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RADIATION CHEMISTRY

Action of Ionizing Radiation on Rat Liver Cell Nuclei

The site of attack by OH radicals on the various components of some cell nuclei has been explored using the radiation-induced carboxylation technique recently described[1]. This involves irradiation of oxygen-free suspensions of the nuclei in the presence of sodium bicarbonate, under conditions where the radicals can react with the nuclear constituents. The negative polarons[2], $(H_2O)^-$, produced from the water react with the bicarbonate according to:

$$(H_2O)^- + HCO_3^- \rightarrow CO_2^- + OH^- + H_2O \qquad (1)$$

In general, carboxylation depends on the formation of a free radical from an organic molecule (RH), for example, by the process:

$$RH + OH \rightarrow R\cdot + H_2O \qquad (2)$$

and association of the free radical with the CO_2^- radical ion according to:

$$R\cdot + CO_2^- \rightarrow RCO_2^- \qquad (3a)$$

$$RCO_2^- + H^+ \rightleftarrows RCO_2H \qquad (3b)$$

In order to assay the carboxylic acids produced in the various components of the nuclei, bicarbonate labelled with carbon-14 was used.

Nuclei were prepared from rat-liver by Dounce's method[3], involving homogenization with dilute citric acid at *p*H 4·0. The isolated nuclei were then suspended in phosphate buffer (4×10^{-3} M, *p*H 8·0) containing labelled sodium bicarbonate (6×10^{-3} M; 5 μc./ml.). The suspensions of the nuclei (0·2 mg/ml.) were evacuated and then irradiated with cobalt-60 γ-rays. After irradiation the nuclei were precipitated with perchloric acid (final concentration 0·4 M). Aliquots were assayed for activity after taking to dryness on planchets. The remaining nuclei were treated as follows. Lipids were extracted with ethanol and ethanol/ether (3 : 1) at 55° C. Nucleic acids (DNA and RNA) were then extracted by heating twice with 10 per cent sodium chloride at *p*H 8·0, and precipitated with ethanol. RNA was removed by treatment with 0·2 N sodium hydroxide at 80° C and the DNA reprecipitated. Removal of small amounts of contaminating protein from the DNA was achieved by incubation with pronase. Aliquots of each component were counted.

Table 1 records some of the results obtained. The actual experimental figures are given in terms of counts/min per mg of each component. The percentage of total activity in each fraction is also shown in Table 1. It can be seen that, of the total extent of carboxylation, only 3–4 per cent go into the DNA fraction; by far the major reaction occurs in the protein and lipids.

Table 1. Irradiation (cobalt-60 γ-rays) of suspensions of rat liver nuclei (0·2 mg/ml.) in phosphate buffer (4×10^{-3} M, *p*H 8·0) and in the presence of ^{14}C-labelled sodium bicarbonate (6×10^{-3} M; 5 μc./ml.); dose-rate $1{\cdot}15 \times 10^4$ rads/min. (The figures in the parentheses give the percentage of total incorporation in each fraction)

Dose (rads $\times 10^{-4}$)	Total* (c.p.m./mg)	Lipids (c.p.m./mg)	Protein (c.p.m./mg)	RNA (c.p.m./mg)	DNA (c.p.m./mg)
3·45	342 (100%)	3,544 (31·2%)	216 (53·6%)	183 (1·1%)	110 (3·2%)
11·5	1,095 (100%)	9,688 (26·7%)	748 (57·8%)	398 (0·8%)	401 (3·6%)

* Recovery was about 90 per cent of the total activity.

The composition (per cent) of these nuclei was approximately: protein 85, DNA 10, RNA 2, lipid 3. Thus, even though the lipid fraction makes up only about 3 per cent of the bulk of the nuclei, it accounts for 30 per cent of the total incorporation; this is demonstrated by the high values of the counts/min per mg for this particular fraction. Such a high reactivity of the lipid material may be associated with its chemical nature as well as with the fact that it is predominantly in the nuclear membrane, and may thus 'trap' OH radicals generated in the ambient medium.

If one assumes that the different proteins present in the cell nuclei have undergone about the same degree of carboxylation, it would follow that in the protein, which is possibly more specifically associated with the DNA, about 7 per cent of the incorporation has occurred, that is, the relative extent of carboxylation of the DNA and of the protein in the complex is in the ratio 1 : 2.

It should be pointed out that there may be certain qualifications on any absolute interpretation of the results obtained by the carboxylation technique. In particular, carboxylic acids produced at certain sites may be unstable and, furthermore, steric hindrance and other chemical factors may affect the reaction of the CO_2^- radical ion. The extent of such interference is, at present, unknown. Nevertheless, this appears to be a useful technique for a preliminary survey of certain features of the effects of ionizing radiations on more complex systems.

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BIOCHEMISTRY

Appearance of New *N*-Terminal Groups by the Action of Rennin on Casein

Wissmann and Nitschmann[1], studying the action of rennin on α-casein by the method of Sanger[2], found, after the action of rennin for 30 min., not only the original *N*-terminal amino-acids arginine and lysine but also a new *N*-terminal amino-acid,