Turkish Journal of Nuclear Sciences, 10 (1983) 112-117

IODINATION OF BOVINE SPLEEN FERRITIN*

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ABSTRACT

Two different methods have been used for iodination of bovine spleen ferritin with ^{125}I by the Chloramine-T and Conjugation labelling (Bolton-Hunter). The percentage efficiencies of iodination have been estimated to be 17 % with the Chloramine-T and 6.2 % with the Bolton-Hunter method. The Chloramine-T method was more efficient, easier and cheaper than the Bolton-Hunter method. The bovine ferritin containes tyrosine residues, thus the Chloramine-T method was convenient for labelling ferritin in order to use it as tracer in a ferritin-RIA.

ÖZET

Sığır dalağı ferritinini ^{125}I ile radyoaktif işaretlemek için iki farklı yöntem kullanıldı. Bunlar Chloramine-T yöntemi ve konjugasyon ile işaretleme işlemi olan Bolton-Hunter yöntemidir. İyotlamanın yüzde verimliliği Chloramine-T yöntemi için % 17 ve Bolton-Hunter yöntemi için % 6.2 olarak hesaplandı. Chloramine-T yöntemi Bolton-Hunter yöntemine oranla daha etkin, kolay ve ucuzdur. Sığır ferritini yapısında tyrosine amino asitleri kapsadığından ferritinin radyoaktif işaretlenmesinde ve radioimmunoassay yöntemi için izleme maddesi (tracer) olarak hazırlanmasında Chloramine-T yöntemi uygun bir işlemdir.

INTRODUCTION

There are several known radicisotopes of iodine but the most useful in biological applications are ^{131}I, a beta-gamma emitter with a half-life of 8 d and ^{125}I, a very low-energy gamma emitter with a much longer half-life of 60 d. Both isotopes are available in a

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carrier free form. Because of its more desirable physical properties, ^{125}I has largely replaced with ^{131}I in RIAs.

The iodination reaction that is generally used involves direct labelling of protein by a substitution of free iodine for hydrogen, principally in the aromatic ring of tyrosyl groups /1/. Some iodine may also react with histidine /2,3/, tryptophane /4/ or sulphydryl groups /5/, but tyrosine is the principal amino acid involved.

Radioactive iodine along with carrier iodide is usually converted to iodine in one of the two ways: either chemically-i.e., by use of strong oxidizing agents, such as nitrous acid /6/ or Chloramine-T /7,8/ followed by addition of reducing agent or enzymically by use of peroxidase /9,10/.

Chloramine-T has replaced nitrous acid because of its milder pH conditions. The Chloramine-T method has been almost universally used to label the ever-increasing number of proteins and polypeptides for which radioimmunoassays have been reported /11-13/. On the other hand, it has a denaturant effect on proteins. This has never well-documented, and now it appears likely that the undesirable effects attributed to Chloramine-T may have been due to radiation damage. The damaging effect to the protein due to iodination include alteration of the molecule due to conformational changes to accommodate the relatively large iodine atom, the side effects from the reaction itself, and the subsequent radiation damage due to the presence of the radioactive iodine. Indirect iodination by the Bolton-Hunter method /14/ avoids some of these effects since the iodination takes place separately in the absence of protein. Reaction with Chloramine-T is used to obtain the iodinated reagent, N-hydroxysuccinimide ester of P-hydroxyphenyl propionic acid. This ester reacts with an amine group on the protein. It is obvious that the molecular structure is much larger than an iodine atom. This conjugated group offers an important alternative labelling method for those proteins and peptides that lack a tyrosyl residue.

The present paper describes a direct iodination method by Chloramine-T and an indirect iodination method by Bolton-Hunter reagent for labelling bovine ferritin with ^{125}I. This iodinated ferritin has been used as tracer in continuing research on "Development of Ferritin Radioimmunoassay and the Study of Iron Stores in Cattle".

MATERIALS AND METHODS

Two methods have been used for iodination of bovine ferritin.

A. The Chloramine-T Method:

Pure ferritin from bovine spleen was prepared in our laboratory and ferritin was radioiodinated by the modification methods /6,7/. Five micrograms of ferritin (measured by Lowry Protein Assay) were added to 0.05 ml of 0.5 M phosphate buffer at pH of 7.5 and 0.85 mCi of Na ^{125}I solution was added and followed by adding 0.025 ml of the Chloramine-T solution that has been freshly prepared (5 mg Chloramine-T/ml in 0.05 M sodium phosphate buffer, pH 7.5) and mixed gently for exactly two minutes. The reaction was performed at room temperature and after two minutes, 0.05 ml of sodium metabisulphite (1.2 mg/ml in 0.05 M sodium phosphate buffer, pH 7.5) was added to stop the oxidizing action of Chloramine-T. Then, 0.1 ml of "transfer solution" (2.0 mg NaI/ml containing several sucrose crystalls) was added to the reaction mixture and transferred to a Sephadex G-25 column that has been equilibrated with 20 ml of 0.05 M phosphate buffered saline containing 0.1 % gelatine (PBS-gel) and eluted by collecting 1 ml fractions into test tubes containing 0.1 ml of the PBS-gel. From each tube 5µl fraction was pipetted into a test tube for counting in a gamma counter. Reaction vial was also counted. With a column of a total volume of 10 ml, the second through fourth fractions contained the iodinated ferritin, so that at least 10 consecutive fractions were collected.

B. The Conjugation Iodination Method, Using N-Succinimidyl 3- (4-hydroxy, 5-(^{125}I) iodophenyl) propionate.

The purified ferritin fraction was radioidinated by the modification method of Bolton-Hunter /14/. The N-succinimidyl 3-(4-hydroxy, 5-(^{125}I) iodophenyl) propionate (NSHPP) is commercially available. 0.030 ml. of NSHPP (1 mCi) was pipetted into a vial and dried with air flow. 5 µg (0.010 ml) of protein in 0.1 M borate buffer at pH of 8.6 was pipetted to the dried iodinated ester and the reaction mixture agitated for 15 min at 0^{o}C in an ice bath. Labelled ester hydrolyses in aqueous media, the half-life of this material being about 9 min in 0.1 M borate buffer, pH 8.6, 0.5 ml of 0.1 M borate buffer with 0.2 M glycine, pH 8.6 was added into the reaction mixture and left 5 min at 0^{o}C. The ^{125}I- labelled ferritin was separated from the other

labelled products of the conjugation reaction glycine conjugate, an 3-(4-hydroxyphenyl) propionic acid, by gel filtration.

RESULTS AND DISCUSSION

A) The Chloramine-T Method:

^{137}Cs was used as standard for calibration of the gamma counter. Counts of fractions eluted from gel filtration column were expressed as cpm/ml after background subtraction and plotted versus fraction number (Fig.1.). Peak 1 is labelled bovine ferritin. Peak 3, low molecular weight material, can be shown to be largely composed of (^{125}I) iodide by its electrophoretic mobility. Fraction three contains iodinated protein and the highest activity. The efficiency of iodination was calculated to be 17 %

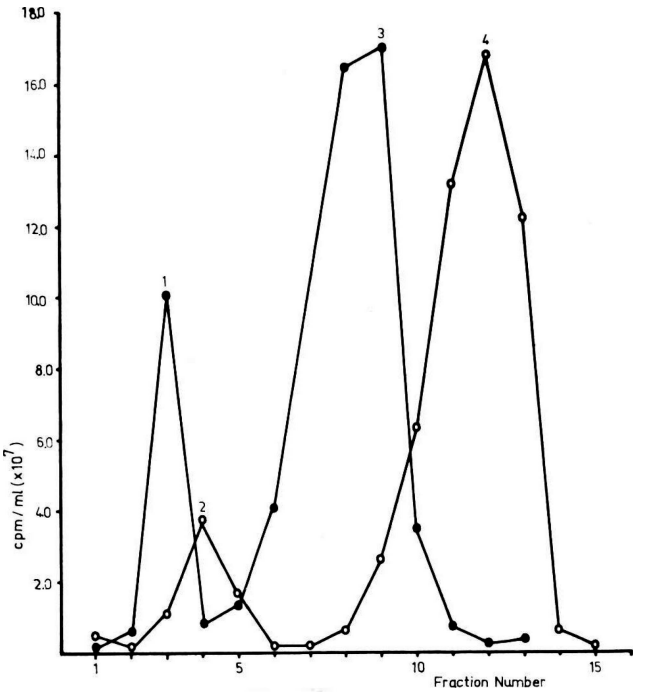


Fig. 1. Gel filtration of radioiodinated bovine spleen ferritin by the Chloramine-T method (o—o) and Bolton-Hunter method (●—●) on Sephadex G-25 column.
1-2: Labelled protein peaks
3-4: Free ^{125}I and ^{125}I labelled moiety

B) The Conjugation Iodination (Bolton-Hunter) Method:

Gamma counter setting was adjusted with ^{137}Cs as in the Chloramine-T method. The count rates of fractions eluted from gel filtration were expressed as cpm/ml after background subtraction and plotted versus fraction number (Fig. 1). Two different peaks were observed on the graph as seen in the figure. Peak 2 is iodinated bovine spleen ferritin and peak 4 is low molecular weight labelled moiety. Fraction 4 contains iodinated protein and the highest activity. The efficiency of iodination was figured out to be 6.2 %.

The labelling of protein with the Chloramine-T procedure is easier than the conjugation labelling method and others, also the best understood method but in this procedure some proteins are damaged by oxidizing and reducing agents.

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