Studies on the Labeling of Streptokinase with ^{99m}Tc for Use as a Radiopharmaceutical in the Detection of Deep-Vein Thrombosis: Concise Communication

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Streptokinase was labeled with ^{99m}Tc using both stannous chloride and stannous pyrophosphate as reducing agents. Sixty to seventy-five percent of the ^{99m}Tc was incorporated into streptokinase using stannous chloride as a reducing agent at pH 1–2, whereas 50–60% was incorporated using stannous pyrophosphate at neutral pH. Increasing the pH from 2 to 7 in the presence of stannous chloride caused the release of 15–20% of the protein-bound ^{99m}Tc. Incorporation of ^{99m}Tc into protein was relatively slow: labeling required 2–3 hr at room temperature. The concentration of stannous pyrophosphate required for optimum labeling varied between 10^{-5} and 10^{-2} M. Polyacrylamide-gel electrophoresis showed that the filler substance in commercial streptokinase was also labeled with ^{99m}Tc. However pure streptokinase gave a homogenous protein band after polyacrylamide-gel electrophoresis. This protein band coincided with the peak of streptokinase-bound ^{99m}Tc. The results obtained may partially explain why ^{99m}Tc-labeled streptokinase lacks the necessary specificity for the satisfactory location of blood clots in vivo.

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A number of reports have described the radioactive labeling of streptokinase for use as a radiopharmaceutical in the detection of blood clots (*1–3*). Although streptokinase has a strong affinity for thrombi, the radiolabeled enzyme has not been as successful in the location of clots (*3,4*) as might be expected from theoretical considerations. This relative lack of success may in part result from unsatisfactory labeling of the enzyme.

We herein describe the effects of varying a number of parameters on the ^{99m}Tc-labeling of streptokinase. The results show that some of the procedures previously reported may not have achieved optimum and specific labeling of the enzyme.

METHODS AND MATERIALS

Technetium-99m was obtained from a ^{99}Mo generator,* and greater than 99% of the technetium eluted was present as pertechnetate ion. Streptokinase,† stannous chloride, and stannous pyrophosphate‡ were procured from commercial firms.

Labeling of streptokinase with ^{99m}Tc. The standard reaction mixture contained the following: streptokinase 25,000–75,000 I.U.; stannous pyrophosphate 1 m*M* in 0.9% NaCl, or 1 ml of stannous chloride 1 m*M* in 0.1 *N* HCl; and ^{99m}Tc. The stannous chloride was dissolved in 0.1 *N* HCl immediately before use. Labeling of the streptokinase was carried out for 2 hr at room temperature.

Separation of free from bound ^{99m}Tc by thin-layer chromatography. At the end of the labeling reaction 10 μl of the reaction mixture was applied to Sepra-chrom™ thin-layer plates. These were dried and developed initially using acetone as solvent. After chromatography the acetone was evaporated and the plates re-run using 0.9% NaCl as solvent.

Separation of free from bound ^{99m}Tc by gel filtration. Gel-column chromatography was carried out using a 0.9 × 30-cm column packed with Sephadex G25. The eluting solvent was 0.9% NaCl with a flow rate of 1 ml/45 sec. Fractions of 1 ml were collected and analysed for radioactivity.

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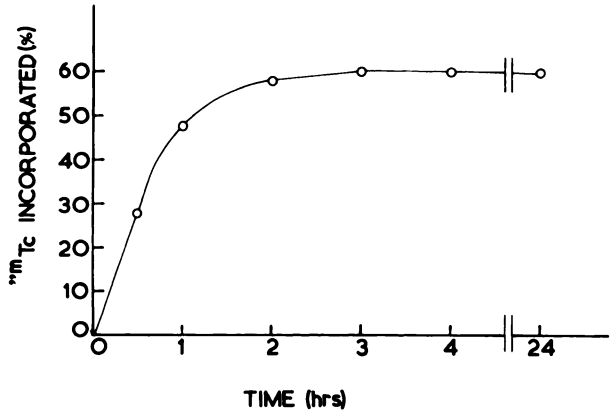

FIG. 1. Time course of ^{99m}Tc incorporation into streptokinase. Reaction mixture contained streptokinase 50,000 I.U., stannous pyrophosphate 1 mM, and ^{99m}Tc. Values are means of three separate experiments each carried out in duplicate.

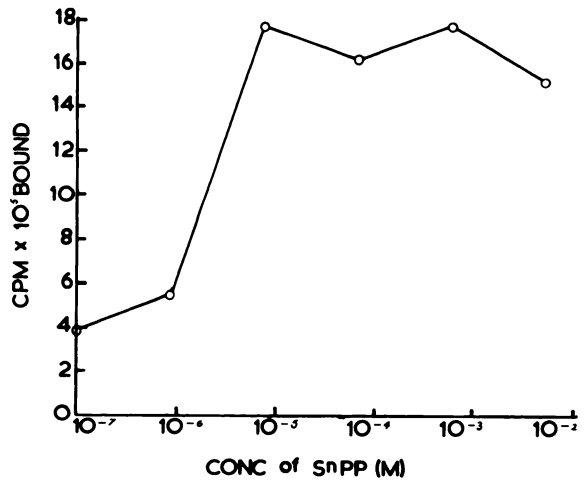

FIG. 2. Effect of varying concentration of stannous pyrophosphate (Sn PP) on ^{99m}Tc incorporation into streptokinase. Values are means of three separate experiments each carried out in duplicate.

Polyacrylamide-gel electrophoresis. Separation gels and stacking gels were prepared as previously described (*5*). Polymerization was accomplished chemically by the addition of 0.25% ammonium persulfate and TEMED. The electrode buffer contained 10 m*M* Tris with 77 mM glycine (pH 8.3 at 25°C) and the gel buffer 0.38 *M* Tris with 0.06 *N* HCl (pH 8.9 at 25°C). Separation was carried out at 2 mA per gel tube.

RESULTS

Assessment of labeling efficiency. Using both thin-layer chromatography and gel filtration we found that between 60 and 75% of the ^{99m}Tc was incorporated into streptokinase (streptase) in the presence of 1 m*M* stannous chloride, and between 50 and 60% using an identical concentration of stannous pyrophosphate. Labeling of streptokinase using stannous chloride was carried out at pH 1–2. If sufficient serum or Tris-HCl buffer was added to bring the pH of the incubation medium up to 7.0, some 15–20% of the protein-bound label was released. Thus, the end result of labeling at pH 1–2 using stannous chloride was similar to the efficiency of labeling at neutral pH with stannous pyrophosphate.

Time course of ^{99m}Tc incorporation into streptokinase. Figure 1 shows the effect of varying time on the incorporation of ^{99m}Tc into streptokinase using stannous pyrophosphate as reduction agent. Maximum incorporation required 2–3 hr at room temperature, and 24 hr produced no significant gain.

Effect of varying concentration of added stannous pyrophosphate. Figure 2 shows the effect of varying the concentration of stannous pyrophosphate on ^{99m}Tc incorporation into streptokinase. Increasing the concentration of stannous ions from 10^{-6} *M* to 10^{-5} *M* caused a sharp increase in the amount of ^{99m}Tc bound to streptokinase. But continued increase from 10^{-5} to 10^{-2} *M* had no significant effect on the percentage tracer incorporated.

Labeling of pure and commercially available streptokinase. Polyacrylamide-gel electrophoresis of commercially available streptokinase followed by protein staining gave a pattern shown in Fig. 3. The lack of discrete protein bands was due to the heterogeneity of the partially degraded gelatin used as a filler in the streptase tablet. The distribution of radioactivity in general correspond to the protein-staining area in the gel. However, in addition to the steptokinase the gelatin protein was also labeled with

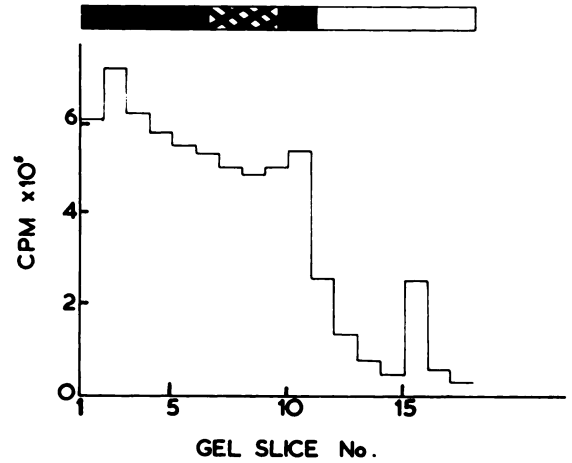

FIG. 3. Separation of proteins in streptase by polyacrylamide-gel electrophoresis. Procedure as described in section on methods and materials. Top part of diagram shows diagrammatic representation of gel as stained for protein. Lower part shows distribution of ^{99m}Tc in different sections of gel.

^{99m}Tc. In contrast, purified streptokinase gave a single protein band coinciding with the peak of radioactivity (Fig. 4).

DISCUSSION

Previous reports using thin-layer chromatography in one solvent system have shown that up to 99% of ^{99m}Tc is incorporated into protein when stannous chloride is used as reducing agent (*6*). However, the results in the present investigation, using both thin-layer chromatography in two solvent systems and gel filtration, show that the percentage of ^{99m}Tc incorporated in the presence of stannous chloride is only between 60 and 75%. Our results are in close agreement with recent findings of Persson and Kempi (*8*) who also used stannous chloride as reducing agent and gel-chromatography column scanning to monitor the efficiency of ^{99m}Tc labeling. However, in vitro methods of determining the amount of tracer incorporated into streptokinase may not be very meaningful, especially if the labeling is carried out at pH 1–2. As shown in the present study, restoration of pH to neutrality releases significant amounts of protein-bound ^{99m}Tc. A similar release of ^{99m}Tc may occur upon injection of the radiolabeled streptokinase into patients, thus giving rise to high background radioactivity in the blood stream.

Our results also show that the labeling of streptokinase is a relatively slow process. Most reports in the literature do not accurately describe the time allowed for the labeling procedure, and when incubation times are discussed they tend to be of relatively short duration—e.g., Dugan et al. use 5 min to label streptokinase (*1*). Our results suggest that an incubation time of at least 1 hr at room temperature is required for near-optimum labeling.

The labeling of the gelatin filler with ^{99m}Tc obtained in the present investigation may explain why radiolabeled streptokinase is not as satisfactory in locating blood clots as might be expected. If the ^{99m}Tc-labeled gelatin were greatly in excess of the ^{99m}Tc-labeled streptokinase, this would contribute to a high background radioactivity in blood and thus impair the effectiveness of the labeled streptokinase in locating clots.

Our results apply specifically to the labeling of streptokinase (streptase:Hoechst). However, the findings may be relevant to the labeling of other compounds with ^{99m}Tc, for instance, (A) filler substances may also be labeled in other impure preparations of pharmaceuticals, and (B) increasing the pH of labeling reaction mixtures to neutrality may cause release of ^{99m}Tc previously bound to proteins at pH 1–2.

In conclusion, if streptokinase is to be successfully used for locating blood clots it is essential to carry out the radioactive labeling at a pH as near to neutrality as possible, to remove excess free ^{99m}Tc by gel filtration, and—most important—to use pure streptokinase.

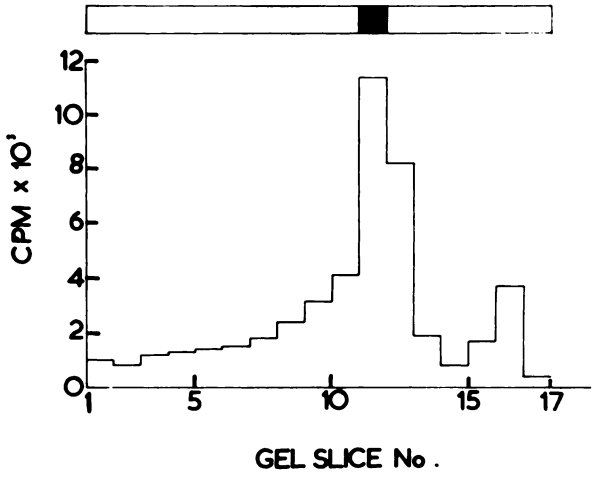

FIG. 4. Polyacrylamide-gel electrophoresis of purified streptokinase. Top part of diagram gives diagramatic representation of gel stained for protein. Lower part shows distribution of ^{99m}Tc in different sections of gel.

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FOOTNOTES

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† Hoechst Ltd., Dublin and Hoechst AG, Frankfurt, Germany.

‡ Mallinckrodt, Inc., St. Louis, Mo.

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