Journal of Radioanalytical and Nuclear Chemistry, Vol. 257, No. 1 (2003) 71–73

Synthesis of radioiodinated labeled peptides

M. Matloobi,[1] H. Rafii,[1] D. Beigi,[2] A. Khalaj,[2] M. Kamali-Dehghan[2]

[1] *Cyclotron Department, Research Center for Agriculture & Medicine, P.O. Box: 31585-4395, Karaj, Iran*
[2] *Faculty of Pharmacy, Tehran University, Tehran, Iran*

(Received November 6, 2002)

This report covers optimization of radioiodination of peptides by both a direct method in which a constituent tyrosine residue is labeled and indirect method by using an iodinated derivative (SIB) of N-succinimidyl 3-(tri-n-butylstannyl) benzoate (ATE) as the intermediate. Radioiodination of IgG and FMLF were performed by direct method using Chloramine-T as an oxidant but since Formyl-Methyl-Leucyl-Phenylalanine, FMLF, does not lend itself for direct radioiodination we performed labeling of FMLF by indirect method via radioiodined SIB at different pH.

Introduction

Radioiodination is the method which has been most frequently employed for labeling proteins in high specific activity for in vitro application. Iodine-131 and iodine-125 labeled compounds are widely used for biochemical function studies. The radioisotopes of iodine offer certain advantages for pre-clinical and clinical studies of labeled monoclonal antibodies[7] for the diagnosis and therapy of tumors.[4]

Antibodies can be "indirectly" radioiodinated via N-succinimidyl (tri-n-butylstannyl) benzoate (ATE) intermediate and the use of this approach for antibody labeling significantly decreases the loss of radioiodine from proteins in vivo.[8] The "indirect" radiohalogenation via prosthetic groups has provided a useful route for labeling proteins, peptides and drug molecules. This method is the only option available for molecules that are not amenable to classical "indirect" radiohalogenation reaction because of the lack of tyrosine groups in their structure.[2]

Radioiodination of IgG by the "direct" method and formyl-methyl-leucyl-phenylalanine, FMLF, by the "indirect" method using ATE/SIB is the objective of our research.[5,6] Once these procedures have been optimized, the method will be applied to other peptides.

Experimental

N-Chlorosuccinimide (NCS), Chloramine-T, KI, $Na_2S_2O_5$, acetic acid glacial and chloroform were obtained from Merck and Aldrich Co. Silica Sep-Pak Cartridges (WAT020520) was purchased from Waters. Iodine-125 was obtained from Amersham and iodine-131 and IgG from our colleagues in Tehran (NRC). Sephadex G-50 and FMLF were purchased from Sigma Chemical Co.

Direct radioiodination of IgG by Chloramine-T

The direct iodination of human IgG was performed as follows:[1] 5 μl Chloramine-T (25 μg) was added to a mixture of 10 μg IgG in 10 μl, 0.2M phosphate buffer (PBS) and 500 μCi Na*I. The solution was mixed at room temperature for 60 seconds before the addition of 25 μl sodium metabisulphite $Na_2S_2O_5$ (125 μg) to the mixture. Then 250 μl NaI or KI (500 μg) was added to the reaction mixture. The [^{125}I or ^{131}I] IgG was isolated from the reaction mixture using a Sephadex G-50 column eluted with PBS, pH 7.4 at a flow rate of 1 ml/min. The eluted 0.5 ml fractions were collected in test tubes and counted to obtain the elution profile. The fractions between "9–13" were labeled IgG (Fig. 1).

Synthesis of [I-125, I-131] N-succinimidyl-3-iodobenzoate, [I-125, I-131] SIB

[I-125, I-131] SIB was synthesized by using NCS as starting agent.[7] Briefly, in a 2 ml conical vial the following reagents were added:

–10 μl ATE dissolved in anhydrous chloroform; 1 μmol of 0.1 ATE; 30 μl Na*I in 0.02N NaOH, about 0.5 to 1.5 mCi; 50 μl acetic acid in chloroform, prepared by dissolving 290 μl of acetic acid in 4.9 ml of chloroform; 50 μl NCS, 1M NCS in anhydrous chloroform (the solubility of NCS in chloroform is low).

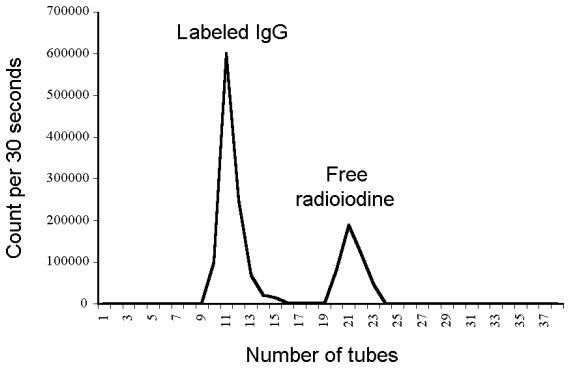

Fig. 1. Yield of direct radioiodination of IgG by Chloramine-T

0236–5731/2003/USD 20.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

The reaction mixture was stirred at room temperature for 30 minutes and radioiodinated SIB was isolated as follows:

A silica gel cartridge was saturated with 25 ml n-hexane; the reaction mixture was loaded on the column with help of 3×100 µl hexane (fraction 1); the column was washed by 8×5 ml hexane (fraction 2–9); 5×5 ml 8% ethylacetate in hexane (fraction 10–14); 6×1 ml (fraction 15–20) and finally 2×5 ml (fraction 21, 22) 30% ethylacetate in hexane. Activity of each fraction was measured. The relative activity per ml of each fraction and the elution profile is shown in Fig. 2. It was seen that the fraction 16–19 contained the desired-labeled SIB. Therefore, the 30% ethylacetate in hexane fractions were pooled and evaporated to a small volume with a steam of nitrogen and transferred to a conical vial and by continuing evaporation of eluent to dryness to obtain the labeled SIB.

Radioiodination of FMLF

The radiolabeling studies were performed by the direct method using Chloramine-T[3] and indirect method[5,7] using [^{125}I and ^{131}I] SIB. Since FMLF does not lend itself for direct iodination, after radiolabeling, most of the unreacted radioiodine was isolated from the reaction mixture by a column packed with Sephadex G-50 eluted with 0.01M PBS, pH 7.4 (Fig. 3).

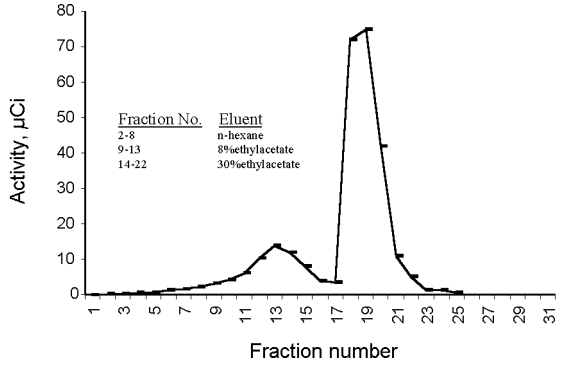

Fig. 2. Sep-Pak elution profile of [^{125}I] SIB

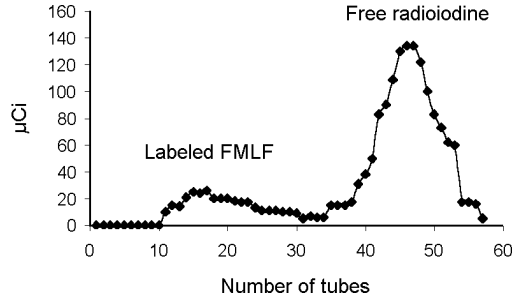

Fig. 3. Yield of direct radioiodination of FMLF by Chloramine

Radioiodination of FMLF via [^{125}I or ^{131}I] SIB was carried out as follows: 50 µl FMLF was added to the labeled SIB (200 µg FMLF/50 µl 0.1M borate buffer) at different pH 8.5–9.0 and 10. The mixture was stirred in ice-water bath for 30 minutes. 300 ml glycine (200 mM in 0.1M borate buffer, pH 7.5) was added to terminate the reaction followed by an incubation of 5 minutes. The labeled product was isolated from reaction mixture using a column Sephadex G-50 eluted with PBS, pH 7.4 at a flow rate of 1 ml/min. The eluate of 0.5 ml was collected in a test tube and counted to obtain the elution profile (Fig. 4).

Discussion

Some peptides such as FMLF do not lend themselves to the direct labeling method. Therefore, the indirect method (SIB) must be used for radioiodination.

Radioiodination of ATE with ^{125}I and ^{131}I was performed successfully and labeled SIB was isolated in high radiochemical purity.

A strong buffer should always be included in the mixture because the reactions are pH-dependent.

Labeling of FMLF via radioiodinated SIB was performed at pH values of 8.5, 9.0, and 10. The best radiochemical yield was found at pH 8.5

With increasing pH, the yield of labeled FMLF decreased, perhaps because of interaction OH to carboxyl of SIB resulting m-iodobenzoic acid.

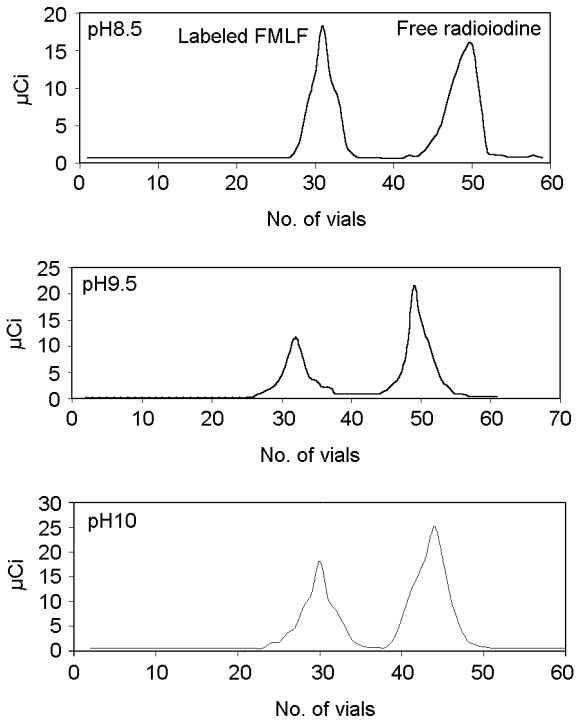

Fig. 4. Yield of indirect radioiodination of FMLF at different pH

Dilution of ^{125}I with non-radioactive iodine (^{127}I) increases the incorporation of ^{125}I by an unknown mechanism.

Radioiodination of IgG was performed successfully by Chloramine-T method.

Conclusions

The indirect method (conjugation method) circumvents the problem of protein damage cause by direct contact between protein and iodination reagent.

The indirect method allows derivatization of residues other than tyrosine.

The indirect method is extremely useful for labeling some unstable proteins and for peptides, which lack tyrosine.

The indirect method is more complex than the direct method.

Radioiodination of some peptides like Vasoactive Intestinal Peptide (VIP) can probably be accomplished by both the direct and the indirect methods because this peptide contains two tyrosine and three lysine residues.

References

1. P. ANGELBERGER, I. VIRGOLINI, O. BUCHHEIT, M. EGGER, R. PROTNER, H. KVATERNIK, J. Label. Compds. Radiopharm., 37 (1995) 502.
2. P. K. GARG, G. E. ARCHER, D. D. BIGNER, M. R. ZALUTSKY, Appl. Radiation Isotopes, 40 (1989) 485.
3. L. A. KHAWLI, A. I. KASSIS, Nucl. Med. Biol, 16 (1989) 727.
4. S. M. LARSON, J. A. CARRASQUILLO, Nucl. Med. Biol., 15 (1988) 231.
5. G. L. TONNESEN, R. N. HANSON, D. E. SEITZ, Intern. J. Appl. Radiation Isotopes, 32 (1981) 171.
6. D. S. WILBUR, D. S. JONES, A. R. FRITZBERG, A. C. MORGAN, J. Nucl. Med., 27 (1996) 959.
7. M. R. ZALUTSKY, A. S. NARULA, Appl. Radiation Isotopes, 38 (1987) 1051.
8. M. R. ZALUTSKY, A. S. NARULA, Cancer Res., 48 (1988) 1446.