A SIMPLE ELECTROLYTIC METHOD OF LABELING HUMAN FIBRINOGEN, IMMUNE GAMMA GLOBULIN AND SERUM ALBUMIN WITH ^{99m}Tc-PERTECHNETATE AT pH 7.4

Dennis W. Wong

Division of Nuclear Medicine, Department of Radiology, Martin Luther King Jr. Hospital and Charles R. Drew Postgraduate Medicine School, 12021 S. Wilmington Avenue, Los Angeles, California 90059, U.S.A.

Received May 17, 1977

SUMMARY

An improved electrolytic labeling technique has been developed for tagging human plasma proteins at physiologic pH condition. The basic principle involves the addition of protein after electrolysis and pH adjustment, thus avoiding harsh treatment of the protein and preserving its biological properties. High binding efficiency has been obtained for human fibrinogen, immune gamma globulin and serum albumin with an average of 77%, 86% and 87% respectively as assayed by paper radiochromatography. The labeling mechanism is not known. Data from protein electrophoresis demonstrate the existance of chemically active ^{99m}Tc-complex species with high protein binding capacity. The entire labeling process requires less than 1 hour. Since plasma proteins are labeled at pH 7.4, the problem of protein denaturation has been significantly reduced.

Key words: Human fibrinogen, immune γ-globulin, albumin, Technetium-99m.

INTRODUCTION

Human fibrinogen and serum albumin have been successfully labeled with ^{123}I, ^{125}I and ^{131}I by chemical, enzymatic or electrolytic methods. [1-7] The labeling

0362-4803/78/0414-0603$01.00

efficiency varies depending upon the method employed. The in vitro as well in vivo properties of these radioiodinated proteins have been extensively investigated and have been reported in the literature.[8-12] Because of the undesirable isotopic characteristics of ^{125}I and ^{131}I and limited supply of ^{123}I, Technetium-99m has become in recent years the isotope of choice in replacing radioiodinated compounds.

Due to the highly sensitive biochemical nature of the human plasma proteins, a reliable chemical method of labeling serum albumin or fibrinogen with ^{99m}Tc without denaturation has yet been developed. Electrolytic methods of tagging serum albumin and fibrinogen with ^{99m}Tc have been reported in the literature.[13-16] However, earlier labeling process requires the addition of the protein to be labeled to an acidic medium during electrolysis which leads to subsequent denaturation of the final labeled product. Attempts to label human fibrinogen in a physiologic condition have met with some success.[17, 18] Later investigation of our earlier labeling methodology suggests that ^{99m}Tc can be bound to human fibrinogen after pH adjustment. Furthermore, the same simple tagging technique can be applied to other plasma proteins with good reproducibility and preservation of their biologic properties.

MATERIALS AND METHOD

The improved labeling process is a modification of our earlier technique.[14] Reduction of ^{99m}Tc-pertechnetate from a stable (+7) state to a chemically active (+4) valence state is accomplished by electrolysis in a weak 0.05 N HCl acidic medium. Electrolysis is maintained at a controlled current of 100 mAmp with a voltage of 5.6-5.7 V for 45-50 sec. Following electrolysis, the pH of the electrolyzed solution is readjusted to 7.4 with 3.5-4 ml 2% (0.068M) trisodium citrate solution previously adjusted to pH 12.4 with 1 N NaOH. Immediately inject the protein to be labeled, 0.2 ml(4 mg) of the reconstituted fibrinogen*, 0.1 ml immune gamma globulin+ or 0.1 ml serum albumin* into the elctrolytic vial with continuous gentle swirling for 2 min. Incubate the contents of the vial at 37°C for 30 min. The final product is clear and ready for use.

---

* Cutter Lab. Berkery, Calif.

+ Hyland Lab. Calif.

Ascending paper radiochromatography with Whatman #1 paper in 85% methanol medium was used to assay the binding efficiency(BE) of the labeled proteins. Although this method could not be used to differentiate hydrated ^{99m}Tc-Zr complex or other types of complexed species from labeled protein, it was quite useful for detecting any unbound or unreduced ^{99m}Tc-pertechnetate. The actual amount of labeled protein present in the final product was determined by protein precipitation method with 20% trichloroacetic acid(TACC).

Qualitative radioactive protein identification was determined by protein electrophoresis with Gelman SepraTek® system using a cellulose polyacetate support medium. Normal human serum was used as standard. ^{99m}Tc-labeled proteins as well as unlabeled proteins were electrolyzed in Tris-Barbital-Na-Barbital pH 8.8 buffer, ionic strength 0.075, for 20 min at 200 volts. The electrolyzed plate was stained with Ponseau-S dye and rinsed with 5% acetic acid solution. Each red-stained band (approximately 3 mm) representing different protein fraction was cut from the plate and assayed for radioactivity.

In order to determine the percent clottable labeled fibrinogen and the degree of denaturation, 0.5 ml ^{99m}Tc-fibrinogen was first precipitated from solution with 20 units of topical thrombin solution (Parke-Davis). After separation and washing procedures, non-clottable protein which remained in the supernatant was removed by addition of 1 ml 20% TCAA solution. The per cent clottability of the ^{99m}Tc-fibrinogen was determined and calculated by the amount of radioactive clot formed from the total amount of the labeled protein present in the samples.

Immunological property of ^{99m}Tc-labeled gamma globulin was assessed with ^{99m}Tc-labeled anti-Staphylococcus aureus antibody. The antiserum was produced in rabbits by multiple subcutaneous innoculation of formalin-killed Staphylococcus aureus culture mixed with Freund's complete adjuvant. After a period of 2-3 weeks, the rabbits were bled and the antibody was extracted from serum by ammonium sulfate precipitation and purified by column chromatography and immunoabsorption technique. Antibody activity determination was performed before and after labeling process by visual assessment of agglutination of the antigen mixed with the antibody in varying dilutions.

RESULTS

The binding efficiency of ^{99m}Tc-labeled human plasma proteins is listed in Table I. Results from analysis of a series of 12 trials indicates an average B.E. of 77% (range 62-86%) is achieved for ^{99m}Tc-fibrinogen. Clottable protein accounts for 47% (68% clottability) of the total labeled fibrinogen as assayed by thrombin/TCAA precipitation method (Table I, column C). Non-clottable protein, presumably denatured radioactive fibrinogen, amounts to an average of 22%. In all cases, results from thrombin/TCAA precipitation method is approximately 10-12% higher than the the data obtained from TCAA protein precipitation assay. The discrepency is probably attributed to additional trapping of ^{99m}Tc-Zr-Citrate complex species during clotting process.

Table I. Binding efficiency of ^{99m}Tc-labeled fibrinogen, gamma globulin and serum albumin as determined by (A) ascending paper radiochromatography in 85% methanol, (B) TCAA protein precipitation and (C) thrombin clottability assay. An average of 12 trials for each labeled protein.

	(A)	(B)	(C)		
RADIOPHARMACEUTICALS	Percent Bound*	Total Labeled Protein*	Clottable Protein*	Non-clottable Protein	Total Labeled Protein
^{99m}Tc-Fibrinogen	77.04 (7.54)	60.43 (7.11)	47.16 (12.41)	22.21 (8.79)	69.36 (9.30)
^{99m}Tc-Immune Gamma Globulin	86.37 (8.12)	77.59 (3.19)	—	—	—
^{99m}Tc-Serum Albumin	86.75 (7.54)	74.90 (1.60)	—	—	—

* Mean % ± (s.d.)

A much higher degree of reproducibility and labeling efficiency is achieved for gamma globulin and serum albumin, an average of 84% (range 74-98%) and 87% (range 80-99%) respectively as assessed with ascending paper radiochromatography. However, TCAA protein precipitation assay, similar to ^{99m}Tc-fibrinogen, yields a lower percentage of total labeled protein content (Table I, column B). This

strongly suggests the existance of a ^{99m}Tc-(Zr)citrate complex which was not separated from labeled protein by paper radiochromtography. Electrolyzed ^{99m}Tc-Zr or ^{99m}Tc-(Zr)citrate complex as well as labeled proteins will not migrate in methanol medium and will remain at the origin. The chemical nature of these complex species has not been determined. ^{99m}Tc-(Sn)citrate complex produced by electrolysis using tin electrodes has been reported in the literature.[21]

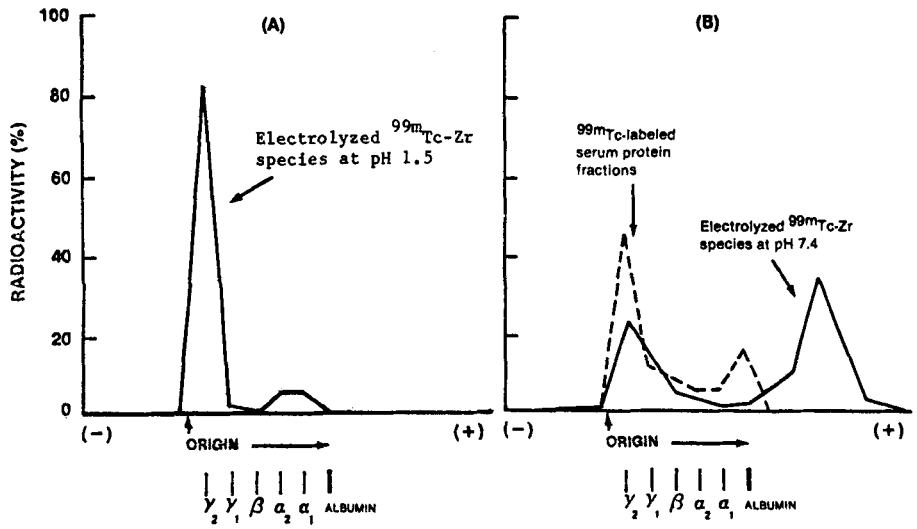

Fig. 1 Migration characteristic of (A) electrolyzed ^{99m}Tc-Zr species at pH 1.5 as compared with (B) at pH 7.4 after pH adjustment with trisodium citrate/NaOH solution and addition of human serum. ^{99m}Tc-pertechnetate will not migrate in electrophoresis but will diffuse into the buffered medium.

Protein electrophoresis performed at various time intervals up to 4 hours after the initial labeling procedure demonstrated that ^{99m}Tc is firmly bound to the protein moiety. Greater than 90% of the radioactivity was found to be associated with the corresponding labeled protein fractions. The same radioactive electrophoretic protein bands were identical with unlabeled proteins.

An average binding efficiency of 82% (range 70-98%) was obtained for ^{99m}Tc-labeled anti-Staphylococcus aureus antibody. Data from immunological assays indicated no significant change in antibody activity after labeling process. The

labeled antibody was effective against the antigen.[19,20]

DISCUSSION

Figure 1. illustrates the migration characteristic of ^{99m}Tc-Zr or ^{99m}Tc-(Zr)-citrate complex species at pH 1.5 and 7.4 conditions after electrophoresis. The labeling mechanism is not known. In the presence of pure plasma protein, these complex species in both pH conditions will quickly disappear with the radioactivity firmly bound to the protein moiety. Protein binding by ^{99m}Tc is assumed to involve the reaction of reduced ^{99m}Tc-Zr or ^{99m}Tc-(Zr)citrate complex with the protein ligand. Further evidence of high protein binding capacity of these complex species is seen by the addition of human serum to the electrolyzed pH 7.4 ^{99m}Tc-(Zr)citrate solution. Afer incubation at room temperature for only 5 min, the radioactivity is quickly redistributed, presumably bound, to different protein fractions. The same results is seen with 1 hour samples (Fig. 1B).

In vivo biological behavior of ^{99m}Tc-fibrinogen and ^{99m}Tc-gamma globulin has been investigated with laboratory animals. Initial findings indicate that the highest uptake of these labeled protein is seen in the liver and kidneys. The biological half-lives are also shorter than the native plasma proteins. The shortened initial half-life indicates that the in vivo behavior of ^{99m}Tc-labeled fibrinogen or gamma globulin is modified.

The biological property of ^{99m}Tc-labeled serum albumin has not been determined in animals.

REFERENCES

1. Welch M.J. and Krohn K.A.-Radiopharmaceuticals, (New Edition), The soc. of Nucl. Med. Inc. New York, (1975).
2. Rosa U. Scasselati G.A. Pennisi F,et al-Biochim.Biophys. Acta. 86:519 (1964)
3. Katz J. Bonorris G. - J. Lab. Clin. Med. 72: 966 (1968)
4. Morrison M. Bayse G. - Biochemistry 9: 2995 (1970)
5. Roberts R.C. Sonnetag C.O. Frisbie J.H. - J. Nucl. Med. 13: 843 (1972)
6. Ingraham S.C. III, Silberstein E.B., Kereiakes J.G. and Wellman H.N. - J. Nucl. Med. 10: 410 (1969)

7. DeNardo S.J., DeNardo G.L., O'Brian T. et al - J. Nucl. Med. 15: 487 (1974)
8. Krohn K.A., Sherman L. and Welch M.J. - Biochim, Biophys. Acta. 285: 404 (1972)
9. Korhn K.A., Koberg M.D. and Welch M.J. -J. Nucl. Med. 14: 420 (1973)
10. Metzer J.M., Secker-Walker R.H. and Krohn K.A. et al - J. Lab. Clin. Med. 82: 267 (1973).
11. Coleman R.E., Krohn K.A., Metzer J.M. et al - J. Lab. Clin. Med. 83: 977 (1974)
12. Metzer J.M., Secker-Walker R.H., Coleman R.E. et al - J. Nucl. Med. 14: 429 (1973)
13. Benjamin P.P. - Int. J. Appl. Rad. Isotopes 20: 187 (1969)
14. Wong D.W. and Mishkin F. - J. Nucl. Med. 16: 347 (1975)
15. Dworkin H.J. and Gutkowski R.F. - J. Nucl. Med. 12: 562 (1971)
16. Russell C.D. - Int. J. Appl. Rad. Isotopes 28: 241 (1977)
17. Harwig J.F., Harwig S.S. Wells L.D. and Welch M.J. - Int. J. Appl. Rad. Isotopes 27: 5 (1976)
18. Hebestreit H.P. and Pryss C. - Nucl. Med. 14: 172 (1975)
19. Wong D.W. and Huang T.T. - Int. J. Appl. Rad. Isotoptes 28: 719 (1977)
20. Huang T.T., Wong D.W., Tanaka T.T. et al - To be published in Clin. Research
21. Schneider P.B. -J. Nucl. Med. 14: 843 (1973)