IN VITRO PREPARATION OF $^{68}$GA-LABELLED TRANSFERRIN

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Systematic investigations into the practical problems of labelling transferrin with $^{68}$Ga in vitro are presented. A chemical purification of the $^{68}$Ga generator eluate is described and the working conditions whereby several millicuries of $^{68}$Ga may be bound quantitatively onto a few milligrams of transferrin are defined.

Introduction

Regional blood volume is an important parameter for "in-vivo" regional metabolic studies by emission tomography. Activity seen in the cross-sectional slices represents the sum of tissue-incorporated tracer activity and blood tracer activity; to eliminate this latter component it is obviously necessary to know the blood volume present in the slice examined.

Using Positron Emission Tomography one accurate way to measure this regional blood volume is to label the circulating blood pool with the vascular tracer $^{11}$C--labelled carboxyhemoglobin$^{1,2}$ but a more convenient method is to label red cells or blood proteins with $^{68}$Ga, one of the most attractive generator products for positron imaging applications.

Red blood cells can easily be labelled with $^{68}$Ga by the standard oxine technique$^{3}$, but once the labelled cells are returned to the natural plasma the tracer is unfortunately very quickly eluted out.

Of blood proteins the molecule able most easily to label with a metallic tracer is transferrin (Tf).

Transferrin is a single-chain glycoprotein with a molecular weight of about 80,000 which, though preferential for Fe (III) can bind most transition metals including Co, Cr, Mn, Zn, Ni, Cu, V, In, and gallium.$^{8}$

It is now well established that for each metal ion attached to the protein, an anion is bound concomitantly. Bicarbonate when available will preferentially occupy the anion binding site but may be replaced by a variety of chelates such as glycinate,$^{9}$ oxalate, malonate, nitritotriacetate or perhaps citrate.$^{10}$
The affinity of transferrin for metals in general and gallium in particular is sufficient theoretically to allow the few nanograms of $^{68}$Ga to be fixed without trouble on a protein mass small enough ($< 10$ mg) not to disturb the circulating transferrin pool (Tf plasmatic concentration : $2.5$ mg/ml).

In reality however $^{68}$Ga is always somewhat contaminated by traces of stable metallic elements. Knowing that only about $8 \mu$g of metal ($12 \times 10^{-2}$ mole) is needed to saturate completely $5$ mg of protein ($6 \times 10^{-2}$ mole) we realize that the chemical purity of generator eluate used play a capital part in the "in-vitro" transferrin labelling yield.

The purpose of this work was therefore to prepare a vascular pool tracer suitable for regional metabolic studies and to this end the working conditions whereby several millicuries of $^{68}$Ga may be fixed quantitatively on a few milligrams of Tf have been defined.

Materials and methods

Preparation of $^{68}$Ga

An SnO$_2$/HCl ionic $^{68}$Ga generator containing $10$ mCi $^{68}$Ge was eluted under vacuum with $1$N HCl. After elimination of the first ml of acid $2.5$ ml eluate containing $7$ mCi $^{68}$Ga were collected.

Purification of the $^{68}$Ga solution

To free the eluate from the metallic impurities always present, whatever the nature of the generator, a specific extraction of gallium by ether in reducing solution was adopted. The generator eluate, evaporated to dryness, was taken up in $1$ ml $6$N HCl then left in contact for $3$ min at $80 \degree$C with about $300$ mg silver wool. $^{68}$Ga was then extracted by two lots of $1$ ml ether which were washed by $1$ ml $6$N HCl.

Preparation of the Tf-$^{68}$Ga complex

The ether extract of $^{68}$Ga (about $6.5$ mCi or $2.6 \times 10^{-3}$ nmole), evaporated to dryness, was taken up in $100$ $\mu$l of a $10^{-2}$ M citric acid solution brought to pH $3.35$ by addition of sodium hydroxide (1 mole of citrate ion). $50$ $\mu$l of a $1.2 \times 10^{-3}$ M transferrin* solution (5 mg or $6 \times 10^{-2}$ umole) were then added, bringing the final solution to a pH between 4.5 and 5.

After $15$ minutes contact at room temperature more than $99\%$ of the radioelement was bound to the protein, as shown by electrophoresis and gel filtration tests.

*Sigma: Apotransferrin