R-II2

LABILE BINDING OF Tc-95M TO PROTEINS IN THE GIANT UNICELLULAR ALGA ACETABULARIA ACETABULUM

F. CAPOT[1], A. BOSSUS[1], G. NUYTS[1], M. COGNEAU[2], D. VAN DER BEN[3] and S. BONOTTO[1]

(1) Department of Biology, C.E.N./S.C.K., Mol (Belgium)
(2) Laboratoire de Chimie Inorganique et Nucléaire, Université Catholique de Louvain, Louvain-la-Neuve (Belgique)
(3) Institut Royal des Sciences Naturelles de Belgique, Bruxelles (Belgique)

ABSTRACT

Molecular sieving-chromatography and electrophoresis on polyacrylamide gels have revealed that Tc-95m binds to Acetabularia proteins having molecular weights ranging from 120 to about 10 kdaltons. However, proteins of relatively low molecular weight (⩽ 15 kdaltons) are the heaviest labeled. In addition, most of the Tc-95m-protein bonds are labile, being rapidly destroyed by acid treatment.

1. INTRODUCTION

The giant unicellular alga Acetabularia acetabulum, by contrast to other green marine algae, is capable of accumulating technetium supplied as Tc-99- or Tc-95m-pertechnetate, the concentration factor attaining values up to 348 (Bonotto et al., 1983). More recent experiments have revealed that Tc-95m, taken up by growing vegetative cells, becomes more or less strongly bound to various cell constituents. Only about 56% of Tc-95m incorporated by the cells can be extracted with buffers, approximately 40% being unextractable (Capot et al., 1985). This paper deals with the incorporation of Tc-95m into proteins.

2. RESULTS AND DISCUSSION

Soluble proteins of vegetative cells of Acetabularia acetabulum were extracted with Tris-buffer (0.02 M Tris-HCl, 0.1 mM Phenylmethyl-sulfonyl Fluoride, PMSF (an inhibitor of proteases), pH 7.2) and chromatographed on Sephadex G-25. The remaining proteins (mainly membrane proteins) were extracted with a higher ionic strength buffer (0.02 M Tris-HCl, 2 M NaCl, 5 M Urea and 0.1 mM PMSF, pH 7.5) and chromatographed on Sephacryl S-300 (Capot et al., 1985). No attempt was made to solubilize proteins associated with the cell walls. The comparison of the optical with the radioactive profiles of the chromatographs revealed that Tc-95m was bound to macromolecules (mostly proteins) as well as to small organic molecules. In addition, evidence was obtained suggesting that part of the technetium taken up by the cells remained in the form of pertechnetate. Electrophoresis on 15% polyacrylamide gels of extracts, obtained with Tris-SDS buffer (0.06 M Tris-HCl, 5 mM Na_2EDTA, 5% β-mercaptoethanol, 2% SDS), from Acetabularia cells supplied with Tc-95m-pertechnetate (50 nCi or 1.8 kBq ml^{-1}) up to 15 days, followed by gel slicing or gel autoradiography, showed that Tc-95m was bound to proteins having molecular weight ranging from 120 to about 10 kdaltons. Moreover, proteins with molecular weight ⩽ 15 kdaltons were the most heavily labeled (fig.1.). However, parallel experiments, have demonstrated that the Tc-95m-protein bonds are rather labile. In fact most of technetium was lost from the proteins after acid precipitation with cold 5% TCA. This finding rises the question whether technetium is dissociated from proteins in the digestive systems of animals grazing on marine algae having incorporated this radionuclide.

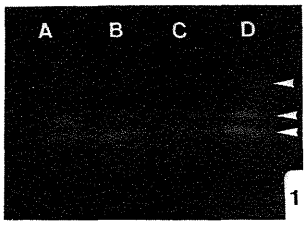

Fig.1. Autoradiograph of Acetabularia proteins extracted from whole vegetative cells supplied with Tc-95m-pertechnetate, and separated by electrophoresis on 15% polyacrylamide gel. Lanes A, B, C and D correspond respectively to proteins extracted from cells labeled during 1, 3, 10 and 15 days. The arrows show bands having molecular weight ⩽ 15 kdaltons.

3. ACKNOWLEDGEMENTS

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R-II3

EXPERIMENTAL INVESTIGATIONS ON TRITIUM INCORPORATION INTO THE MARINE GREEN ALGA ACETABULARIA ACETABULUM

G. ARAPIS[1], Y. BELOT[1], G. NUYTS[2], A. BOSSUS[2] and S. BONOTTO[2]

(1) Département de Protection, Centre d'Etudes Nucléaires, Fontenay-aux-Roses (France)
(2) Département de Biologie, Centre d'Etude de l'Energie Nucléaire, C.E.N.-S.C.K., Mol (Belgique)

ABSTRACT

The unicellular marine alga Acetabularia acetabulum L. (Silva), Dasycladaceae, is a useful organism to study the impact of tritium on the aquatic environment. The plant is capable of converting HTO to OBT, under light as well as under dark conditions, although to a lower extent. Acetabularia actively incorporates supplied amino acids (H^3-leucine) into cell and chloroplast proteins, having molecular weights ranging from 140 to 12 kdaltons. HT/HTO and HT/OBT conversion is under investigation.

1. INTRODUCTION

Recent work suggested that in fusion reactor facilities and in recovery systems, tritium inventories could attain very high values (10^8 Ci or 3.7 EBq) (Peterman et al., 1985). The possibility, thus, exists that workers shall be exposed to high concentrations of tritium gas (HT or T_2). Oxydation of HT to HTO and incorporation of tritium into organic materials (OBT) could provoke a contamination of the foodchains leading to man. This last aspect is of particular importance since OBT in human foods could increase the total body dose by a factor of 1.7-4.5 times the free body water dose alone (Travis, 1985).

Tritium may be taken up by aquatic organisms, directly from the surrounding water (HTO, dissolved tritiated organic molecules or HT), or indirectly through contaminated foods. Micro- and macro-algae are of particular interest for investigations on the behaviour of tritium in the aquatic environment. In previous studies (Bonotto et al., 1982 ; Arapis et al., 1984a,b), we have shown that tritium, supplied as tritiated water, was incorporated into the organic matter of various green algae. This paper deals with the incorporation of tritium supplied to Acetabularia, under light and/or dark conditions, in different chemical forms (tritiated water, tritiated amino acids and tritium gas).

2. RESULTS AND DISCUSSION

When vegetative Acetabularia cells are supplied with HTO (10 μCi/ml or 3.7 x 10^5 Bq/ml) during 7 days, tritium is incorporated into their organic matter. Microcombustion analyses have shown that under dark conditions the amount of organic tritium (OBT) present in the cells is lower than that fixed under light conditions by a factor of about 4. Nevertheless, it is of interest to notice that HTO/OBT conversion occurs even in darkness.

Tritiated leucine (1 μCi or 3.7 x 10^4 Bq/ml) was taken up at a higher rate under light than under dark conditions. At the end of the experiments (24 h), however, the uptake of H^3-leucine in darkness was reduced only by about 20%. The ratio between acid soluble and acid insoluble tritiated compounds, after a 5 h incubation period, was respectively 1.8 and 2.4 in light and in darkness. The incorporation of tritium from tritiated water (HTO) or from tritiated leucine (OBT), into the proteins of whole and/or isolated chloroplasts (labeled in vivo), was studied also by electrophoresis on 15% polyacrylamide gels. Protein markers were used to determine the approximate molecular weight of the tritiated molecules. The gels were sliced and counted, as reported before (Arapis et al., 1984b), or submitted to autoradiography (Kodak X-Ray Ortho G Film, using a fluorographic procedure). Labeled proteins had molecular weights ranging from 140 to 12 kdaltons in whole cells as well as in chloroplasts. Since very recent work has shown that algal chloroplasts (Chlamydomonas reinhardii) have an hydrogenase activity (Majone and Gibbs, 1986), the incorporation of tritium gas into Acetabularia (one cell contains about 10 millions of chloroplasts) is being studied.

3. ACKNOWLEDGEMENTS

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