On the Mechanism of Radiosensitization by Iodine Compounds at the Molecular Level

Many compounds have been found which sensitize living systems to the action of ionizing radiation. The best known of them are some halogenated analogues of DNA bases[1]; compounds like iodoacetamide, iodoacetic acid[2], N-ethylmaleimide and phenylmercuric acetate[3], which react with mercapto groups; and, finally, inorganic halides[4], chloroform[5], and chloral hydrate[6].

Recently it has been reported that also the enzyme alcohol dehydrogenase from yeast (ADH) is inactivated at higher yield when irradiated in solutions containing non-alkylating iodine compounds like sodium iodide (NaI) and 3-iodopropionic acid (IPA)[7]. These observations have been extended with the aim of elucidating the mechanism involved.

ADH was prepared from baker's yeast according to the RACKER procedure (1950)[8]. In $6 \cdot 10^{-3} M$ pyrophosphate buffer pH 8.5, at 30 °C, with NAD^+ $1.2 \cdot 10^{-3} M$ and ethanol $10^{-3} M$, the specific activity of the enzyme preparations varied from 290–340 μmoles NAD^+/min/mg of protein. The enzyme solutions, in phosphate buffer $0.05 M$ pH 7, were irradiated, at 0 °C, with X-rays generated at 200 kvp with 0.2 mm Cu filtration, at a dose-rate of 770 rad/min, as measured by ferrous sulphate dosimetry[9]. Under such conditions, the ADH inactivation was found to be logarithmically related to radiation dose over the range of concentrations studied. The yield of inactivation, calculated from the slope of the line resulting from the plot of the measured D_{37} dose against the enzyme concentration (from 0.16–1.2 mg/ml[10]), was G = 0.2.

When ADH was irradiated in solutions containing NaI in a molar ratio of 80 with the enzyme, this was still inactivated as a logarithmic function of radiation dose, but at a higher yield than in plain buffer. The relationship D_{37} versus enzyme concentration was still linear, the G value being 0.4.

3-Iodopropionic acid (IPA) and methyl iodide (MEI) have also been tested for their radiosensitizing effect on ADH at a single enzyme concentration of $1.1 \cdot 10^{-6} M$. Table I shows the value of the ratio of D_{37} in presence of different molar excesses of modifier substances and D_{37} in plain buffer (dose modifying factor, DMF). Results relative to NaI are included.

It can be seen from Table I that NaI and IPA display sensitizing activities which are in the same order of magnitude, being greater than that of NaI. MeI is less active by at least 1 order of magnitude.

The relationship between sensitization and molar excess of NaI and IPA, from 0–64 moles of modifier/mole of enzyme, has been studied on ADH solutions $1.2 \cdot 10^{-6} M$ irradiated with a dose of 3 Krad of X-rays, which caused, under control conditions, a 31% inactivation. The results, reported as % sensitization (= 100 × % inactivation in the presence of modifier − % inactivation in plain buffer) are shown in Table II.

Both NaI and IPA sensitize ADH to X-ray even at a very low molar excess, the sensitizing effect being almost linearly related to the molar excess of modifier in the range of 0–16 moles of modifier/mole of enzyme.

The results reported above are consistent with the hypothesis that radiolytic products of modifier compounds may be responsible for the radio-sensitizing effect[11]. Now, considering the structure of the compounds involved, it is obvious that the first radiolytic product which comes to mind is iodine. It is, in fact, well known that iodine atoms are formed by radiation from iodine ions as well as from organic iodine compounds[12]. On the other hand, it has been shown that detectable amounts of iodine are actually formed when NaI, IPA and MeI are irradiated in the phosphate buffer used in the present investigation. The effect of increasing concentrations of iodine on ADH activity has therefore been investigated, with the results shown in Table III.

Table I. Dose-modifying factors by iodine compounds

Substance	moles of modifier/moles of enzyme	DMF
Sodium iodide	80:1	0.40
	8:1	0.59
Iodopropionic acid	80:1	0.56
	8:1	0.80
Methyl iodide	360:1	0.59
	90:1	1.00

Table II. % sensitization by iodine compounds

moles of modifier/moles of enzyme	% sensitization	
	NaI	IPA
0.5	32	7
1.0	58	11
2.0	69	22
4.0	87	40
8.0	128	63
16.0	160	103
32.0	183	135
64.0	224	152

Table III. ADH inactivation by iodine

moles of iodine/moles of enzyme	% residual activity after 30 min contact at 0 °C
4:1	68.8
8:1	53.5
12:1	42.0
16:1	35.4
20:1	26.9

[1] B. DJORJEVIC and W. J. SZYBALSKY, J. exp. Med. *111*, 509 (1960).
[2] C. J. DEAN and P. ALEXANDER, Prog. Biochem. Pharmac. *1*, 48 (1965).
[3] B. A. BRIDGES, Radiat. Res. *16*, 232 (1962).
[4] M. R. BIANCHI, M. BOCCACCI, M. QUINTILIANI and E. STROM, Prog. Biochem. Pharmac. *1*, 384 (1965).
[5] I. M. COTTON and L. S. LOCKINGEN, Proc. natn. Acad. Sci. U.S.A. *50*, 363 (1963).
[6] J. BURNES, J. P. GARCIA, M. LUCAS, C. MONCRIEF and R. F. PITTILLO, Radiat. Res. *25*, 460 (1965).
[7] M. QUINTILIANI and G. GORIN, Radiat. Res. *25*, 231 (1965).
[8] E. RACKER, J. biol. Chem. *184*, 313 (1950).
[9] J. WEISS, A. O. ALLEN and H. A. SCHWARZ, Int. Conf. peaceful Uses atom. Energy *14*, 179 (1956).
[10] F. HUTCHINSON and D. A. ROSS, Radiat. Res. *10*, 477 (1959).
[11] D. L. DEWEY and B. D. MICHAEL, Biochem. biophys. Res. Commun. *21*, 392 (1965).
[12] J. W. T. SPINKS and R. J. WOODS, *An Introduction to Radiation Chemistry* (J. Wiley and Sons Inc. 1964).

Another series of experiments was carried out where ADH was irradiated in solutions containing sodium iodide labelled with I^{131}; the enzyme was then separated and tested for any bound radioactivity. The experimental technique can be summarized as follows: carrier free NaI^{131}, was diluted with inert NaI to achieve a molar excess of 100:1 with respect to ADH, whose concentration was adjusted to $6.5 \cdot 10^{-6} M$. The samples were then irradiated with a dose of 15.4 Krad of X-rays (residual enzymatic activity less than 1%), or left unirradiated in ice for the same time as control. Thereafter the enzyme was separated from the radioactive solution either by passage over Sephadex G-25 (Pharmacia, Sweden) or by precipitation with 60% ammonium sulphate or 10% trichloroacetic acid and washed by centrifugation until no radioactivity was detectable in washings. In no case did the separated enzyme reveal any radioactivity. This finding does not essentially contradict the hypothesis that iodine may be involved in the sensitizing effect, because the lack of iodine binding could be due to oxidation of sulphydril groups to disulphides. Experiments are at present in progress to control whether this may actually be the case[13].

Riassunto. L'alcool deidrogenasi del lievito irradiata con raggi X in soluzioni contenenti ioduro di sodio, acido 3-iodopropionico o ioduro di metile, è inattivata con un rendimento più elevato che in assenza di tali sostanze. L'effetto sensibilizzante dello ioduro di sodio e dell'acido iodopropionico è proporzionale alla loro concentrazione nell'ambito di 0–16 molecole di essi per molecola di enzima. L'alcool deidrogenasi irradiata in presenza di ioduro di sodio marcato con I^{131} non rivela la presenza di iodio legato alla sua molecola.

M. Quintiliani and L. Bernardini

Laboratori di Chimica Biologica, Istituto Superiore di Sanità Rome (Italy), 10th March 1967.

[13] The assistance of Mr. V. Puccio is gratefully acknowledged.

The Effect of Microcytosis on Red Cell Constituents

The onset of iron deficiency anaemia in human subjects is first marked by the development of microcytic red cells with a normal hemoglobin concentration and later by the appearance of hypochromic cells with a low mean corpuscular hemoglobin concentration (MCHC)[1]. This situation is in accord with the hypothesis of Stohlman and coworkers that the total maturation time of the red cell is dependent on the rate of hemoglobin synthesis. When a critical MCHC is reached this triggers a feed back mechanism which stops further DNA synthesis and cell division[2]. If hemoglobin synthesis is impaired the time taken to achieve the critical MCHC is prolonged and an increased number of cell divisions occur, resulting in microcytosis. The critical MCHC is never exceeded whatever the duration of red cell development.

If the MCHC plays this crucial role in determining the end of nuclear activity then it must also be a limiting factor for other cell functions. Estimation of red cell riboflavin in iron deficiency anaemia shows that when microcytosis occurs the intracellular concentration is increased[3]. This suggests that if a normal concentration is attained before hemoglobinization is complete a further

[1] M. E. Conrad and W. H. Crosby, Blood *20*, 173 (1962).
[2] F. Stohlman, D. Howard and A. Beland, Proc. Soc. exp. Biol. Med. *113*, 986 (1963).
[3] I. A. J. Cavill and A. Jacobs, Clinica chim. Acta, in press.

Correlation of red cell concentration (a) and total red cell content (b) with mean cell volume for 5 constituents

Constituent measured	No. of cases	Correlation coefficients r	p	Regression coefficients
Riboflavin (a) µg/100 ml	74	− 0.52	< 0.001	− 0.15
(b) µg/10^{14}RC's		+ 0.32	< 0.01	+ 0.06
Cholinesterase (a) units/100 ml	36	− 0.432	< 0.01	− 0.87
(b) units/10^{10}RC's		+ 0.407	< 0.05	+ 0.66
Glutamic-oxaloacetic transaminase (a) µmole/100 ml	81	− 0.475	< 0.01	− 2.75
(b) µmole/10^{12}RC's		+ 0.015	n.s.	+ 0.06
Glutamic-pyruvic transaminase (a) µmole/100 ml	79	− 0.369	< 0.01	− 0.94
(b) µmole/10^{12}RC's		− 0.120	n.s.	− 0.22
Folate (a) µg/ml	49	− 0.486	< 0.01	− 4.99
(b) µg/10^{14}RC's		− 0.164	n.s.	− 0.11

n.s., not significant.