DETERMINATION OF INORGANIC RADIOIODINE IN SOLUTIONS OF ^{125}I-LABELLED PROTEINS

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SUMMARY

A rapid paper chromatography procedure using 33% w/v aqueous solution of ammonium sulphate as a solvent has been applied for routine determination of the proportion of ^{125}I present inorganic iodine in solutions containing ^{125}I-labelled proteins. R_f values for protein, iodide and iodate correspond to 0.0, 0.5, and 0.9, respectively.

The amount of inorganic iodine in the iodination mixture or in the iodinated protein preparation can be determined with good precision in less than 30 minutes.

Key Words: Radiochemical Purity, Labelled Proteins, Iodine-125

INTRODUCTION

Manufacturing of radioiodinated proteins requires routine determination of unreacted inorganic iodine in the reaction mixtures in order to evaluate the iodination yield and eventually the specific activity of the product. The determination of inorganic iodine impurities in radioiodinated protein preparations is also used for the quality control of the final product.

Various techniques of paper electrophoresis, paper chromatography and thin layer chromatography are used as routine control methods /1-6/, however the time of analysis is rather long ranging from several to more than ten hours.

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By applying the paper chromatography technique described below it is possible to determine the radioiodination yield before purification of the labelled protein in a much shorter time. By using aqueous ammonium sulphate solution as solvent, which acts simultaneously as a precipitating agent of the iodinated protein and as a developing agent for inorganic iodine compounds, a highly effective separation has been obtained.

EXPERIMENTAL

Materials

$/NH_4/_2SO_4$ analytical reagent grade was supplied by POCH-Gliwice. Protein preparations were labelled in this Laboratory using $Na^{125}I$ supplied by Radioisotope Production and Distribution Centre - Swierk, or by The Radiochemical Centre - Amersham.

Procedure

Since the radioactive concentration of the reaction mixtures was high the samples were diluted before analysis with 0.05M phosphate buffer of pH 7.5 containing 0.3% w/v bovine serum albumin and 0.1% w/v NaN_3. Labelled protein preparations in phosphate buffer solution containing albumin as protective protein were analysed directly.

The chromatographic separation was carried out using strips of Whatman 3MM paper 1.5cm wide. 33% w/v aqueous $/NH_4/_2SO_4$ solution of pH 7.5 was used as solvent. A sample volume of $0.001cm^3$ containing activity in the range 0.7-7kBq /0.02-0.2uCi/ was applied to the origin and allowed to dry at room temperature for 5 minutes. The chromatogram was then developed for a distance of 5cm during 10-12min. After drying for 3min at 130°C

the strips were sprayed with hair lacquer and dried again for 1min at the same temperature. The strips were then cut into 0.8cm sections in such a way as to locate the chromatograph origin in the centre of the first section.

The radioactivity of each section was measured with an automatic gamma counter. The activity of the first section corresponds to the labelled protein and the activity of the other sections - to inorganic iodine.

The total time of analysis does not exceed 30 minutes.

Characteristics of the method

The above procedure was used for analysis of reaction mixtures from protein radioiodination as well as for the analysis of solutions of labelled protein preparations. All the proteins examined viz pork insulin, human growth hormone, alfafetoprotein, carcinoembryonic antigen and human chorionic gonadotropin exhibited similar chromatographic behaviour. Additives usually present in the reaction solutions /like chloramine-T and sodium metabisulphite or lactoperoxidase, hydrogen peroxide and cysteine/ shown to have no effects on the results of analysis. Bovine serum albumin which is usually added as carrier or protective protein in concentrations up to $10^{-2}g/cm^3$ also did not affect the efficiency of separation.

The resolution of the method is illustrated by a typical chromatogram /Fig.1/. The labelled protein undergoes complete precipitation at the starting point at R_f 0.0. The peak at R_f 0.5 corresponds to iodide. It has been found in a separate experiment that the R_f value for iodate under these conditions is 0.9.

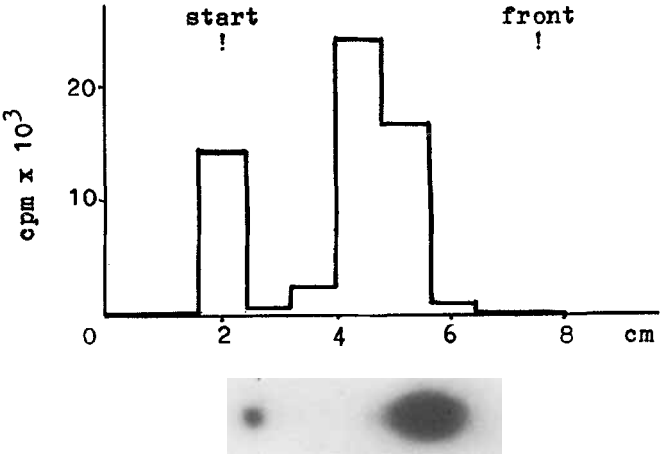

Fig.1 Chromatogram of a reaction mixture after iodination of human chorionic gonadotropin by $Na^{125}I$

In a control experiment it was shown that when $Na^{125}I$ is applied to the chromatogram 5% of the activity is adsorbed to the paper. The results for the determination of inorganic iodine are therefore corrected for this effect.

The losses of the ^{125}I activity in the course of analysis can be neglected because they do not exceed 0.3% as found by comparing the activity applied to the starting point with the total activity of the developed chromatogram.

It has been observed that the presence of carrier protein in the reaction mixture is essential to achieve optimum precision therefore reaction mixtures should always be diluted with a suitable protein solution, for example phosphate buffer containing albumin, prior to analysis.

The reproducibility of the method is illustrated by the data shown in Table 1. The mean values were in agreement, within the limits of the experimental error, with the data obtained by paper electrophoresis in a routine quality control procedure /7/.

Table 1 Results of the determinations of inorganic iodine in a single sample

A - human chorionic gonadotropin iodination mixture

B - human chorionic gonadotropin labelled preparation

Sample	Number of determinations	Mean /%/	Standard deviation /%/	Coefficient of variation /%/
A	18	67.38	0.74	1.10
B	20	8.47	0.38	4.49

/%/ In percent of total ^{125}I activity

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