NEWER 99m Tc RADIOPHARMACEUTICALS USING BIFUNCTIONAL CHELATES AND THEIR POSSIBLE BIOMEDICAL APPLICATIONS*

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ABSTRACT

Linkage of 99m Tc and other radionuclides to biologically significant macromolecules for biomedical applications has been a subject of interest. Direct radiolabelling of the biological moiety involves several chemical manipulations which impair the functional integrity of the protein. In addition the products obtained have inadequate *in vivo* and *in vitro* stability for use as radiopharmaceuticals. The use of bifunctional chelating agents which act independently as chelator for the metallic nuclide and conjugators for the proteins, not only provides greater *in vivo* stability to the radiolabelled protein but also offers a distinct separation of cold chemistry from hot chemistry making the preparation of radiopharmaceuticals convenient.

Various bifunctional chelates including derivatives of EDTA and DTPA and Deferoxamine are discussed for synthesis of radiopharmaceuticals for use in imaging and/or therapy.

INTRODUCTION

The ready availability and near ideal radionuclidic properties (T½-6.5 hr, no beta emission and abundant photon emission at 140 keV) of 99 m technetium (Tc) has led to its widespread use for imaging purposes. With the advent of commercial generator systems, innovations in chelation and new chelating agents, there has been a marked expansion in the use of 99m Tc radiopharmaceuticals. Chemical forms of 99m Tc are presently the most widely used radiopharmaceuticals for radionuclidic imaging of the brain, liver, lung and skeleton and to a lesser extent in thyroid scintigraphy.

It is now considered important to be able to link radionuclides with proteins particularly immunoglobulins which in the form of polyclonal or monoclonal antibodies are extensively being explored today for imaging[1]. Of these radionuclides technetium immunoglobulins have attracted special study.

DIFFICULTY IN DIRECTLY LINKING TECHNETIUM TO BIOLOGICALLY SIGNIFICANT PROTEINS

As regards the use of technetium directly labelled protein there are certain problems *viz.*

(i) In the reduced valency state Tc requires an octahedral coordination structure *i.e.* upto six coordination sites in a target molecule would be directly bound to the radionuclide and this can prevent the expected interaction of the biological molecule with the active site responsible for localization.

(ii) Technetium in reduced states is easily oxidizable when weakly chelated and this hampers studies of radiochemical purity and *in vivo* metabolism.

(iii) The technetium protein bond must be strong enough to withstand *in vivo* dilution or ligand substitution. Many technetium-labelled antibodies used in early work dissociated and the resultant free 99 m Tc enhanced the background during scan procedures (K. E. Britton, Personal Communication, 1981). Technetium-labelled albumin also forms a weak combination but since it is used for blood pool studies where one needs to follow it only for a few hours, this dissociation does not pose problems[2].

(iv) Khaw *et al*[3] report that methods used in linking 99m Tc directly to macromolecules usually require very harsh conditions such as electrolysis using Zirconium electrodes[4] or reduction in an acidic medium[5,6] and these frequently result in excessive denaturation of the labelled biomacromolecules.

Alternative modes of 99m technetium linkages have therefore been a desirable goal for R&D efforts.

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INTRODUCTION OF BIFUNCTIONAL CHELATE CONTAINING DERIVATIVES

To avoid the problems encountered with direct labelling of biologically active molecules, derivatives have now been formed by covalent bonding of a chelating agent because covalent linking offers the possibility of far stronger bonding between the antibody and radioisotope. These molecules contain both powerful chelating groups *e.g.* EDTA or DTPA and a reactive group capable of forming covalent or other bonds with biomolecules, hence the name 'bifunctional'. A bifunctional chelating agent acts independently as a chelator for the metallic nuclide and a conjugator for the protein. This type of approach is considered a logical step to combat the difficulties encountered in synthesis by direct labelling.

There are various methods of linking the chelating agents to proteins out of which diazonium coupling and acylation are the most commonly used methods.

Use of bifunctional chelates introduces a rather large-charged foreign group into the molecule in comparison to conventional radioiodination. In general although the chelate usually have a smaller charge than the metal ion, the chelate will add a polar group to the biologically active molecule. Because of the nonpolarity of most of these molecules there will be an alteration in the biologically active molecule with the addition of this large polar group. Because of the size and charge of labelling group it is important to determine whether the biological properties of the labelled molecule are significantly altered by the foreign substituent. This question must be answered for each labelled species of molecules by *in vivo* observation of its behaviour, rates and route of metabolism, compared to standard radioiodination derivatives[7].

Further the metal chelate should be shown to be stable *in vivo* at 'infinite' dilution. The transchelation rate at which the radioligand dissociates from the chelate to link with other naturally existing metal binding molecules (*e.g.* transferrin) must be slow as compared to the biological process being observed (*i.e.* rate of uptake by tumour imaging). Transferrin bound radioligands circulate in the blood pool and then get deposited in reticuloendothelial system resulting in non-target background radioactivity 'degrading' the image signal. The rate of transfer of radioligand from the chelate to transferrin or other metal binding molecules may depend upon steric properties of the chelate, competition with low molecular weight metal binding agents (*e.g.* alpha amino acids), pH, carbonate ion concentration and the number and types of ligands coordinated to the metal ions[8]. Various investigators have published their work in this area of chelate derivative approach.

Sundberg *et al*[9] synthesized an EDTA derivative containing diazonium group 1-p benzenediazonium) ethylene diamine N, N, N, N, tetracetic acid. This chelating agent could be reacted with a molecule containing an activated benzene ring *i.e* phenol or aniline and has formed chelate derivative of fibrinogen, albumin, bleomycin. Although these compounds were labelled with indium their potential applicability to technetium is evident.

Loberg *et al*[10] has produced an analog of anti-arrythmic drug, Lidocain. Their chelating agent was imino disetic acid (IDA) which characteristically binds metal strongly and easily reacts with functional groups on the biologically active molecule. To produce this lidocain analog IDA was reacted with *w* chloro *w'* 2, 6-di-methyl acetanilide.

Tronter *et al*[11] and Davison *et al*[12] published their work on cyclic compounds (cyclans) and tetradentate ligand specifically designed to co-ordinate technetium. These compounds have been prepared with the purpose of either forming useful derivatives or coupling them to biologically active molecules for use as radiopharmaceuticals and are reported to form strong 99m Tc complexes that are stable *in vivo*.

Khaw *et al*[13] reported the use of diethylenetriamine pentaacetic acid to label antimyosin specific FAb fragments with 111 In for imaging purposes and in the same experimental model they also obtained gamma camera images with 99m Tc labelled antimyosin.

DeReimer *et al*[14] described the use of 1 (*p*-bromoacetamidophenyl) EDTA (BAPE) as label to produce alkyl albumin.

Scheinberg and Strand[15] described derivitization through chelation of monoclonal antibodies with a variety of radionuclides using ^{67}Ga, ^{68}Ga, ^{111}In and ^{99}mTc. They also studied (i) the effect of conjugation on the biological activity of antibody (ii) the maximum number of chelates that can be conjugated per antibody (iii) the biological half-life of the radioactive metal chelate on the antibody *in vivo* and (iv) the technical ease of covalently attaching several different chelates to immunoglobulins.

The chelates used include 1-(*p*-benzyl diazonium) EDTA, the *p*(hydroxybenzimidate of the 1-(*p*-benzyldiazonium) EDTA[16] 1-(*p*-carboxymethoxy-benzyl EDTA[17] and carboxy carbonic anhydride of DTPA[18]. They emphasized that rapid and specific imaging without computer enhancement or subtrac-

Table 1 *Bifunctional chelates currently under study*

Chelate	*Reference*
1-(*p* benzenediazonium) tetra acetic acid	Sundberg *et al*[9]
Iminodiacetic Acid (IDA) (HIDA & MIDA)	Loberg *et al*[10]
Cyclans	Tronter *et al*[11] and Davison *et al*[12]
1(-*p* bromoacetamidophenyl) EDTA (BAPE)	DeReimer *et al*[14]
Carboxycarbonic anhydride of DTPA	Krejcareck & Tucker[18]
1-(-*p* Carboxymethoxy Benzyl) EDTA	Yeh *et al*[17]
Deferoxamine	Yokoyama *et al*[22]
Metallocenes (Ferrocenes and Ruthenocenes)	Wenzel *et al*[26]
Cyclic Anhydride of DTPA	Hnatowich *et al*[27]

tion technique is possible with radioactive metal chelates conjugated to monoclonal antibodies. The other reported advantage which we feel is very important is the excretion of protected isotope immediately if the chelate during metabolism gets unconjugated which as contrasted to the unprotected iodine isotopes constitutes a real advance[19,20]. Indium chelate of carboxy carbonic anhydride of DTPA coupled to albumin was studied by Yeh *et al*[21] but they report that this method does not give the kinetic inertness of C1 substituted EDTA analogues and one is limited to reactions involving the carboxyl group.

Yokoyama *et al*[22] very recently reported a different bifunctional chelating agent namely deferroxamine which was used for labelling various biologically significant proteins with radiogallium. A good chelating agent for the radiopharmaceutical purpose must have low toxicity, be easy to obtain and have no influence on biological properties of the protein. Deferroxamine is commercially available and is already used *in vivo* for treatment of iron overload[23,24]. Because it is a noncharged drug of compact structure its influence on parent protein is minimal. Unlike EDTA or DTPA derivatives it does not have high affinity for $Ca+2$ or $Mg+2$ which are so essential for biological reactions.

CONCLUSION

Bifunctional chelates thus are very promising compounds from the point of view of linking technetium or other radionuclides to immunoglobulins derived from monoclonal antibodies. Because of their long shelf life they can be developed in kit form for linkage with radionuclides just before use.

Versatility of the radionuclides and using non specific immunoglobulins as well as specific antibodies should permit subtraction techniques to remove the reticuloendothelial image. Alternatively, the non-specific image can be allowed to decay and the early work scan subtracted from the late scan to show up the target. Finally it is possible that using bifunctional chelate and sufficiently specific monoclonal antibodies one may achieve imaging even without subtraction scanning.

We have elsewhere described the desirability of many labels other than technetium or indium for applications other than imaging[25] *e.g.* autoradiography, therapy or assay. It is therefore desirable that the bifunctional chelate be designed to be capable of linking not only technetium and Indium but other radionuclides as well. Metallocenes (Ruthenocens and Ferrocenes) have recently been suggested as easily derivatized compounds that bind certain radioactive metals strongly (^{59}Fe, ^{109}Ru) masking their chemistry and permiting their use as labels for radiopharmaceuticals[26]. We are at present engaged in certain studies pertaining to development of such general purpose radiopharmaceuticals both for imaging and possibly therapy.

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ANNOUNCEMENT

NATIONAL LEVEL INSTITUTE ON RECENT ADVANCES IN PLANT TISSUE CULTURE

Osmania University and University Grants Commission, Bahadur Shah Zafar Marg, New Delhi, will conduct a three week National Level Institute in Plant Tissue Culture. The Institute will commence from 10 December and will conclude on 31 December, 1984.

Each participant will be allotted a project work and must submit a report comprising of results of the project work besides special practicals. The topics to be covered by the Institute are: 1. Tissue and cell culture in crop improvement, 2. Clonal propagation of forest and fruit trees through tissue culture. 3. Selection for disease free and disease resistance through Tissue culture techniques. 4. Somatic cell Genetics. 5. Cryopreservation, 6. Genetic engineering of higher plants, 7. Transgenosis, and 8. Eukaryotic gene expression in bacteria and its application.

Last date for receiving applications is *1 November 1984*. For detailed information please write to: Prof. G. M. Reddy, Director, National Level Institute, (Advances in Plant Tissue Culture) Department of Genetics, Osmania University, Hyderabad 500 007.