Adenylate Cyclase-the More Membrane Associated, 
the Less Radiosensitive

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A dose dependent but not parallel decreases were observed both in SH content and catalytic activity of “free” catalytic subunit after irradiation (0–3200 Gy), while SH groups of membrane-associated adenylate cyclase were insensitive (under 3200 Gy). An initial “radioactivation” of membrane-associated enzyme was found under 800 Gy, then an inhibition above 1600 Gy.

The SH alkylating agent, N-ethylnmaleimide resulted in a complete inactivation, both of membrane associated form of adenylate cyclase and “free” catalytic subunit with similar inactivation profiles. These data indicate that in the radiosensitivity or “radioprotection” of adenylate cyclase, its membrane association/integration might play a more important role than the SH groups themselves.

INTRODUCTION

Radiation modification of plasma membranes and membrane associated components seems to be more complex, than simple peroxidation of membrane lipids and/or oxidation of SH groups of membrane proteins. Inactivation of membrane-associated adenylate cyclase of chicken embryo brain by N-ethylnmaleimide, but not by gamma irradiation (between 0–800 Gy) has been found earlier. The aim of this work was to investigate the effect of gamma irradiation on SH content and on the catalytic activity both in the cases of membrane associated and “free” catalytic subunit of adenylate cyclase of chicken embryo brain and to compare it with the effect of N-ethylmaleimide.

It is known that the catalytic subunit of adenylate cyclase contains SH groups, e.g. in bovine brain more than 30 per molecule. Using gamma irradiation we have investigated the potential role of SH groups in radio-inactivation of the membrane-associated and the “free” catalytic subunit of adenylate cyclase of chicken embryo brain.
MATERIALS AND METHODS

The whole brains of 19 day old chicken embryos were homogenized in a 50 mM Tris-HCl buffer (containing 5 per cent saccharose, 5 mM MgCl₂, 2 mM EDTA, pH 7.5). Supernatant of the 15000×g was layered on a discontinuous saccharose gradient and was centrifuged at 88000×g for 90 min. The interface of 25 per cent and 40 per cent saccharose was dissolved in 50 mM Tris-HCl buffer (pH 7.5) and dialyzed against the same buffer. It was used as membrane associated enzyme³. It was solubilized with Lubrol PX/mixing with 0.5 per cent Lubrol PX at 4°C for 1 hour. The soluble form was precipitated from the supernatant (145000×g) with 33 per cent saturation of ammonium sulphate (at 0°C) and was centrifuged at 34000×g for 30 min. The pellet containing the “free” catalytic subunit was dissolved in 50 mM Tris-HCl buffer (pH 7.5) and dialyzed against the same buffer. The protein content was determined by Bradford’s method⁶. Adenylate cyclase activity was measured by the method of Brooker et al.⁷ with some modifications. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 0.4 mM Na EDTA, 1 mg/ml BSA (bovine serum albumin), 12 mM creatine phosphate, 1 mg/ml creatine phosphokinase, 0.5 mM 3-isobutyl-1-methylxanthine 1 mM ATP, 5–8 μg membrane protein in a final reaction volume of 100 μl. The reaction was started by the addition of membrane fraction and the mixture was incubated at 32°C for 20 min with constant shaking. The reaction was stopped by adding 500 ml of cold Na acetate buffer (50 mM, pH 4.75) and the amount of other adenine nucleotides was decreased by addition of Al₂O₃. Cyclic AMP radioimmunoassay was carried out by the modified method of Brooker et al.³,⁷. The assay solution contained 100 μl sample or standard, 50 μl of (¹²⁵I)-Sc-cAMP-TME (2'-O-Monomosuccinyl-Adenosine 3':5'-cyclic Monophosphate Tyrosyl Methyl Ester) (10000 cpm assay tube) as the labelled ligand, 150 μl of goat antiserum (prepared according to Brooker et al.³,⁷), final dilution 1:180000. The range of the cAMP standard curve was between 0.1 and 25 pmol. After incubation for periods of 18–20 hours the bound radioactivity was measured in a gamma counter (GAMMA Co., Budapest).

The SH content was measured according to Sedlak and Lindsay⁸ using glutathione (10–100 μM) as the standard. Gamma irradiation was carried out at 0°C, with a dose rate of 0.36 Gy/sec in NORATOM Gamma 350C. The samples were air saturated, and their protein content was 3.5–7.0 mg/ml depending on the preparation. The controls were kept under the same circumstances (except the irradiation). Enzyme activity was measured within 3 hours after irradiation.

RESULTS

Several papers have been published though with contradictory results about the effect of irradiation on adenylate cyclase, or on cyclic AMP level both in vivo and in vitro⁹–¹⁵. Basal activity (measured without any activator) of membrane-associated enzyme was 260±40 pmol/min/mg protein and was elevated to 424±40 pmol/min/mg protein in the presence of 5 mM NaF. Basal activity of the “free” catalytic subunit was 168±18 pmol/min/mg protein and was elevated to 1126±41 pmol/min/mg by 1 mM MnCl₂ (NaF was without effect)/Table I./.
Table 1. The Absolute Values of the Controls

<table>
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<tr>
<th></th>
<th>cyclic AMP production*</th>
<th>SH content</th>
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<tbody>
<tr>
<td>Membrane associated enzyme</td>
<td>424±40</td>
<td>158±33</td>
</tr>
<tr>
<td>“Free” catalytic subunit</td>
<td>1126±41</td>
<td>98±7</td>
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* Cyclic AMP production was measured in the presence of activators (5 mM NaF in the case of membrane bound enzyme, and 1 mM MnCl₂ in the case of catalytic subunit).

![Graph A](image1.png)

**Figure 1.** Radiosensitivity of the membrane-associated (part A.) and the “free” form (part B.) of chicken embryo brain adenylate cyclase. The enzyme assay was carried out in the presence of activators (5 mM NaF for the membrane-associated enzyme and 1 mM MnCl₂ for the “free” catalytic subunit. In the case of MnCl₂ activation, MgCl₂ was omitted.) according to Brooker (3, 7) method (see the text). The SH content was measured according to Sedlak and Lindsay (8). Three independent experiments (with two parallels for each points) were carried out. Gamma irradiation was carried out at 0°C, with a dose rate of 0.36 Gy/sec in NORATOM Gamma 350C.

- ○-----○ = enzyme activity
- ○-----○ = SH content.

The effect of gamma irradiation on the activity of adenylate cyclase was followed in the presence of activators mentioned above (Fig. 1.). An initial activation (under 800 Gy) followed by inhibition above 1600 Gy was found in the case of the membrane-associated enzyme. This observation is in good agreement with the observations on stimulatory effect of low dose irradiations upon adenylate cyclase both in vivo and in vitro. A similar effect has been observed with Pb²⁺, i.e. activation of chicken embryo brain adenylate cyclase at lower concentrations (10⁻⁸–10⁻⁵ M) and inhibition at higher ones (above 10⁻⁴ M). The activity of
Figure 2. Effect of N-ethylmaleimide on the catalytic activity of the membrane-associated enzyme (part A.) and the "free" catalytic subunit (part B.). The enzyme assay was carried out in the presence of 0–200 μM NEM, either without activators /○○○○○/ or in the presence of activators /□□□□□/, (5 mM NaF for the membrane-associated enzyme and 1 mM MnCl₂ for the "free" catalytic subunit). For more details see the legend of Fig. 1.

"free" catalytic subunit decreased as a function of the dose of irradiation, and the remaining enzyme activity was 1–2 per cent of the control after irradiation with 3200 Gy.

The difference in the radiosensitivity of SH groups of membrane-associated and "free" catalytic subunit was also quite remarkable. The total amount of SH groups of the membrane-associated enzyme was higher (158±33 nmol/mg protein) than that of the "free" catalytic subunit (98±7 nmol/mg protein) /Table I/. There was no detectable decrease in the SH content
of membrane-associated enzyme (within 95 per cent confidence) after irradiation up to 3200 Gy. The decrease in the SH content of the “free” catalytic subunit was dose dependent at lower doses (less, than 800 Gy), but did not decrease below 50 per cent between 800 and 3200 Gy. Neither the decrease of catalytic activity of membrane-associated adenylate cyclase nor that of “free” enzyme was parallel with the decrease of their SH contents. The SH groups proved to be very “radioreistant” in both cases.

Both forms of adenylate cyclase were inhibited by N-ethyl-maleimide /Fig. 2/. Fifty per cent inhibition was found at 40–50 μM concentrations. The “free” catalytic subunit was more sensitive than the membrane associated enzyme, and the presence of activators (NaF in the case of the membrane bound enzyme and MnCl₂ in the case of the “free” catalytic subunit) decreased the effect of NEM. The effect of NEM on the SH content of both forms of adenylate cyclase was less expressed (Fig. 3) and at 100 μM NEM (where the catalytic activity was very low) about 50 per cent of SH content could be measured.

DISCUSSION

There are two types of discrepancies concerning the radiosensitivity of adenylate cyclase. One is the increased radiosensitivity of purified, “free” enzyme, the other is the difference between the radiosensitivity of SH groups and the catalytic activity. Many factors can play role in the “radioprotection” of catalytic subunit of adenylate cyclase when it is membrane-associated. It can be protected by the regulatory subunits (G protein complexes), or other proteins. The connection of G proteins with the catalytic subunit may be an explanation of the activating effect of irradiation, or that of low concentrations of lead acetate⁵, which can inhibit/ damage G₁ protein (inhibitory/regulatory component of adenylate cyclase) causing indirect activation of the less sensitive catalytic subunit. The possibility of the scavenger effect of membrane lipids can not be excluded either¹,². It is known that a part of the catalytic subunit is integrated into the membrane structure⁴, and this can cause a kind of protection. If the level of integration depends on the actual functional state of membrane or the enzyme¹⁷, adenylate cyclase could have a “function-dependent” radiosensitivity. After low doses of X-irradiation some ultrastructural changes involving translocation of adenylate cyclase in the plasma membrane of fibroblasts have been found previously¹⁰. Also the role of the tissue-specific organization of plasma membranes in the radiosensitivity cannot be excluded, too. There are some preliminary results (data not shown) about the higher radiosensitivity of adenylate cyclase of chicken embryo liver, than that of brain. The possibility of the tissue specific and function-dependent membrane integration of the catalytic subunit can explain the contradictory results (activation/inactivation) with the irradiation of adenylate cyclase⁹–¹⁶,¹⁸.

There is the possibility of the existence of some radiosensitive SH groups responsible for the activity of adenylate cyclase. On the basis of N-ethylmaleimide inhibition the probability of specific radio-oxidation of these undetectable SH groups might be rather small as N-ethylmaleimide decreases both the SH content and catalytic activity, as it is seen on Fig. 2, 3.

The “SH-group-independent” inactivation of adenylate cyclase can be explained by
radiosensitivities of some amino acids (Phe, Tyr, His, Met) as it has been described in the case of yeast invertase\textsuperscript{19}. The catalytic subunit of adenylate cyclase is a glycoprotein\textsuperscript{20} and the radiolysis can play a role in its inactivation similarly to some gastric peptides\textsuperscript{21}. Notwithstanding, the damage of the structure of the “free” catalytic subunit, or some oxidation of its SH groups cannot be excluded, they could not explain its elevated radiosensitivity. These data suggest that SH groups do not play important role (if any) in the radiosensitivity of adenylate cyclase.

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