Inactivation of Aldolase by X-Rays

This note reports measurements of the inactivation yield of rabbit-muscle aldolase (E.C. 4.1.2.7) in solution and of the effect of various added substances, that are protectors or sensitizers in vivo. Previous determinations made on this enzyme have given values of 0.03[1] (in anaerobic conditions), 0.11[2] and 0.13[3]; in this work, the yield has been determined at various concentrations and with different samples of enzyme, to insure that the results would not be vitiated by impurities in the medium or in the enzyme samples. There is at present considerable uncertainty and controversy concerning the susceptibility of enzymes in solution to inactivation by ionizing radiation and also concerning the mechanism of protection[4-6]; it is hoped that the present investigation will contribute to our understanding of these problems.

The general procedure, apparatus and dosimetry were as described in another paper[7]. Three enzyme samples were examined: WI and WII from Worthington, BI from Boehringer. WI and WII gave a single, symmetrical peak on analytical ultracentrifugation, WII contained some 5–10% of a faster-sedimenting component. The initial enzyme concentration E was estimated from the absorbance at 280 nm ($E^{1\%}_{1\,cm}$ 0.91); the M.W. was taken as 149,000[8]. The irradiations were conducted in $0.05M$ phosphate buffer, unless otherwise specified. Assay was by BECK's modification of the method of SIBLEY and LEHNINGER[8]; samples and control were assayed at the same time, soon after irradiation (< 2 h); the samples were diluted as needed with phosphate containing $5 \times 10^{-4}M$ ethylenedinitrilotetraacetate and the assay was conducted immediately after dilution.

Samples WI and WII were measured at a single initial concentration. Four or five sets of measurements were made on each sample, to 70–80% inactivation, and the results averaged. The fraction of activity α remaining decreased logarithmically with the dose D: $\log \alpha = -KD/2.303$ (eq. 1). The Table reports the mean values of D_{37}, the dose giving 37% of the original activity: $D_{37} = 1/K$; these values were computed by the method of least squares and the standard deviation of the mean is reported also. The apparent yield $G = EK = E/D_{37}$ (eq. 2).

HUTCHINSON and ROSS[10] have pointed out that the values of G given by eq. 2 would be low if reaction with impurities or other processes not involving the enzyme consumed an appreciable fraction of the dose. These authors therefore suggest that G be determined for various E values and the results plotted; one might expect to obtain a straight line, the slope of which would be the 'true' yield, G'. The Figure shows the results obtained in this way with sample BI. The average value of G' found for this sample is given in the Table. If one assumes that there would be the same difference between G and G' for all 3 samples, one obtains an overall average of 0.10 ($\pm$ 0.02) for the radiochemical yield.

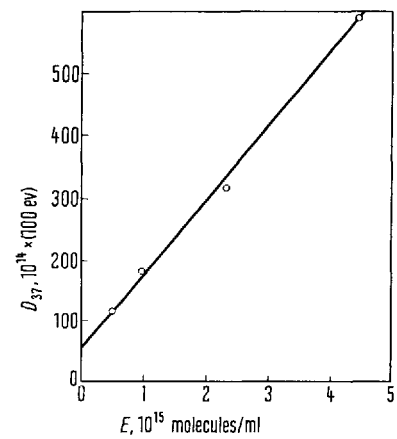
It is difficult to estimate the accuracy of this result because we do not as yet understand well enough what factors influence the yield, and by how much. However, the degree of agreement obtained with different samples, 2 from entirely different sources, gives us confidence that the possible impurities could not greatly affect the result. Other experiments show that the value of the yield does not depend very critically on the conditions used. The values of G' determined respectively in $0.05M$ phosphate buffer, in $0.01M$ buffer and in doubly-distilled water were in the ratio 1:1.1:1.25. Also, the yields found in phosphate buffers of pH 6 and 8 were within 10% of the value at 7.

We are led by these results to the conclusion that many primary ionizations occur in the solvent per molecule of aldolase inactivated – some 30 ionizations, if one takes 33 ev as the average energy required per ionization. In water, the primary ionization products are quickly converted into radicals, e^-_{aq}, H·, OH·, and OOH·, and it is generally agreed that inactivation is caused mostly by reaction with these radicals. The low inactivation yield indicates that only a small fraction of the radicals produced are effective in causing inactivation. Two possibili-

Inactivation yields

Enzyme sample	Specific activity[a]	Concentration $\times 10^6 M$	D_{37} Krad	G or G' molecs/100 ev
WI	113	5.8	65.2 ± 1.02	0.086
WII	93	6.5	53.5 ± 1.03	0.12
BI	131	0.8–7.5	–	0.10

[a] Expressed as suggested by SWENSON and BOYER[9] as the ratio of the absorbance of the product at 540 nm to the absorbance of the enzyme at 280 nm.



D_{37} at various initial concentrations E for sample BI. The slope of the line equals G' in molecules/100 ev.

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ties are that: (a) reaction can occur at many sites in the enzyme without causing inactivation; or (b) repair can occur after an initial attack by radical, restoring the enzyme to its pristine condition[7].

The dose-modifying effect F of added substances is defined as follows: $F = D_{37}^{m}/D_{37}^{c} = K^{c}/K^{m}$, in which the superscripts m and c denote respectively the values found in the presence of modifier and for the control. E was $5 \times 10^{-6} M$ and the modifier was in 50-fold greater molar concentration, unless otherwise specified. For cysteamine, F was 6.4, for cysteine 5.9, i.e. these substances had a pronounced protective effect. For the derivative disulfide cystamine F was 3.3, for cystine 3.9; i.e. these substances were somewhat less effective, distinctly so, if compared on the basis of weight. Experiments were done with cystine-S^{35} to determine the mechanism of protection[7]: the enzyme and cystine were mixed, allowed to react for some time, then separated by chromatography on Sephadex-G25. No appreciable radioactivity was incorporated into the protein in 6 h, showing that the protection realized in the present conditions was not due to formation of a mixed-disulfide product[6]; it might be due to radical-scavenging and/or to repair by the sulfur compound.

The inactivation yield was little affected by added sodium iodide at 1:50 molar ratio, but at 1:600 ratio F increased to 6.4. Presumably the protection is due to competition that converts a reactive radical to a less damaging one, e.g., OH· to I·. Since iodide is a sensitizer in vivo, at least in many cases[11], the result indicates that sensitizing action cannot be directly related to some simple effect at the molecular level. F for iodoacetic acid was 1.5, for 3-iodopropionic acid 1.6. Of some interest is the fact that ethyl methanesulfonate, a mutagenic and radiomimetic agent, also had little effect: F was 1.0 at 50:1 ratio and only 1.1 even at very high concentration, 8000:1.

The behavior of aldolase with respect to protection and sensitization is in marked contrast to that of alcohol dehydrogenase, although this has a very similar inactivation yield[7]. Although the data so far available are not sufficient to sustain any generalization, it should be anticipated that protective and sensitizing effects may be more specific than had been suspected hitherto[12].

Riassunto. È stata studiata in vitro l'inattivazione da raggi x dell'aldolasi dal muscolo di coniglio. Il rendimento di inattivazione corrisponde a 0,10 (molecule/100 ev) per concentrazioni comprese tra 10^{-5} and $10^{-6} M$ in tampone di fosfati $0{,}05 M$, pH 7. Il rendimento non subisce modificazioni di rilievo variando il pH da 6 a 8 o sostituendo al tampone l'acqua bidistillata. Il rendimento é ridotto da 4 a 6 volte in presenza di cisteamina, di cisteina o dei corrispondenti disolfuri in concentrazioni corrispondenti a 50 molecule per molecola di enzima. Coll'ioduro di sodio in concentrazione di 600:1 si ottiene una riduzione di 6 volte.

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Mannosidohydroxystreptomycin from *Streptomyces* sp.

Although the formation of mannosidostreptomycin together with streptomycin in the fermentation broths of *S. griseus* is a well-known phenomenon[1], a mannosido derivative of hydroxystreptomycin has never been described. We wish to report the isolation of mannosidohydroxystreptomycin together with hydroxystreptomycin, streptidine and toyokamycin from the cultures of *Streptomyces 86*[2].

The filtered broth (20 l) was worked up by standard absorption-elution procedures on cation exchange resins to give a mixture of sulphates which were separated by chromatography on a column of activated charcoal and filter-aid. The elution was carried out with distilled water followed by $0.1N$ sulphuric acid in 50% aqueous methanol. The yields were the following: 1.6 g of streptidine sulphate, identified by direct comparison with an authentic specimen prepared from streptomycin[3], 5.8 g of hydroxystreptomycin sulphate, identical in all respects to an authentic sample[4], 6.4 g of sulphate of the new antibiotic. The latter was further purified through the crystalline β-naphthalenesulfonate, $C_{27}H_{49}O_{18}N_7 \cdot 3\,(C_{10}H_8O_3S) \cdot 3\,H_2O$, m.p. 182–184°, and isolated as the hydrochloride $C_{27}H_{49}O_{18}N_7 \cdot 3\,HCl \cdot {}^3/_2\,H_2O$, $[\alpha]_D^{23} -55°$ (H_2O), $-49°$ (methanol). Hydrogenation of this product (Pt catalyst) gave a dihydro derivative $C_{27}H_{51}O_{18}N_7 \cdot 3\,HCl \cdot 3\,H_2O$, $[\alpha]_D^{23} -62°$ (H_2O).

Paper chromatography of the hydrolyzate obtained by treatment of the antibiotic with N aqueous sulphuric acid on the boiling water bath showed spots corresponding to streptidine, N-methylglucosamine and mannose. Treatment of the dihydro derivative of the antibiotic with $1.6N$ hydrogen chloride in methanol gave, after 130 h at room temperature, streptidine, α-methyldihydrohydroxystreptobiosaminide (isolated as the exaacetate which was identical in all respects with the same product obtained from

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