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ON THE FORMATION OF $^{125}IO_3^-$ IONS IN THE CHLORAMINE-T LABELING MIXTURE

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The formation of $^{125}IO_3^-$ ions in the course of labeling proteins with ^{125}I by the use of the Chloramine-T method has been investigated. It was demonstrated that one of the components of the labeling reaction mixture is $^{125}IO_3^-$. The identification of radioiodate ions was performed by the use of paper electrophoresis.

INTRODUCTION

Proteins or peptides labeled with ^{125}I are frequently used as tracers in radioimmunoassay. The introduction of the radioiodide atom into the molecule to be labeled can be performed by several methods, e.g. iodine monochloride[1], Chloramine-T[2], Iodo-Gen[3], etc. The Chloramine-T method was used in the experiments presented here. The mechanism of the labeling procedure is as follows:

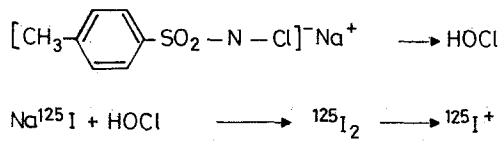

Fig. 1.

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As can be seen from Fig. 1, Chloramine-T oxidizes the $^{125}I^-$ ions to electrophilic species, e.g. I^+, H_2OI^+, etc., which can be incorporated into molecules with phenolic hydroxy group via aromatic electrophilic substitution. The labeled molecules can be separated from free radioiodide, from the labeled by-products and the reagents used in the Chloramine-T method by adsorption[4] or gel filtration chromatography[5]. Either the first or the second method is used for the separation, an elution peak near that of the free radioiodide often appears[6]. It was anticipated that this peak can be attributed to $^{125}IO_3^-$ formed in the course of the labeling procedure.

EXPERIMENTAL

TSH (Calbiochem, iodination grade) and human IgG (Sigma) were labeled with ^{125}I by the use of Chloramine-T method. To 20-40 nmol of TSH and IgG dissolved in 100 l of phosphate buffer (pH=7.4), a slightly alkaline solution of 1-2 mCi (37-74 MBq) carrier-free $^{125}I^-$ was added followed by the addition of 25-50 l of an aqueous solution containing 100-200 g of Chloramine-T. The labeling reaction was quenched with 50 l of an aqueous solution containing 350 g of sodium metabisufite. The components of the labeling reaction mixture were separated on a Sephadex G-75 column. The Sephadex G-75 was swollen in phosphate buffer (pH=7.4) prior to being packed in the column (length 500 mm, diameter 10 mm). The sample (0.1-0.5 ml) from the labeling reaction mixture was placed on top of the column and was allowed to soak in it. The elution was performed with 0.05M phosphate buffer (pH=7.4). The effluent was passed over a

NaI(TI) scintillation crystal and the count rate was monitored by a ratemeter and registered by an X-Y plotter.

The eluent was delivered by a peristaltic pump (flow rate 22-44 ml h^{-1}).

The identification of free radioiodide was performed by paper electrophoresis (Whatman 1 chromatographic paper, 0.01M borate buffer (pH=9.1), 30 v cm^{-1} voltage gradient, 60 min).

So as to make the formation of radioiodate unambiguous, blank labeling was also carried out when the protein was omitted from the labeling reaction mixture, i.e. the Chloramine-T reacted with $^{125}I^-$ only, under the same conditions as in the case of the labeling TSH or IgG with ^{125}I.

RESULTS

The elution curves obtained when the reaction mixture of the TSH and IgG labeling was chromatographed using Sephadex G-75 column are shown in Figs 2-3.

As can be seen from these figures the ^{125}I-labeled TSH and IgG were eluted first followed by an elution peak which could be attributed to free radioiodide. The paper electrophoretic analysis of the second elution peak has revealed that it consists of two components, viz. $^{125}I^-$ and $^{125}IO_3^-$. The paper electrophoretic pattern of the second elution peak from the gel filtration of the TSH labeling mixture on Sephadex G-75 column is shown in Fig. 4. The first peak has been attributed to $^{125}IO_3^-$, while the second one to $^{125}I^-$. The identification of radioiodide and radioiodate was performed using authentic.

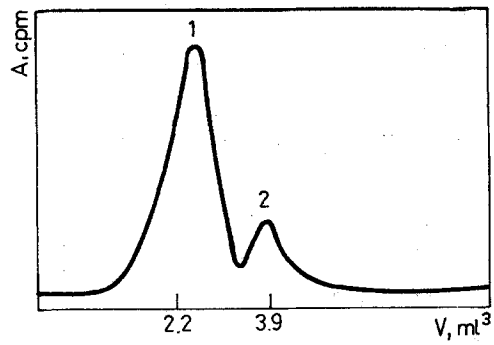

Fig. 2. Elution curve of the TSH labeling reaction mixture. (Sephadex G-75 column, eluent: 0.05M phosphate buffer pH=7.4, flow-rate 22-44 ml h^{-1})

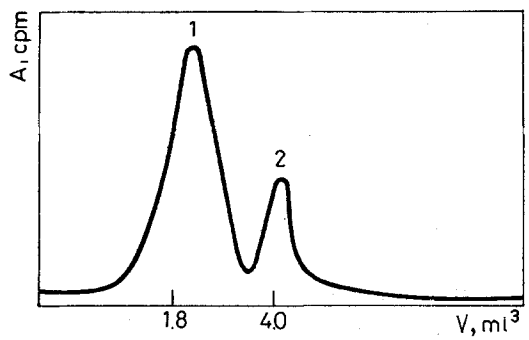

Fig. 3. Elution curve of the IgG labeling reaction mixture. (Sephadex G-75 column, eluent: 0.05M phosphate buffer pH=7.4, flow-rate 22-44 ml h^{-1})

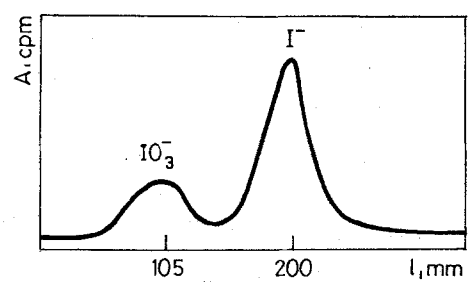

Fig. 4. Paper electrophoretic pattern of the second elution peak from the TSH labeling reaction mixture. (Whatman 1 chromatographic paper, 0.01M borate buffer (pH=9.1), 30 V cm^{-1} voltage gradient, time 60 min)

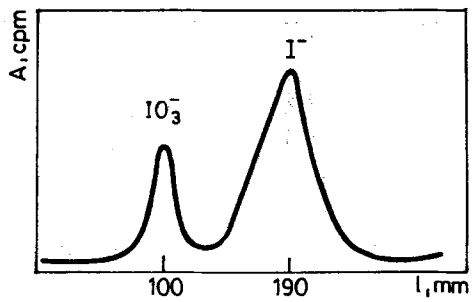

Fig. 5. Paper electrophoretic pattern of the second elution peak from the IgG labeling reaction mixture. (Whatman 1 chromatographic paper, 0.01M borate buffer (pH=9.1), 30 V cm^{-1} voltage gradient, time 60 min)

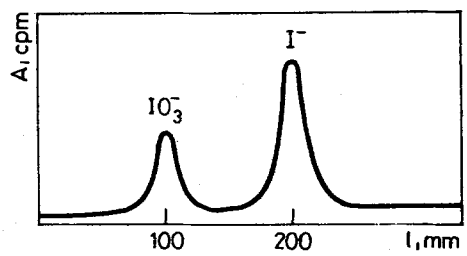

Fig. 6. Paper electrophoretic pattern of the "blank" labeling reaction mixture. (Whatman 1 chromatographic paper, 0.01M borate buffer (pH=9.1), 30 V cm^{-1} voltage gradient, time 60 min)

$^{125}IO_3^-$ and $^{125}I^-$. Similar results were obtained when the second elution peak from gel filtration chromatographic separation of the human IgG was analyzed by paper electrophoresis. The activity distribution is shown in Fig. 5. As in the former case the first peak has been attributed to $^{125}IO_3^-$ and the second one to $^{125}I^-$. As in the case of TSH and IgG labeling, both $^{125}IO_3^-$ and $^{125}I^-$ appeared in the labeling mixture in which no protein was present. The paper electrophorogram of the "blank" labeling mixture is shown in Fig. 6.

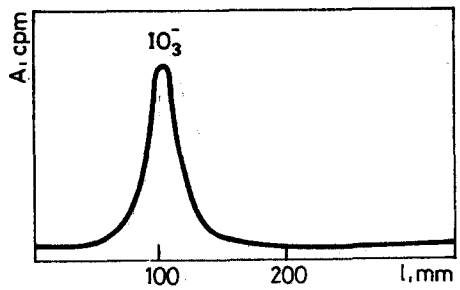

Fig. 7. Paper electrophoretic pattern of the "blank" labeling reaction mixture at reaction times longer than 3-5 min. (Whatman 1 chromatographic paper, 0.01M borate buffer (pH=9.1), 30 V cm^{-1} voltage gradient, time 60 min)

The radioiodide/radioiodate ratio depends on the reaction time. In the case of "blank" labeling the radioiodide has been converted quantitatively to radioiodate at reaction times longer than 3-5 min (Fig. 7).

SUMMARY

It has been demonstrated that in the course of the Chloramine-T labeling procedure besides the radioiodide-labeled proteins $^{125}IO_3^-$ is also formed.

REFERENCES

1. E. Regoeczi, Iodine-labeled Plasma Proteins (1984) Vol. 1, Chapter 3.

2. A.S. McFarlane, Nature 182 (1958) 53.

3. P.J. Farker, J.C. Speck, Biochem. Biophys. Res. Commun., 80 (1978) 849.

4. G. Toth, J. Chrom., 267 (1983) 420.

5. R. Hall, Clinical Endrocrinology, 1 (1973) 115.

6. A. Taurog, Endrocrinology, 69 (1961) 126.