PREPARATION OF SOME MERCURI-DERIVATIVES OF IODINATED FLUORESCEIN LABELLED WITH IODINE-^{131}I.

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SUMMARY

Mercurisation reaction of the iodinated fluorescein-^{131}I has been studied. Individual products formed in the reaction mixture were isolated with both paper chromatography and chromatography on alumina column. It was found that in contrast to unsubstituted fluorescein the yield of mercuri-derivatives is considerably lower in case of iodinated fluorescein-^{131}I.

INTRODUCTION

In our previous papers /1 - 3/ we studied the preparation of mercuri-derivatives of fluorescein labelled with ^{203}Hg or ^{197}Hg by substitution reactions, exchange reactions and recoil-labelling. These substances were used for study of accumulation of substances in injured tissues. The accumulation of radioactivity of derivatives of fluorescein partially mercurized in injured tissues proved to be so high that it was possible to work out a method for detection of heart-attack in vivo (4). Further experiments have shown, how-

ever, that clinical use of these methods has some drawbacks. The most serious argument against the routine usage of these substances may be a relatively large hold-back of activity in kidneys, which is accompanied with a rather high radiation dose absorbed in the body.

Therefore, we tried to modify the original molecule of mercurifluorescein by additional substitution with ^{131}I. When introducing this substance into the organism one can expect either increased excretion by liver (in which way iodinated derivatives of fluorescein are mostly excreted), or degradation of the molecule in kidneys, which results in increasing excretion of ^{131}I both by liver and kidneys. Biological behaviour of these substances is the object of another our study, the results of which will be published elsewhere.

Experimental

Chemicals:

Fluorescein (reagent grade, Lachema, Prague) was purified as a sodium salt on an alumina column. Distilled water was used as elution agent. Only first coloured fraction was collected.

Iodofluorescein-^{131}I and di-iodofluorescein-^{131}I were prepared as follows: To a carrier-free solution of sodium iodide-^{131}I the solution of inactive sodium iodide was added, acidified with dilute hydrochloric acid and elemental iodine was afterwards extracted into chloroform after releasing by addition of hydrogen peroxide. After separation of aqueous phase from the chloroform layer, aequimolar solution of fluoresceine (10 mg/ml) was added to the latter. The mixture was shaken for 1 hour. The aqueous phase containing unreacted fluoresceine and its iodinated derivatives

was separated and evaporated to dryness at 100°C in vacuo. The residue was dissolved in a solution of 5% acetic acid in chloroform and this solution was poured onto a column of silica gel G which was prepared by mixing dry silica gel in the same solution as mentioned above (5% acetic acid in chloroform). To separate 50 mg of the residue resulting from the crude reaction mixture, a column of 3 cm in diameter and 15 cm high was used. The solution of 5% acetic acid in chloroform was used as the elution agent. The first fraction was tri-iodofluorescein, the second and the third ones were di-iodo- and iodofluorescein, respectively. The purity of iodo- and di-iodofluorescein-^{131}I was checked by thin-layer chromatography on silica gel G. The chromatograms were developed by a 3% solution of acetic acid in chloroform (5). The inactive iodofluorescein used for preparation of mercurized iodofluorescein-^{203}Hg was prepared in the same way as labelled iodofluorescein-^{131}I. Silica gel G used for column and thin-layer chromatography was a Mercks product, similarly as mercuric oxide, from which mercuric acetate was prepared. Aluminium oxide (according to Brockman) used for column chromatography was a product of Reanal (Budapest, Hungary).

Determination of mercury in the products:

Mercuric acetate-^{203}Hg of known specific activity was used for preparation of reaction mixture. The latter was applied on the column of aluminium oxide (Reanal) and individual fractions were eluted by 0.2M solution of potassium bicarbonate. The content of the product in the eluate was determined gravimetrically after precipitation by diluted acetic acid. The activity of the products was measured under exactly the same conditions as the activity

of the stock solution of mercuric acetate-^{203}Hg. The content of mercury in individual fractions was calculated from the ratio of specific activity of the former to that of the latter.

The course of mercurization reaction:

The aqueous solution of chromatographically pure sodium salt of iodo- and di-iodofluorescein-^{131}I (the concentration was 10 mg/ml) was mixed with the solution of mercuric acetate (the concentration 10 mg/ml) which resulted in formation of a dark-red precipitate. The reaction mixture was then heated at 100°C under reflux for 1 hour. First sample for chromatographic separation was removed immediately after mixing the reaction mixture at room temperature. Then, in short intervals, individual samples were removed from boiling mixture and after dissolution of the precipitate by addition of 0.1 M solution of sodium hydroxide and applied to chromatographic paper.

Separation of the products of mercurization reaction:

Paper chromatography:

The separation was carried out on chromatographic paper Whatman No 3 in the system 6% ammonia-methanol (1 : 1).

Column chromatography:

The separation of products can be carried out preparatively on the alumina column with 0.2 M solution of potassium bicarbonate as elution agent. The first eluted fraction is pure iodofluorescein-^{131}I and the second one is hydroxymercuriiodofluorescein-^{131}I. Bis(hydroxymercuri)iodofluorescein-^{131}I, which remains on the column can be eluted by 0.1 M potassium hydroxide. Mercurization products of di-iodofluorescein-^{131}I can be separated in the same way.

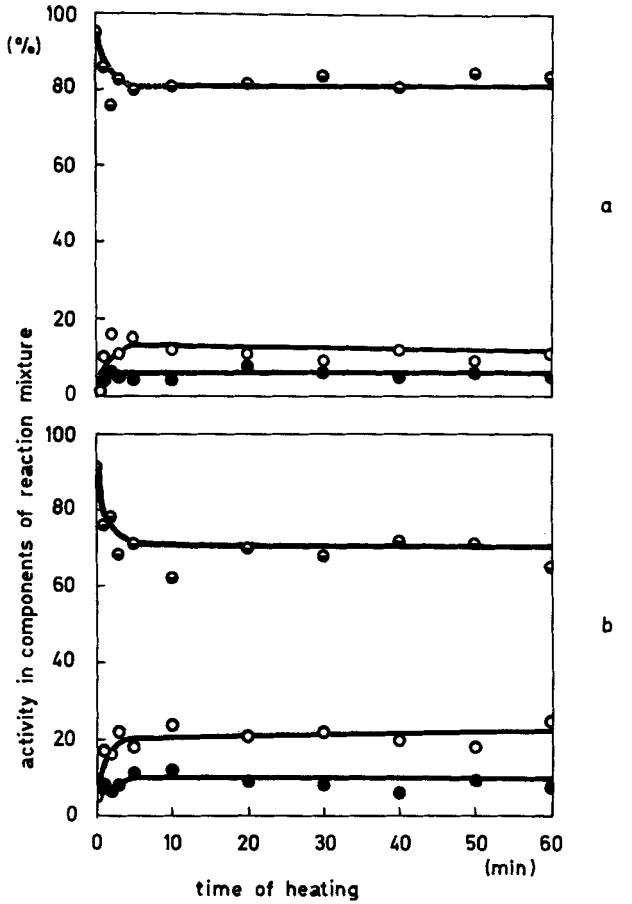

Fig. 1. Yields of individual products of mercurization of iodofluorescein-^{131}I as a function of heating time . The molar ratio of iodofluorescein-^{131}I to mercury:
a) 3: b) 2:1

◒ - J F ○ - J F Hg ● - J F Hg_2

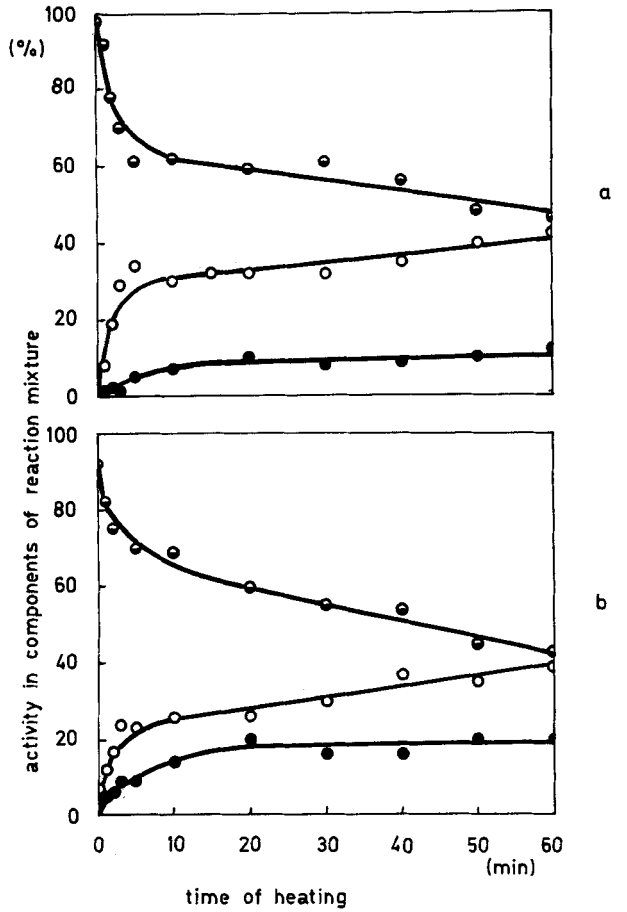

Fig. 2. Yields of individual products of mercurization of iodofluorescein-^{131}I as a function of heating time. The molar ratio of iodofluorescein-^{131}I to mercury:
a) 1:1 b) 1:2

◒ - J F ○ - J F Hg ● - J F Hg_2

Activity measurements:

Radioactivity of chromatograms was measured by means of a GM-tube and a Frieseke-Hoepfner scaler.

Results and discussion

A number of derivatives is formed in the reaction mixture during mercurization of fluorescein (1 - 3). It was therefore possible to anticipate the presence of several products also in the reaction mixture during mercurization of iodinated fluorescein. Preliminary chromatographic experiments showed that on chromatograms of the reaction mixture of iodofluorescein-^{131}I and mercuric acetate, inactive spots of mercuriderivatives of fluorescein as well as active spots of mercurized derivatives of iodo- and di-iodofluorescein-^{131}I can be found. If chromatographically pure iodofluorescein-^{131}I is used, only two active spots of mercurized derivatives of iodofluorescein-^{131}I are apparent. The respective R_F values are given in the Table 1. The analysis has shown that the product with the R_F = 0.04 corresponds to bis(hydroxymercuri)iodofluorescein ($IFHg_2$). The other spot with R_F = 0.42 corresponds to hydroxymercuriiodofluorescein (IFHg). The mercurization reaction was studied in some more detail using reaction mixtures with varying molar ratios of iodofluorescein-^{131}I to mercury. The results of this study are shown in Figs. 1 and 2. In the presence of excess of iodofluorescein-^{131}I in the reaction mixture, the overall amount of the products is small as can be seen from Fig. 1 a,b while bis(hydroxymercuri)-fluorescein is present only in minute quantities. At aequimolar ratio or at excess of mercury, larger amounts of the products are found. All four Figures show that mercurization reaction takes

place mostly in first 20 minutes of heating and afterwards the distribution of products practically does not vary.

In contrast to unsubstituted fluorescein (2) the yield of mercuriderivatives is considerably lower in case of iodofluorescein-^{131}I. Even at excess of mercury (Fig. 2 b) the reaction reaches an equilibrium between the components without the reaction of iodofluorescein-^{131}I being complete.

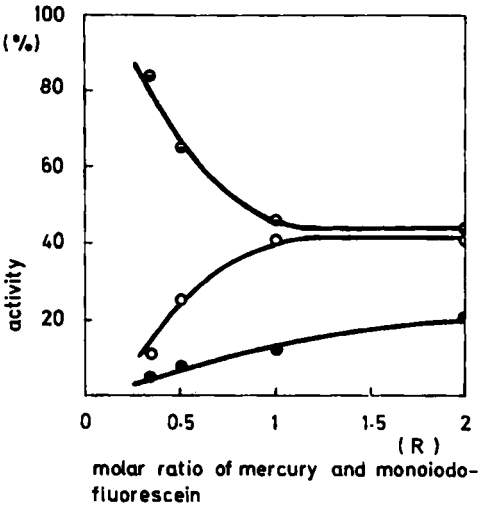

Fig. 3. Yields of individual products of mercurization of iodofluorescein-^{131}I after 1 hour heating as a function of molar ratio of iodofluorescein-^{131}I to mercury.

◒ - J F ○ - J F Hg ● - J F Hg_2

So one can see that additional substitution of iodofluorescein is much more difficult than mere iodination or mercurization of fluorescein. The plot of the yield of mercurization products of iodofluorescein-^{131}I against the molar ratio of reactants for 1 hour's heating is shown in Fig.3. Here one can see that the largest quantities of products can be obtained in the presence of an excess of mercury. However, the disadvantage is that both hydroxymercuri- and bis(hydroxymercuri)iodofluorescein-^{131}I are formed under these conditions. Additional chromatographic separation is then necessary to produce these compounds pure.

Substitution of di-iodofluorescein-^{131}I (I_2F) with mercury is even more difficult than in the case of iodofluorescein-^{131}I as is shown in Fig. 4 a. Here is shown the formation of hydroxymercuri-di-iodofluorescein-^{131}I (I_2FHg_2) at the molar ratio of 1 : 1 of di-iodofluorescein to mercuric acetate as a function of heating time. In the presence of excess of mercuric acetate (see Fig. 4 b) larger quantities of hydroxymercuri-iodofluorescein and partly also bis(hydroxymercuri)di-iodofluorescein-^{131}I are produced (I_2FHg_2). Besides, probably a part of iodine-^{131}I is eliminated from di-iodofluorescein-^{131}I while hydroxymercuri-iodofluorescein and mercuric iodide-^{131}I are formed. The former can be found on chromatograms as separate fraction, while mercuric iodide-^{131}I remaining at the start contributes probably to the activity of bis(hydroxymercuri)di-iodofluorescein-^{131}I. The respective R_F values are shown in Tab.2.

Similarly as in case of iodofluorescein-^{131}I also for preparation of pure mercurized derivatives of di-iodofluorescein additional chromatographic separation is necessary.

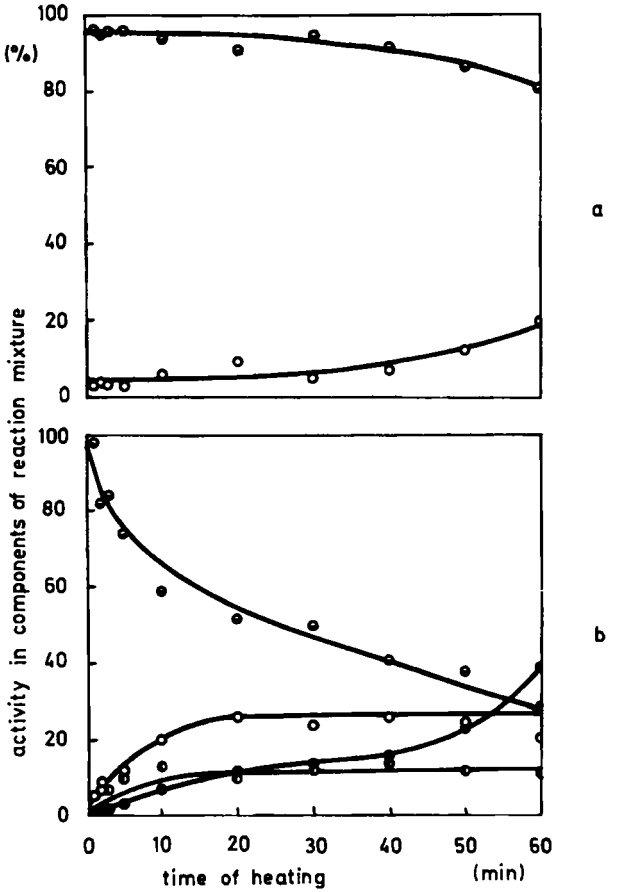

Fig. 4. Yields of individual products of mercurization of di-iodofluorescein-^{131}I as a function of time. The molar ratio of di-iodofluorescein-^{131}I to mercury a) 1:1 b) 1:2

◒ - J_2 F ○ - J_2 F Hg ● - J_2 F Hg_2 ◐ - J F Hg

Table 1

R_F-values of products in the reaction mixture of iodofluorescein-^{131}I and mercuric acetate (6% ammonia-methanol, 1 : 1).

Product	J F Hg_2	J F Hg	J F
R_F	0.04	0.42	0.64

Table 2

R_F-values of products in the reaction mixture of di-iodofluorescein-^{131}I and mercuric acetate (6% ammonia-methanol, 1 : 1).

Product	J_2 F Hg_2	J_2 F Hg	J_2 F
R_F	0.07	0.31	0.58

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