TECHNICAL NOTE

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Determination of ABO Blood Groups by Radioimmunoassay Using ^{125}I-Protein A

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ABSTRACT: Since the crystallizable fragment (Fc) portion of the immunoglobulin G (IgG) molecule is the binding site of Protein A, a radioimmunoassay procedure using ^{125}I-Protein A was developed for identification of the ABO blood groups. The isotope level bound to Group A, B, or AB red cells decreased with the dilution of anti-A or -B, respectively. After sensitization by anti-A plus B in Group O serum, the isotope bindings were observed in Groups A, B, and AB cells, while no significant radioactive count appeared in Group O cells. Furthermore, there was little significant isotope binding in both Group A and B red cells sensitized by the serum from Group A or B blood containing mainly IgM anti-A or -B. A radioimmunoassay using ^{125}I-Protein A is an excellent method for identifying ABO blood groups.

KEYWORDS: pathology and biology, blood, antigen systems, radioimmunoassay, ^{125}I-Protein A, ABO blood group

Many methods have been reported for the determination of red cell blood groups. Isotopic techniques permit the study of red cell antibody binding independent of visual agglutination [*1,2*]. Recently, we have reported a new method for identifying the Rh 1 (Rho, D) antigen using radioimmunoassay (RIA) [*3*]. RIA using ^{125}I-Protein A was sensitive enough to detect anti-D sensitized red cells.

In this paper, we demonstrate a rapid and highly sensitive RIA method using ^{125}I-Protein A for determination of ABO red blood groups.

Materials and Methods

Blood samples were collected from healthy humans of Groups A, B, O, and AB. A tube containing 50 μL (5×10^6) of washed red cells was incubated with 50 μL of anti-A (or anti-B) diluted with saline containing 1% bovine serum albumin (BSA). Following incubation, red cells were washed three times in saline and then once in phosphate-buffered saline (PBS)

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containing 1% BSA. The supernatant was completely aspirated. A volume of 100 μL of varying amounts of ^{125}I-labelled Protein A (5-μm Ci/μg, Radiochemical Center, Amersham, U.K.) was pipetted into the tubes containing the cell buttons; the tubes were capped and gently shaken approximately every half hour at 25°C. After washing three times with PBS buffer, the collected cells were counted using Packard γ-counter. Cold Protein A (UCB-Bioproducts S.A. Belgium) was added to the ^{125}I-Protein A (30-m Ci/mg) to be adjusted to 5-m Ci/mg. For preparation of anti-A or -B serum, Group O serum was absorbed with red cells of Group A or B after inactivation at 56°C for 30 min. The titers of the anti-A and -B were 1:128 and 1:32.

Results and Discussion

In a first reaction step, red cells were incubated for 30 min with anti-A (or anti-B) at 37°C. Thirty minutes of incubation was sufficient for binding of antiglobulin in a preliminary experiment. The time course of the binding of ^{125}I-Protein A to the sensitized Groups A and B cells is shown in Fig. 1. The binding of ^{125}I-Protein A to the sensitized red cells was completed by 60 min at 25°C. One or two hours at 25°C was chosen as a standard incubation time. In contrast, no significant radioactive count appeared in Group O cells. Nonspecific absorption of ^{125}I-Protein A by red cells was reduced to a minimum by addition of 1% BSA [*4*].

Effects of dilution of anti-A and anti-B serum on the total ^{125}I-Protein A bound to Groups A, B, and AB are shown in Fig. 2. The results obtained were plotted using a logarithmic scale for cycles per minute. The relative amounts of ^{125}I-Protein A bound to Groups A, B, and AB cells decreased in parallel with the dilution of anti-A or anti-B. The overall rate of isotope binding to Group AB cells was around 90% that of Group A or B cells, except for the binding at high concentration serum (×1).

The isotope counts bound to Groups O, A, B, and AB cells sensitized by the serums from each blood group are shown in Fig. 3. As most of Group O sera contains both immunoglobu-

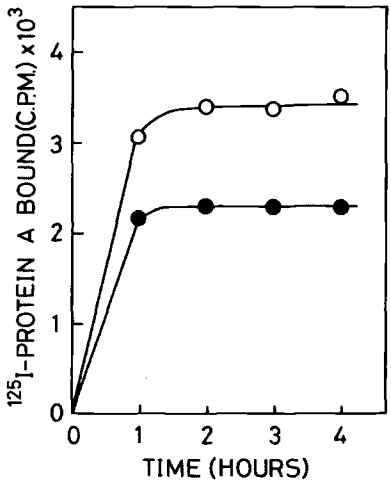

FIG. 1—*Time course of the binding of ^{125}I-Protein A to the sensitized Groups A and B cells. The tubes containing 50 μL (5 × 10^6 cells) of 2% red cells were incubated with anti-A or anti-B. After incubation of 30 min and washing, 100 μL (15 × 10^4 cpm) of ^{125}I-Protein A was added. The reaction was carried out at 25°C for the designated amount of time. Anti-A and B sera were prepared as described under Materials and Methods.* (○): *A cells,* (●): *B cells.*

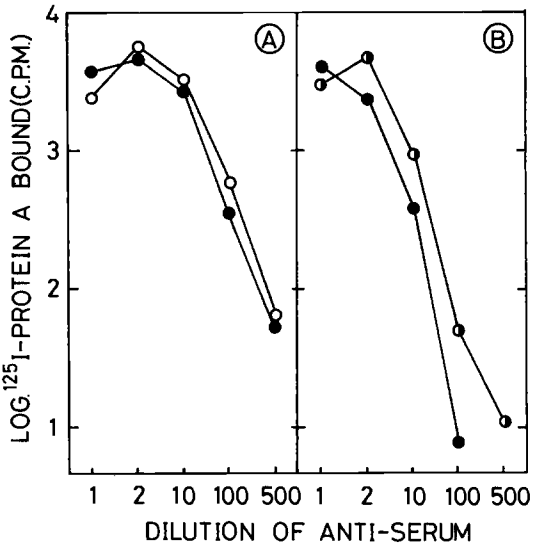

FIG. 2—*Effect of the concentration of anti-A and B on the binding of ^{125}I-Protein A to the sensitized Groups A, B, and AB cells. The tubes containing 50 μL (5 × 10^6 cells) of red cells were incubated with the various concentrations of anti-A or B. After incubation of 30 min and washing, 100 μL (15 × 10^4 cpm) of ^{125}I-Protein A was added. The reaction was carried out at 25°C for 3 h. Anti-A and B sera were prepared as described under Materials and Methods.* (○): *A cells,* (◑): *B cells,* (●): *AB cells.*

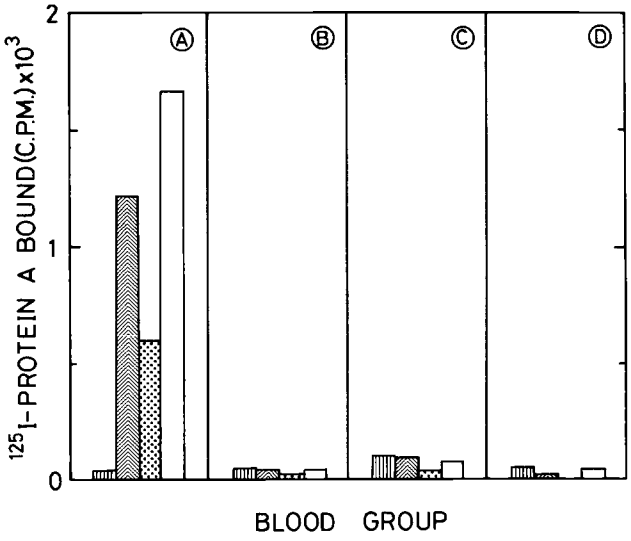

FIG. 3—*ABO grouping. The tubes containing 50 μL (5 × 10^6 cells) of 2% red cells were incubated with 1 : 50 diluted each serum (Groups O, A, B, or AB) in saline containing 1% BSA for 30 min at 37°C. After incubation and washing, 100 μL (15 × 10^4 cpm) of ^{125}I-Protein A was added. The reaction was carried out at 25°C for 2 h. Each serum from Groups O, A, B, and AB was heated at 56°C for 30 min.* (a): O serum, (b): *A serum,* (c): *B serum,* (d): *AB serum,* : *O cells,* : *A cells,* : *B cells, and* : *AB cells.*

lin G (IgG) as well as IgM anti-A and anti-B antibodies, isotope binding activities were detected on Groups A, B, and AB red cells sensitized by the Group O serum (Fig. 3, *a*). On the other hand, no significant radioactive count appeared in Group A or B red cells sensitized by the serum from Group A or B blood (Fig. 3, *b* and *c*). These observations derive from the fact that normal Group A and B sera contains mainly IgM anti-A or -B rather than IgG [*5*]. Protein A has been shown to interact with a variety of IgG molecules from several species [*6*]. Since the crystallizable fragment (Fc) portion of the IgG molecule is the binding site of Protein A [*7*], presence of free hemoglobin did not interfere with the binding of Protein A.

Conclusion

This RIA using commercially available ^{125}I-Protein A is useful for the detection of various blood group antibodies and antigens and would be applicable to identification of medicolegal materials, such as bloodstains, saliva, and semen.

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