# ENZYMATIC LABELLING OF CARCINOEMBRYONIC ANTIGEN WITH IODINE-125

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## SUMMARY

Carcinoembryonic antigen /CEA/ was labelled with iodine-125 using lactoperoxidase. The radiochemical yield of /$^{125}$I/CEA was about 25% at the specific activity of about 30μCi/μg /1.1MBq/μg/. The percentage binding of /$^{125}$I/CEA with specific anti-CEA serum was determined in lyophilized samples during storage. The obtained /$^{125}$I/CEA preparations can be utilized in radioimmunoassay kits for at least two months.

Key Words: Carcinoembryonic Antigen, Iodine-125 Labelling

## INTRODUCTION

Carcinoembryonic antigen /CEA/ is a glycoprotein with molecular mass of 200 000 daltons. The CEA molecule contains only about 2% w/w of tyrosine and about 1% w/w of histidine residues which can be iodinated /1/.

The labelled antigen is used as a marker for the radioimmunological determination of CEA. Increased level of CEA in human blood serum may accompany some neoplastic diseases /2/.

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Labelled CEA is generally prepared by radioiodination in the presence of chloramine T /3-9/. In this work the enzymatic method of labelling /10,11/ was applied. The reaction conditions of labelling CEA with iodine-125 were established and the radioimmunological properties of the product during storage were studied.

## EXPERIMENTAL

### Materials

Immunological reagents were provided under the National Cancer Programme /12/. CEA was isolated from human hepatic metastases of colonic carcinoma /13/ at the Medical Academy in Wrocław and was used in phosphate buffer solution. Goat anti-CEA serum, horse anti-IgG/goat/ serum and normal goat serum were produced at the Agricultural Academy in Wrocław. The CEA and anti-CEA serum were tested by radioimmunoassay /9/ at the Medical School in Poznań by comparison with First British CEA Standard and anti-CEA 6G3 serum from Chester Beatty Cancer Research Institute showing similar immunoreactivity.

$Na^{125}I$ /IMS.300/ was supplied by The Radiochemical Centre, Amersham. Lactoperoxidase, B grade /LPO/ was supplied by Calbiochem, USA and used in phosphate buffer. Bovine serum albumin, Cohn´s fraction V, was obtained from Biomed, Kraków. Sephadex G-200 and Sephacryl S-200 Superfine supplied by Pharmacia, Uppsala were used as gel filtration media.

0.05M phosphate buffer of pH 7.5 /PB/ was prepared using reagents obtained from POCH, Gliwice.

Methods

Labelling was carried out in a glove box using a cylindrical polystyrene vessel 1.5cm high and 1.0cm in diameter at room temperature. The reaction solution was stirred with a magnetic stirrer.

Purification of the labelled /$^{125}$I/CEA was performed by gel permeation using chromatographic columns of dimensions of 0.9x55cm. Labelled products were eluted with PB solution containing 0.3% w/v bovine serum albumin and 0.1% w/v sodium azide. Fractions of 1cm$^3$ were collected at elution rates of 6cm$^3$/h and 12cm$^3$/h for Sephadex G-200 and Sephacryl S-200, respectively.

Radiochemical yield of the labelling reaction was determined by the paper chromatography technique using ammonium sulphate as solvent /14/. The same analytical technique was utilized for the determination of inorganic iodide in final preparations.

Specific activity of the labelled product was calculated from the mass of CEA, the radioactivity of Na$^{125}$I and the radiochemical yield of the iodination reaction.

Samples of the purified /$^{125}$I/CEA of a radioactivity of about 5μCi /0.2MBq/ in PB solution containing 0.3% w/v bovine serum albumin and 0.1% w/v sodium azide were frozen in dry ice and then lyophilized.

Immunoreactivity of the /$^{125}$I/CEA preparations were determined by double antibody radioimmunoassay /9/ using goat anti-CEA serum of a titer in the range 1/100 000 - 1/50 000 diluted with PB solution containing 0.3% w/v bovine serum

albumin, 2.5% v/v normal goat serum and 0.1% w/v sodium azide and precipitation horse anti-IgG/goat/ serum of a titer 1/1 diluted with the above buffer without normal goat serum. The radioactivity was measured in a well type automatic gamma counter /LKB Rack-Gamma/.

The immunoreactivity was expressed as a percentage ratio of the precipitate radioactivity to the total radioactivity of /$^{125}$I/CEA. Blank value, i.e. the radioactivity precipitated with the proteins in the absence of antiserum was 1-3%. Standard deviation of the analytical procedure was 1%.

## RESULTS

### Labelling

The conditions of the iodination procedure described below were based partly on our previous experience concerning the enzymatic labelling of several proteins /15/.

Equal volumes /0.02cm$^3$/ of PB, CEA, LPO, $H_2O_2$ and Na$^{125}$I solutions were successively placed in a reaction vessel. The molar amounts of the reagents in the reaction mixture of total volume 0.1cm$^3$ were as follow: CEA - 100pM /2.0x10$^{-5}$g/, LPO - 20pM /1.6x10$^{-6}$g/, $H_2O_2$ - 2000pM /6.8x10$^{-8}$g/, and Na$^{125}$I - 800-1300pM /1.2-1.9x10$^{-7}$g/. The amount of Na$^{125}$I corresponded to 1.6-2.6mCi /59-96MBq/ of iodine-125 activity.

It was observed that when the amount of $H_2O_2$ was only 1000pM the radiochemical yield of /$^{125}$I/CEA was twofold smaller than that at 2000pM $H_2O_2$ concentration. However, if the amount of $H_2O_2$ was increased up to 4000pM the radiochemical yield remained constant.

The reaction was stopped after 3min by addition of 0.02cm$^3$ of 1% w/v cysteine in PB. The mixture was then diluted with 0.5cm$^3$ of PB solution containing 0.1% w/v sodium azide.

It was observed that the iodination of CEA under the employed conditions proceeded quickly. Most of CEA reacted during the first minute of the reaction time. The yield after 3min was only about 5% higher than that after 1min.

The molar concentration of CEA in the reaction mixture was relatively low. The radiochemical yield under employed conditions was in the range 23-28%, whereas the corresponding specific activity of /$^{125}$I/CEA was 21-37μCi/μg /0.8-1.4MBq/μg/.

Purification

/$^{125}$I/CEA was purified by gel permeation chromatography. A typical chromatogram of radioiodination mixture on a Sephadex column is presented in Fig.1.

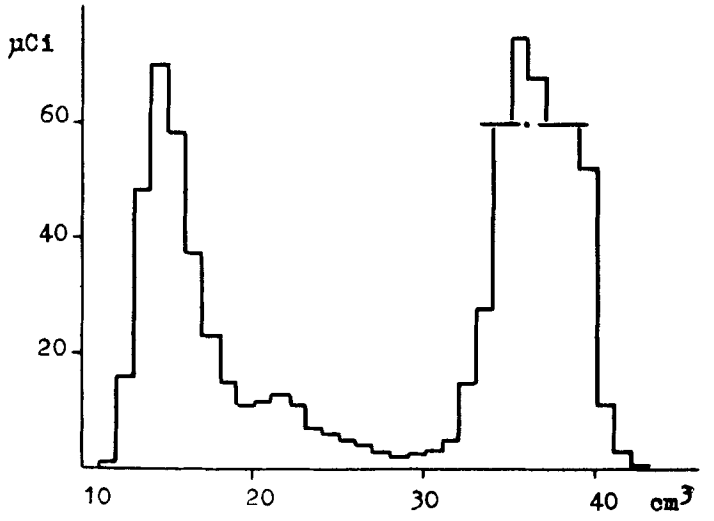


Fig.1 Column chromatogram of an iodination mixture on Sephadex G-200

/$^{125}$I/CEA was eluted as the first peak while $^{125}I^-$ as the last one. It was found that the small peak between them was not immunoreactive, presumably corresponding to iodinated LPO and degradation products of CEA.

The immunoreactivity of individual fractions of the /$^{125}$I/CEA peak as determined by radioimmunoassay is presented in Table 1. It can be seen from comparison of Table 1 and Fig.1

Table 1. The immunoreactivity of individual fractions of the /$^{125}$I/CEA peak eluted from a Sephadex G-200 column

| Fraction No. | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|
| Bound$^+$ % | 48.0 | 57.9 | 61.5 | 56.5 | 39.6 |

$^+$to anti-CEA serum of a titer 1/50 000

that the three fractions of high radioactivity show similar immunoreactivity. Hence, to obtain /$^{125}$I/CEA preparation the fraction of the highest radioactivity /No.14/ was combined with the adjacent ones /Nos.13 and 15/. The percentage binding of fresh /$^{125}$I/CEA preparations to excess anti-CEA serum of a titer 1/1000 was about 90%.

Isolation of /$^{125}$I/CEA from the reaction mixture was also performed using a Sephacryl column /Fig.2/. The first peak eluted was identified as /$^{125}$I/CEA while the last one as $^{125}I^-$. The two peaks in between were not immunoreactive to anti-CEA serum. It can be seen that the resolution achieved on Sephacryl /Fig.2/ is better as compared to Sephadex /Fig.1/. Moreover, the elution rate on Sephacryl under atmospheric pressure is twice as fast as on Sephadex resulting thereby in a shorter purification time. Nevertheless, no significant difference in the immunoreactivity between the /$^{125}$I/CEA preparations purified

on both types of columns was observed.

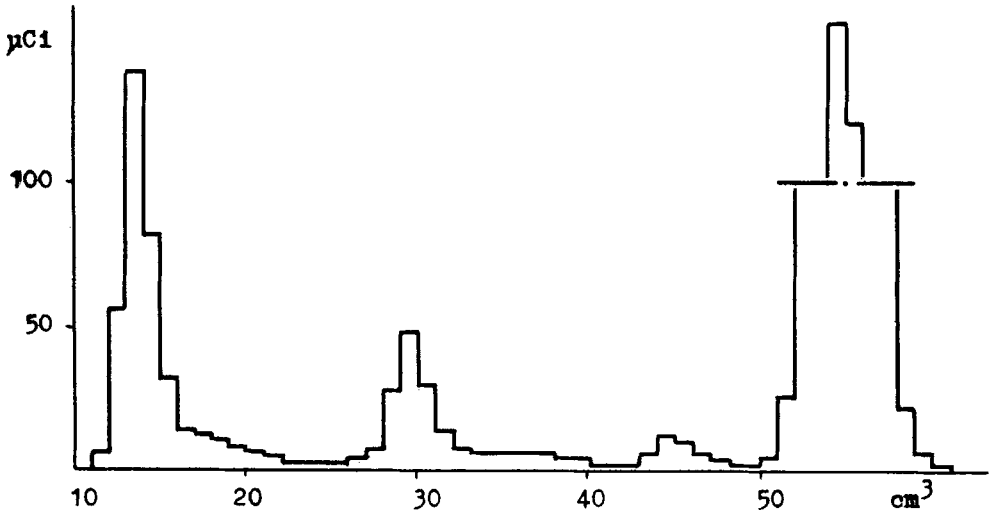


Fig.2 . Column chromatogram of an iodination mixture on Sephacryl S-200

Stability

The lyophilized /$^{125}$I/CEA preparations of a specific activity in the range 22-37µCi/µg /0.8-1.4MBq/µg/ were stored for several weeks. The immunoreactivity of /$^{125}$I/CEA during storage was determined by radioimmunoassay at the same dilution of anti-CEA serum. The samples were reconstituted with PB containing 0.3% w/v bovine serum albumin just before analysis. As it is shown in Fig.3 the fraction of the original binding decreased slowly achieving values 0.8 and 0.6 after one and two months, respectively. No differences were observed between the samples stored at 4°C and those stored at room temperature. The content of inorganic iodine in these preparations after two months of storage increased to about 15% /Fig.4/. The /$^{125}$I/CEA was also used as a marker in routine radioimmunoassay for even more than

two months.

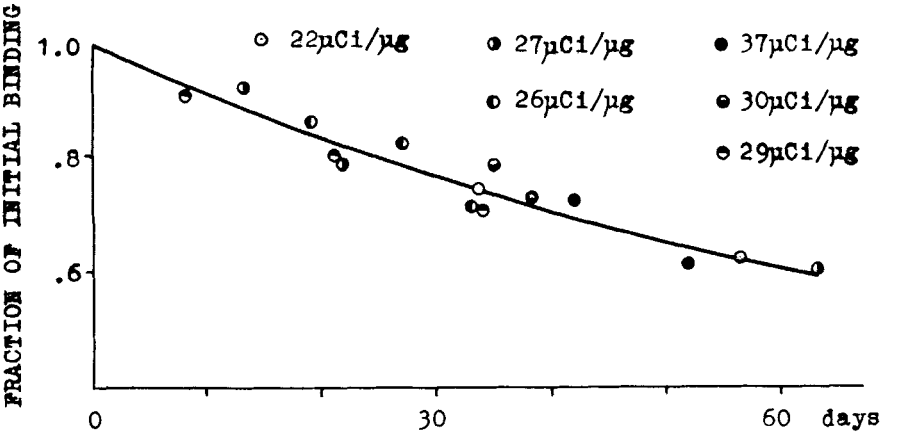


Fig.3 Immunoreactivity of /$^{125}$I/CEA during storage at 4°C /For comparison, the sample of a specific activity 22μCi/μg was stored at room temperature/

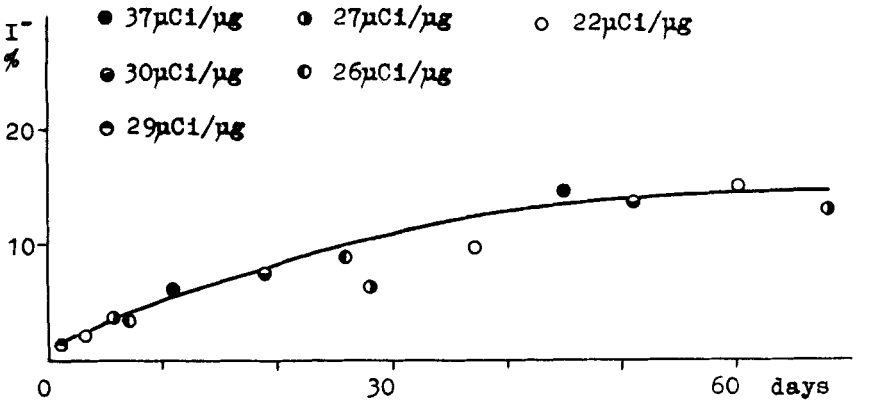


Fig.4 Increase of inorganic iodine content during storage at 4°C. /See note to Fig.3/

## DISCUSSION

In our experiments the average number of iodine atoms incorporated into CEA molecule by the LPO method was about 3 as estimated from the specific activity of the product.

The /$^{125}$I/CEA retained 0.6 of its original immunological activity after two months. The product obtained by the enzymatic method was not directly compared with that iodinated by the conventional chloramine T technique. However, literature data show that the original immunoreactivity of the /$^{125}$I/CEA prepared by the chloramine T method decreases faster than of the product labelled by the LPO method described here. The /$^{125}$I/CEA solution obtained by the chloramine T method, purified by gel permeation chromatography and stored at -20°C can be used in radicimmunoassay for only two weeks /9/. The shelf-life of available commercial kits containing lyophilized /$^{125}$I/CEA labelled by the same technique and purified by electrophoresis on polyacrylamide gel is four weeks /16/ and according to the instruction sheet its percent binding decreases from 30% to 15%, i.e. to 0.5 of the original value, after five weeks of storage at 2-6°C.

The preparations obtained by the LPO method are stable also at room temperature. This is of advantage for use in commercial kits because elevated temperatures, e.g. during transportation, should not affect the properties of the radioimmunoassay.

Stability of the /$^{125}$I/CEA labelled by the LPO method may be due to mild conditions of enzymatic iodination because of the lack in the reaction mixture of aggressive chemical species which can damage the protein being labelled. On the other hand, longer shelf-life of the preparations may be caused by different location of iodine atoms in /$^{125}$I/CEA molecule, particularly with regard to the molecular fragments being immunologically active. The enzymatic labelling proceeds probably through large enzyme-iodine complex /17/ which can not react with tyrosine and

histidine groups located internally the protein molecule due to steric hindrance.

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