Site of Tc-99m Binding to the Red Blood Cell: Concise Communication

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The increasing interest in the use of Tc-99m-labeled red blood cells encourages exploration into the site and mechanism of binding of Tc-99m to the RBC components. The components isolated from the labeled red cells were the lipid and protein of the membrane, heme, and the α and β chains of the globin. The binding occurs mainly to the protein moiety, and preferentially to the β chain of the globin, where the specific activity was found to be 28 $\pm$ 6 times that of the α chain. The mechanism of binding is discussed. The role of heme in the binding is not clear.

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There has been increasing interest in the use of Tc-99m-labeled red blood cells during the last decade, particularly with the advent of in vivo labeling techniques (*1–4*). However, the precise distribution of Tc-99m among the red blood cell (RBC) components is not known. Only one available report, based on studies on a single sample, attributes the preferential site as β chain of hemoglobin (*5*). The extent of binding to the membrane, and the relative preponderance of β-chain to α-chain binding is still to be worked out. In an attempt to explain the mechanism of binding we quantitated the extent of Tc-99m binding to the membrane (protein and lipids), to heme, and to globin (α and β chains) of normal RBC hemoglobin. Understanding the quantitative aspects of distribution in normal Hb may have implications in studying abnormal Hb (*6*).

MATERIALS AND METHODS

Ten milliliters of blood, using heparin as an anticoagulant, were drawn from the antecubital vein of six subjects with normal adult hemoglobin (HbA). The red blood cells, separated from the lower layer after centrifugation for 10 min at 3000 rpm, were labeled with Tc-99m using 0.1 mg of freshly prepared and filtered stannous chloride solution, added 15 min after the pertechnetate (*7*). Labeling efficiency in our study was of the order of 76–88%. The cells were washed three to four times with 10 ml normal saline each time to remove the detachable Tc-99m and also leukocytes and platelets. The activity removed during this process ranged between 15–23%. The radioactivity in the suspension of washed cells with irreversibly bound Tc-99m was taken as 100%, and these cells were used for the following studies.

Separation of hemolysate. The cells were lysed with 0.83% NH_4Cl Tris buffer or with a mixture of 2 volumes water and 0.5 volume toluene. The membranous debris was separated by filtration through Whatman No. 40 filter paper and washing with distilled water, yielding the hemolysate. The washings were continued until two consecutive washings of 2 ml each yielded negligible radioactivity. Filtration was found to be more effective than centrifugation in separating small pieces of membrane and getting hemolysate-free membranes. The radioactivity in the membrane and the hemolysate was measured in a well scintillation counter in constant geometry. The lipids and proteins in the membrane were extracted using ethanol:ether (3:1) (*8*) and the radioactivity in each fraction assayed.

Separation of heme and globin (*5,9*). The hemolysate was treated with 20 volumes excess of 2% concentrated HCl in acetone and the globin precipitate repeatedly washed with cold acetone to remove any trace of acid. The globin precipitate was separated by repeated centrifugation at 5000 rpm for 10 min, and was then washed with ether. The radioactivity in the globin and heme fractions was assayed by counting suitable aliquots.

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Separation of α and β chains of globin (*9*). The starting buffer for chromatography consisted of 0.05 *M* urea in 2-mercaptoethanol and 0.005 *M* in Na_2HPO_4 and adjusted to pH 6.7 with H_3PO_4.

The Tc-99m globin pellet was dissolved in the above buffer solution to a concentration of 4–10 mg/ml and purified by Sephadex G-25 column (10 × 150 mm) using the same buffer. The purified Tc-99m globin was applied to an equilibrated CM-cellulose column (8 × 130 mm) freshly prepared in the above buffer. The globin chains were then eluted at a flow rate of 1 ml/min by means of a linear Na^+-ion gradient formed by mixing 100 ml of the starting buffer with 100 ml 0.05 *M* urea in 2-mercaptoethanol, 0.03 *M* in Na_2HPO_4, and adjusted to pH 6.7 with H_3PO_4. Three-milliliter fractions were collected. The Tc-99m activity profile of the eluted fractions was obtained by gamma counting, and the optical-density profile for the protein peaks by uv spectrophotometry at 280 nm. The protein concentrations in the α and β bands were determined by Lowry's method (*10*). The specific activities in the α and β chains were then calculated.

The calculations of the distribution of radioactivity were based on the sum of individual parts so as to find relative distribution in two components. The calculation method was conservative for the derivation of activity in the heme, globin, membrane protein, and lipids. The calculations for radioactivity in the chromatography fractions were based on ratio of count rate to the protein content rather than the activity in the applied volume, thus obviating the need to account for activity lost in the process of elution or trapped in the column.

RESULTS

The distribution of Tc-99m in various RBC components, obtained from six experiments, is given in Fig. 1. As can be seen, most of the activity is bound to the intracellular components, with membrane binding accounting for merely 5.1 ± 3.8% of the total activity bound irreversibly to the RBC. Within the membrane, Tc-99m activity is divided between protein and lipid fractions in the ratio 12:1. The loss of radioactivity during the separation process was insignificant, being within the statistical variation of count rate. This is known because all activity in washings was accounted for.

Figure 2 shows the profile of Tc-99m activity and the optical density in fractions, eluted from the CM-cellulose column, indicating α- and β-chain peaks. Most of the Tc-99m activity appears confined to the β chain. The specific activity in β chain is 28 ± 6 times that in the α chain.

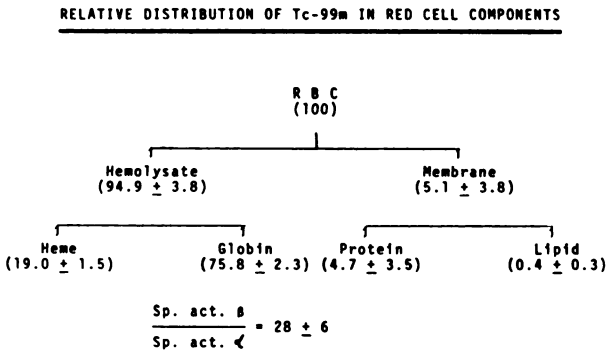

FIG. 1. Relative distribution of Tc-99m in red-cell components.

DISCUSSION

The distribution pattern of the Tc-99m in the red cell components (Fig. 1) indicates that the major part is bound to the globin, with heme and membrane binding contributing much less. The relative binding with the membrane lipids is negligible (0.4 ± 0.3%). The preferential site of binding in the polypeptide chain of the globin is the β chain, where the specific activity of Tc-99m was found to be 28 ± 6 times that in the α chain. This is somewhat similar to the factor of 30 reported for Cr-51 (*11*) indicating similarity in binding sites. The specific-activity profile in the β chain has a pattern like that of the count-rate profile (Fig. 2), with a peak at an elution volume of 21 ml. Thus the labeling in the chain is heterogenous. These observations on six subjects with normal $\alpha_2\beta_2$ hemoglobin may be helpful in further investigations on the mode of technetium-99m binding (or lack thereof) in individuals with abnormal hemoglobins—particularly those involving the β chain.

The process of pertechnetate binding to the RBC essentially involves passive diffusion of pertechnetate into the cell. There is no mechanism inside the cell to reduce pertechnetate, since intracellular $^{99m}TcO_4^-$, in the absence of a reducing agent, readily diffuses out when the RBC is suspended in normal saline, because of the concentration gradient (*13*). The role of stannous ion in the

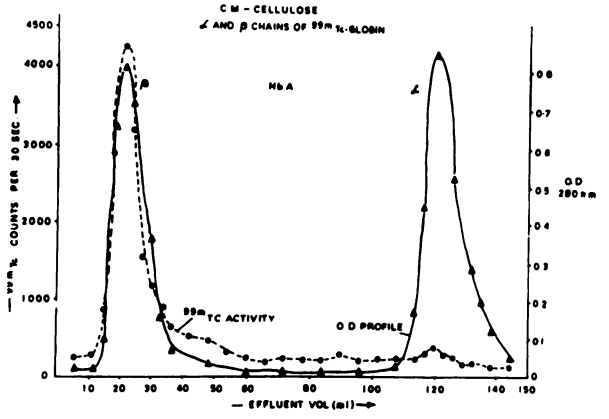

FIG. 2. Separation of α and β chains of globin by CM-cellulose column chromatography. Profiles are shown for Tc-99m activity and optical density.

intracellular reduction of pertechnetate, which results in binding the Tc-99m to the hemoglobin, has been well documented (*5,7,14*). Both pretinning and posttinning have been used with success, with theory favoring pretinning (*15*), but experimental evidence not upholding it. The formation of stannous oxide from stannous compounds has been implicated for larger amounts of technetium bound to the cell membrane (*16*). For this reason, freshly prepared and filtered stannous chloride, or a lyophilized source of tin, favors a better yield of intracellular label. Although a lower concentration of stannous ion has recently been shown to give satisfactory yields, the addition of excessive stannous ion does not affect the yield (*17*) and is unlikely to alter the binding site.

The studies of reaction kinetics with chromate binding to Hb, using first-order kinetics and evaluation of the overall equilibration constant, have shown that the heme group may participate in a rate-limiting step in the reduction of CrO_4^{2-} to Cr^{3+} (*18*). Further, Hb binds CrO_4^{2-} 6-10 times faster than the nonheme proteins and peptides or amino acids (*18*). In the case of pertechnetate binding, however, this situation seems different, since no reduction mechanism inside the cell is evident. We are uncertain about the role played by heme in pertechnetate binding. Understandably, the site of binding is the same for both Cr and Tc. Possibly there is a difference in the reduction mechanism and the rate and kinetics of the reaction.

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REFERENCES

1. STOKELY EM, PARKEY RW, BONTE FJ, et al: Gated blood pool imaging following ^{99m}Tc stannous pyrophosphate imaging. *Radiology* 120: 433-434, 1976
2. THRALL JH, FREITAS JE, SWANSON D, et al: Clinical comparison of cardiac blood pool visualization with technetium-99m red blood cells labeled in vivo and with technetium-99m human serum albumin. *J Nucl Med* 19: 796-803, 1978
3. HEGGE FN, HAMILTON GW, LARSON SM, et al: Cardiac chamber imaging: a comparison of red blood cells labeled with Tc-99m in vitro and in vivo. *J Nucl Med* 19: 129-134, 1978
4. PARKER JA, UREN RF, JONES AG, et al: Radionuclide left vetriculography with the slant hole collimator. *J Nucl Med* 18: 848-851, 1977
5. DEWANJEE MK: Binding of ^{99m}Tc ion to hemoglobin. *J Nucl Med* 15: 703-706, 1974
6. PEARSON HA: The binding of Cr^{51} to hemoglobin. I. In vitro studies. *Blood* 22: 218-230, 1963
7. ECKELMAN W, RICHARDS P, HAUSER W, et al: Technetium labeled red blood cells. *J Nucl Med* 12: 22-24, 1971
8. SPERRY WM: Lipid analysis. *Methods Biochem Anal* 2: 83-111, 1955
9. CLEGG JB, NAUGHTON MA, WEATHERALL DJ: Abnormal human haemoglobins. Separation and characterization of the α- and β-chains by chromatography, and the determination of two new variants, Hb chesapeak and Hb J (Bangkok). *J Mol Biol* 19: 91-108, 1966
10. LOWRY OH, ROSENBROUGH NJ, FARR AL, et al: Protein measurement with a folin phenol reagent. *J Biol Chem* 193: 265-276, 1951
11. EBAUGH FG JR, SAMUELS AJ, DOBROWOLSKI P, et al: The site of CrO_4^- hemoglobin bond as determined by starch electrophoresis and chromatography. *Fed Proc* 20: 70, 1961 (abst)
12. PEARSON HA, VERTRESS KM: Site of binding of chromium-51 to haemoglobin. *Nature* 189: 1019-1020, 1961
13. REHANI MM, SAPRU RP, CHOPRA VK, et al: Evaluation of a simpler technique for in vivo labeling of red blood cells with Tc-99m. *Nucl Med* In press
14. MCRAE J, VALK PE: Alteration of ^{99m}Tc-red blood cells. *J Nucl Med* 13: 399-400, 1972
15. ECKELMAN WC: Technical considerations in labeling of blood elements. *Semin Nucl Med* 5: 3-10, 1975
16. ECKELMAN WC, SMITH TD, RICHARDS P: Labeling blood cells with ^{99m}Tc. In *Radiopharmaceuticals*, Subramanian G, Rhodes BA, Cooper JF, Sodd VJ, eds. New York, The Society of Nuclear Medicine, 1975, pp 49-54
17. HAMILTON RG, ALDERSON PO: A comparative evaluation of techniques for rapid and efficient in vivo labeling of red blood cells with [^{99m}Tc] pertechnetate. *J Nucl Med* 18: 1010-1013, 1977
18. SAMUELS AJ, EBAUGH FG JR, WATSON F: Studies on the mechanism of chromate binding to hemoglobin. *Fed Proc* 20: 70, 1961 (abst)