither the production of cysteic acid nor the groups that react with PCMB can be correlated linearly with loss in enzymic function. There is little if any loss of indole groups (as determined by *N*-bromosuccinimide) produced by the γ -irradiation.

The γ -irradiation of the amino acid mixture corresponding to the composition of trypsin causes destruction of the six compounds listed in Table 1. The disruption of cystine in this mixture may be resolved into two exponential components with greatly differing radiation-sensitivities (D₃₇ = 12 and 85 Mrads).

DISCUSSION

Radiation-sensitivity of amino acids irradiated separately and as residues in trypsin. There have been differing viewpoints over the years as to how much the radiation-sensitivity of a given amino acid residue is changed when it is incorporated into

a protein. In particular, there have been conflicting views on the importance attached to energy transfer. For example, Alexander (1962) concluded from his work on the γ -irradiation of bovine serum albumin that 'energy-transfer processes' to particular amino acid residues occur but that the effect is not very selective, since he argues that many residues should be good 'energy sinks' (Alexander & Hamilton, 1960; L. D. G. Hamilton; see Alexander, 1962). By contrast, Augenstein (1962b), on the basis of his much earlier hypothesis, predicted that the radiation-sensitivity of various amino acids should be greatly modified by incorporation into a protein: he stressed that the internal organization of most proteins is such that it should cause preferential 'funneling' of excitation energy, or charge, or both, to certain preferred amino acids, particularly to specific cystine residues (Augenstein, 1953, 1956, 1962a; Augenstein & Ray, 1957). Hayden & Friedberg (1964) measured yields for the destruction of individual amino acid residues in γ -irradiated ribonuclease, which were not the same on either an absolute or a relative basis as those reported by Alexander (1962). These changes indicated either that the surrounding micro-environment greatly modified the photochemical behaviour of excited amino acids in an enzyme, or else that energy transfer to certain residues was fairly extensive and important. A comparable conclusion could be drawn from the work of Stevens, Henderson & Tolbert (1964) on lysozyme and α -chymotrypsin. From these few results with γ -rays it was clear that there can be big differences between the sensitivity of an amino acid irradiated separately and as a residue in a protein. Further, recent studies of ribonuclease irradiated with four wavelengths of u.v. light have shown that: (a) the changes in the sensitivities of the constituent cystine residues are

Fig. 3. Residual trypsin activity and amino acid content as a function of the dose from 1 Mev electrons. (a) Trypsin; (b) amino acid mixture corresponding to the composition of trypsin. $\times$, BAEE; Δ , BAEE+DTNB; $\circ$, BAEE+urea; $\bullet$, BAEE+urea+DTNB. Methionine was poorly resolved, but apparently little or no loss occurred.

not just the result of incorporation into a polypeptide chain; (b) at two of the wavelengths disruption of cystine must result from the transfer of energy absorbed at chromophores elsewhere in the molecule (Rathinasamy & Augenstein, 1968).

The present results strongly support these conclusions. In particular, it seems doubtful that the widely differing patterns of damage with the three types of radiation could be accounted for simply on the basis of environmental modifications in the sensitivities of excited residues; rather, the changes

must reflect extensive energy transfer both within trypsin and within the amino acid mixture. For example, u.v. irradiation of trypsin produces disruption of cystine with high yield, whereas in the amino acid mixture corresponding to the composition of trypsin, tyrosine and tryptophan are also inactivated to significant extents in addition to cystine. On the basis of those photons that are presumably absorbed only in cystine, the quantum yield for disruption of cystine in trypsin is more than 10 times that for disruption of cystine in the

Fig. 4. Destruction of tryptic activity and individual amino acids by ^{60}Co γ -rays. (a) Trypsin; (b) amino acid mixture corresponding to the composition of trypsin. Results for methionine showed poor reproducibility. $\times$, BAEE; Δ , BAEE+PCMB; $\square$, BAEE+PCMB+urea; $\blacksquare$, BAEE+DTNB+urea.

amino acid mixture. This is not too surprising, since the enhanced yields for destruction of half-cystine in trypsin in solution have been attributed to photons absorbed in other residues that eventually cause disruption of cystine (Risi *et al.* 1967). In fact, it has been proposed that there may be interactions between certain excited tryptophan residues and specific cystine residues within trypsin (Augenstein & Ghiron, 1961; Augenstein, 1962a,b), and between neighbouring tyrosine residues and specific cystine residues in ribonuclease (Rathinasamy & Augenstein, 1968).

Such a possibility is supported by a comparison of the kinetics of destruction of half-cystine by u.v. radiation in trypsin and in the amino acid mixture. In the former, a plot of the log[residual half-cystine] versus dose can be resolved into two exponential components having yields that differ by a factor of 35. By contrast, the disappearance of half-cystine in the amino acid mixture follows simple exponential kinetics.

Both the γ -rays and the 1 Mev electrons destroy half-cystine, tryptophan, histidine and methionine with remarkably high yields in the amino acid mixture, whereas in trypsin we could not detect significant amino acid loss with the same exposures to the radiation, even though those doses destroy practically all of the enzymic activity. The yields of approx. 1 molecule of a given amino acid affected/ev absorbed anywhere in the mixture implies that there must be very extensive and efficient migration of energy or charge within the mixture of amino acids. It is of course possible that this could occur from one amino acid to another. In fact, electron-spin-resonance studies have already shown that quite efficient intermolecular charge migration can occur within mixtures so as to give localization of free radicals on those molecules that appear to be the most efficient electron traps: actually this appears to occur in mixtures produced by drying from solution (as were ours), but not in those produced by grinding and mechanical mixing of powders (Gordy & Miyagawa, 1960). At the same time these large yields could reflect migration of energy or charge, or both, from the cellulose support material to the critical amino acids: previous studies of the modification by X-irradiation of various small molecules adsorbed on to gels indicated that there must be transfer of both electrons and protons from the gels to the adsorbed molecules (Gevantman, 1960; Allen, 1960). Such a possibility might also be consistent with the report by Bayly & Evans (1967) of the extreme lability of sugars adsorbed on to cellulose bases. By contrast, the adsorption of macromolecules on to (or into) porous substrata appears to decrease their radiation-sensitivity (Augenstein, Augenstein & Lippincott, 1960;

Augenstein, 1962a; Holladay, Augenstein & Augenstein, 1966; James & Augenstein, 1966).

Nature of inactivation of trypsin by the three types of radiation. On the basis of his weak-link hypothesis, Augenstein (e.g., 1953, 1956, 1962) predicted that the preferential funnelling to certain critical cystine residues of effects initiated elsewhere in the protein should follow excitation by various types of radiation. He and his collaborators, using u.v. radiation, not only demonstrated that there is a correlation between the extent of disruption of cystine and the loss of biological function in a number of enzymes, but that disruption of cystine is non-random (Grist, Taylor & Augenstein, 1965), and, further, that few other amino acids are disrupted with doses that destroy most of the activity. Further, the activation energies for thermal enhancement of enzyme inactivation in the range 10–400°K by ionizing particles are very similar to those for inactivation by u.v. radiation, suggesting that there may be a number of mechanisms common to such diverse types of radiation as u.v. light, X-rays, protons, deuterons, carbon nuclei, argon nuclei etc. (Augenstein, Brustad & Mason, 1964a). In addition, Brustad (1961) found that the inactivation yields of trypsin depended on the assay conditions after irradiation of dried films with 123 Mev neon ions. His report is similar to the earlier observations after u.v.-irradiation in solution that were ascribed to so-called 'damaged' trypsin molecules (Augenstein & Ghiron, 1961).

With all three types of irradiation we find some results that, at least in first approximation, are consistent with the general weak-link idea. In particular, the presence of 5.5 M-urea in the assay mixture increases the yields by factors of 1.5–1.7 (see Table 2), and the addition of thiol reagents before assay significantly increases the yields for u.v. and electron irradiation. Presumably these treatments prevent the 'damaged' trypsin molecules from reassuming an active conformation. Further, there is disruption of cystine accompanying the trypsin inactivation. However, at least with γ -rays we do not find a linear correlation between disruption of cystine and inactivation of trypsin.

Certainly, the big differences between the destruction of individual residues in trypsin and in the amino acid mixture suggest that in trypsin there is migration of energy or charge, or both, with specific localization that is determined by the internal organization of this globular protein. However, the differences in the products from cystine and in the effects of thiol reagents or urea, or both, on the inactivation yields suggest that the three types of radiation produce similar but not identical changes in trypsin. A decision on just how common or dissimilar the mechanisms may be

will require extensive characterization of the 'damaged' and 'inactivated' trypsin produced by the various types of radiation.

Comparison of the effects of γ -rays and fast electrons. On the basis of theoretical considerations, it has been concluded normally that most of the energy transferred to an absorbing medium by X-ray photons will give rise to fast electrons having average energies of many kev (e.g., Fano, 1954; Augenstein, 1962a, 1963). On this basis most of the excitations in condensed systems should be characteristic of those for fast electrons, i.e. optically allowed transitions obeying the Bethe equation (Bethe, 1933). If so, then both the spectroscopic and biological effects of γ -rays should be essentially identical with those produced by high-energy electrons. However, from considerations of the nature of the X-ray-induced thermoluminescence (Carter, Nelson & Augenstein, 1963; Nelson, Carter, Birkhoff, Hamm & Augenstein, 1967) and also of the phosphorescence/fluorescence ratios produced in aromatic amino acids by various types of radiation (Augenstein, Carter, Nag-Chaudhuri, Nelson & Yeagers, 1964b), it has been concluded that far more of the effects are produced by slow electrons than had been previously expected (Augenstein, Yeagers, Carter & Nelson, 1967; E. Yeagers & L. Augenstein, unpublished work).

It is clear from the present results that γ -rays and 1 Mev electrons do not have the same biochemical effect. For example, the D_{37} dose for destruction by γ -rays of enzymic activity is only half that for inactivation by the fast electrons. Of course, this could reflect actual differences in the true dose absorbed, since our materials were thin films and thus it is possible that electron homogeneity within the sample was different with the fast electrons than with the γ -rays, even though every attempt was made to provide electron homogeneity in the latter case by the use of the glass vials for holding the samples during irradiation. A simple difference in actual dose does not explain the whole spectrum of effects, however, since some of the amino acid yields differ by a factor of 30 and further there is no constant factor by which it is possible to relate the D_{37} doses for the different amino acids, i.e. the patterns of destruction are different for the two types of irradiation. Unfortunately, it was impractical to vary the dose rates for the electrons and γ -rays very much. Thus, we cannot rule out completely that some of the observed differences reflect the 700 times larger dose rate with electrons or the possibility that there is greater diffusion and thus greater availability of oxygen during the much longer γ -irradiations.

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