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RAPID PREPARATION AND PURIFICATION OF ALKALINE PHOSPHATASE AND ^{125}I-LABELED ANTIBODY BY MINICOLUMN GEL CENTRIFUGATION CHROMATOGRAPHY

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SUMMARY

Gel centrifugation chromatography was used for the preparation, purification and concentration of enzyme and ^{125}I-labeled antibody. Low-molecular-weight reactants were rapidly and efficiently removed from either ^{125}I-labeled antibody or enzyme–antibody conjugates by centrifugation of the reaction mixture through a minicolumn of Sephadex. Further purification of both radiolabeled and enzyme labeled antibodies were possible by the application and elution of each labeled antibody through a minicolumn of Protein A Sepharose. Minicolumns were constructed from readily available inexpensive components and allowed the rapid preparation and purification of antibody of high specific enzyme, and radioactivity. No difference between the ^{125}I, or enzyme labeled antibody detection limit could be detected by a direct solid-phase immunoassay.

INTRODUCTION

Enzyme immunoassay (EIA) procedures are increasing in popularity for the detection of many antigens and antibodies. Although many EIA systems are quite similar to radioimmunoassay (RIA) the increased usage of EIA can be attributed to the fact that the antibody is labeled with an enzyme rather than a radioisotope thereby eliminating the hazards and problems associated with the preparation, measurement and disposal of radioactive material.

Many methods for coupling enzymes to antibodies have been described, and these have been recently reviewed[1]. A widely used procedure is the one-step glutaraldehyde linkage described for the conjugation of alkaline phosphatase to the antibody[2]. The method involves the mixing of enzyme with antibody in the presence of glutaraldehyde. Although effective this method requires two extensive time consuming dialysis steps, one prior to the conjugation reaction to remove the ammonium sulphate used to stabilize the enzyme during storage, and a second to remove the excess glutaraldehyde after the conjugation reaction. Gel filtration using Sephadex G-50 has also been used to remove excess glutaraldehyde from the enzyme–antibody

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complexes. Although less time consuming than dialysis this procedure invariably results in extensive dilution of the labeled antibody preparation. Additional dilution occurs during subsequent purification of the labeled antibody using either gel filtration or affinity chromatography[3].

Industrial and large scale preparative desalting of macromolecular solutions has been achieved by basket centrifugation. The gel filter, devoid of liquid in the void space, is prepared by centrifugation of a gel slurry in a perforated centrifuge basket, lined with a porous polyethylene filter[4]. Although this procedure requires the use of a specialized centrifuge, the method is effective resulting in the rapid efficient removal of unwanted contaminating low-molecular-weight components from large amounts of viscous proteinous solutions with a high yield recovery of the macromolecular products without significant dilution.

Utilizing the principle of gel centrifugation, Neal and Florini[5] have described a rapid method for separating low-molecular-weight solutes from protein solutions using conventional bench top centrifuge. Christopherson *et al.*[6] have subsequently improved this system using a small column for the removal of ammonium sulphate from several enzyme preparations. This application, together with the development of a minicolumn system for the removal of free ^{125}I from ^{125}I-labeled proteins[7], and the practical and theoretical aspects of gel centrifugation chromatography presented by Andersen and Vaughan[8] indicates that these methods may have wide application and potential for the rapid preparation of labeled antigens and antibodies used as reagents for diagnostic and research immunoassays.

We have devised a rapid micro-method for the preparation and purification of enzyme and ^{125}I-labeled antibody. The procedures involve the centrifugation of a small reaction volume of enzyme and/or ^{125}I-labeled antibody through a minicolumn of hydrated Sephadex gel. Unwanted low-molecular-weight reactants rapidly enter the gel matrix and are excluded from the protein fraction which upon centrifugation passes through the void space, and is collected without dilution. The labeled antibody may be further purified by centrifugation through a Protein A Sepharose minicolumn followed by concentration and removal of the eluting agent using a column of partially dehydrated Sephadex.

METHODS

Sephadex G-50 minicolumn preparation

Minicolumns were prepared from 5 × 1 cm plastic disposable syringe barrels. A 6-mm glass fiber disc presoaked in 10% fetal calf serum (FCS) in phosphate buffered saline (PBS), pH 7.2, was placed in the bottom of the syringe barrel and centered over the needle attachment aperture. The assembly was filled with 5.0 ml of a thick slurry of Sephadex® G-50 (coarse grade) (1.0 g suspended in 10 ml of 10% FCS in PBS). A 1.5-ml conical polypropylene micro-centrifuge tube was dropped into a 10 × 1.5 cm glass centrifuge tube and the minicolumn assembly positioned with the syringe adapter tip in the microcentrifuge tube as shown in Fig. 1. The combination was centrifuged at 100 *g* for 1 min in a table top clinical centrifuge with a horizontal head. After centrifugation, additional Sephadex was added as necessary to maintain a total bed height of 3.0 cm. The column of hydrated Sephadex was then washed three times by the centrifugation of 2-ml aliquots of PBS, through the column. Before

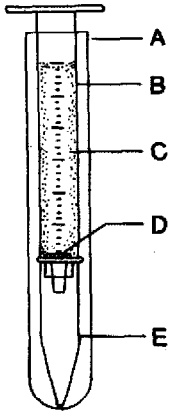

Fig. 1. The minicolumn assembly. A = Glass centrifuge tube (10 × 1.3 cm); B = plastic syringe barrel (3.0 ml); C = chromatographic gel; D = glass fiber disc (6.0 mm diameter); E = plastic conical microcentrifuge tube (1.5 ml).

loading a sample onto the gel bed, the column was centrifuged for an additional 2 min at 100 *g* to ensure complete removal of all buffer from the gel bed void space. Optimum centrifugation times and speeds were established by the addition and centrifugation of 200 μl of a 0.2% aqueous solution of phenol red and dextran blue 2000 through the gel bed. The minimum hydrated gel bed height required for the complete removal of both 3 *M* $(NH_4)_2SO_4$ and 3 *M* potassium thiocyanate from a 200-μl sample volume by centrifugation was determined by experiment. Ammonium sulphate was detected by the addition of one drop of 1 *M* $BaCl_2$ to the fraction recovered after centrifugation. Similarly KSCN was detected by the addition of a drop of a solution containing 5.0% ferric nitrate in 2.5% nitric acid to the eluted fraction.

Protein A Sepharose CL 4B minicolumn preparation

A 1-g amount of Protein A Sepharose® CL 4B was suspended in 5 ml of 10% FCS in PBS. The slurry was loaded into a minicolumn centrifugation assembly, and centrifuged for 1 min at 100 *g*. The protein A Sepharose bed height was adjusted to a total of 0.3 cm and washed three times with PBS. Protein A bound FCS proteins were eluted by the addition of 1.0 ml 3 *M* potassium thiocyanate (KSCN) followed by three washes with PBS. Prior to the addition of sample, the column was centrifuged at 100 *g* for 2 min to remove all buffer from the gel bed.

Labeling antibody with ^{125}I

Fluorescent labeled antihorse IgG (rabbit) were labeled with ^{125}I (Amersham Radiochemical Center) using the chloramine T method originally described by Hunter and Greenwood[9] with modifications[10]. Commercially available fluorescein-labeled antibody has previously been shown to be a readily available source of high quality antibody suitable for radioimmunoassay[11]. To 0.5 mCi sodium iodide was added 25 μl 0.25 *M* phosphate buffer, pH 7.5, followed by 100 μl of antibody in PBS containing 2 mg protein. Twenty five microliters of freshly prepared chloramine T (3.5 mg/ml in phosphate buffer) was added. After 60 sec incubation at 28°C, 100 μl sodium metabisulphite (2.4 mg/ml in phosphate buffer) were added. Free ^{125}I was

separated from ^{125}I-labeled antibody by minicolumn gel centrifugation. The mixture (total volume 225 μl) was applied to the top of 3.0-cm Sephadex column and centrifuged for 2 min at 100 *g*. The total volume, total protein[12] and radioactivity of the labeled antibody fraction collected in the conical microcentrifuge tube were determined.

Labeling antibody with alkaline phosphatase

Iodinated antihorse IgG (^{125}I) prepared as described above, was doubly labeled with alkaline phosphatase ([^{125}I]AP), using a modification of the method of Engvall and Perlmann[2]. Glutaraldehyde was used as the cross linking agent.

A 0.2-ml volume of alkaline phosphatase suspension in 3 *M* $(NH_4)_2SO_4$ (Sigma) containing 1.0 mg total protein was centrifuged in a 1.5-ml polypropylene micro-centrifuge tube at 3500 *g* for 2 min. The supernatant was discarded and 2.0 mg ^{125}I-labeled antibody in a total volume of 200 μl was added to the enzyme precipitate. After resuspension of the enzyme, remaining $(NH_4)_2SO_4$ was detected by the addition of one drop of 1 *M* $BaCl_2$ to the eluted fraction.

Conjugation of alkaline phosphatase to the antibody was carried out by the addition of glutaraldehyde to the eluted fraction to give a final concentration of 0.2%. After incubation at 25°C for 2 h the glutaraldehyde was removed from the antibody conjugate mixture by minicolumn centrifugation as described above. The total volume, total protein and radioactivity of the eluted fraction at each step were measured. Measurement of the enzyme activity was also carried out using the kinetic assay method of Bowers and McComb[13].

Purification of labeled antibody by Protein A Sepharose CL 4B minicolumn centrifugation

A 200-μl volume of labeled antibody (^{125}I or [^{125}I]AP) was layered onto a Protein A Sepharose CL 4B minicolumn (IgG binding capacity, 23.0 mg/ml) with a bed height of 0.3 cm. After incubation of the column at 28°C for 10 min the column was centrifuged at 100 *g* for 2 min. Unbound proteins were washed from the column by four 200-μl PBS washes. Protein A bound labeled antibody was removed by the addition of successive 200-μl aliquots of 3 *M* KSCN. The KSCN was removed from the peak fractions by gel centrifugation as described above, and pooled.

Direct solid-phase immunoassay

The activity of ^{125}I and [^{125}I]AP were tested by solid phase RIA and EIA. Assays were standardized as previously described[10].

Horse and fetal bovine IgG were diluted to provide a standard antigen series ranging from 12.48 to 196 ng IgG/ml. A 25-μl volume of each antigen was added to triplicate microplate wells and were air dried. The plates were fixed using cold methanol for 10 min at 4°C. Non-specific binding sites were blocked by flooding each plate with 0.1% gelatin in PBS, and incubating the plates at 25°C for 1 h. Excess gelatin was removed by washing the plates in PBS with 0.05% Tween 20 (PBS-T). Two hundred and fifty nanograms of labeled antibody were added to all wells and the plates were incubated at 25°C for 4 h. Unbound antibody was removed by washing the plates in PBS-T four times. For RIA each well was cut from the plate and placed in a gamma scintillation tube and counted for radioactivity. By RIA and EIA, wells

were placed in 75 × 12 mm plastic disposable test-tubes and the enzyme activity was detected by the addition of 0.3 ml *p*-nitrophenol phosphate, 1 mg/ml in 0.2 m*M* sodium carbonate buffer (pH 9.8). After incubation at 25°C for 1 h, the reaction was stopped by the addition of 50 μl 3 *M* NaOH. The tubes were then measured for radioactivity in a gamma spectrophotometer. After counting the absorbance at 405 nm of each tube was measured using a flow-through Gilford Instruments Model 240 spectrophotometer.

The efficiency of each labeled antibody to detect antigen was determined by calculation of a specific binding ratio using the following equation:

$$\text{Specific binding ratio} = \frac{\text{Mean O.D. or cpm}}{\text{Mean plus 3 S.D. of the mean control O.D. or cpm}}$$

The antibody detection limit was defined as that dilution of antigen at which the specific binding ratio just exceeded 1.0.

RESULTS

Characterization of the Sephadex minicolumn

Optimum centrifugation times and speeds were determined by the centrifugation of 200 μl of an aqueous solution of phenol red and dextran blue through a 3.0-cm Sephadex column. Centrifugation at 100 *g* for 1 min resulted in the recovery of 200 μl (±5.0%) of a solution containing only dextran blue and the retention of the phenol red in the top 0.75 cm of the gel bed.

A major step prior to the conjugation of alkaline phosphatase to an antibody is the removal of the 3 *M* $(NH_4)_2SO_4$ used to stabilize the enzyme during storage. The volume of hydrated gel required for the removal of this concentration of $(NH_4)_2SO_4$ was determined by the centrifugation of 200 μl of 3 *M* $(NH_4)_2SO_4$ through Sephadex minicolumns of varying gel bed heights. A minimum gel bed height of 2.0 cm completely retarded this concentration of $(NH_4)_2SO_4$ as well as 3 *M* KSCN from a 200-μl sample volume. In subsequent experiments a standard gel bed height of 3.0 cm was used.

Labeling antibody with ^{125}I

The efficiency of the Sephadex minicolumn to separate free ^{125}I from iodinated antibody by centrifugation was determined by the addition of 200 μl of antibody labeled with ^{125}I and free ^{125}I to separate 3.0-cm minicolumns. Prior to use, the labeled antibody was purified by gel filtration and $(NH_4)_2SO_4$ precipitation[11] to ensure the removal of free ^{125}I. Both minicolumns were centrifuged and the radioactivity retained in the gel bed and collected in the eluted fraction were measured. Greater than 99% of the free ^{125}I was retarded in the top 1/3 of the gel bed and as much as 97% of the labeled antibody was recovered in the eluted fraction.

Two 2.0-mg aliquots of antihorse IgG were labeled with ^{125}I, and the iodinated antibody was separated from ^{125}I by minicolumn gel centrifugation (Table I). At least 90% of the total protein, and as much 85.4% of the total radioactivity could be recovered regularly, with no dilution of the original sample volume. Of the remaining 14.6% of the total radioactivity in the gel bed, 14.0% was located in the top 1/3 of the

TABLE I

RECOVERY OF ^{125}I-LABELED ANTIBODY BY SEPHADEX MINICOLUMN CENTRIFUGATION

Two 2.0-mg aliquots of antihorse antibody were labeled with ^{125}I and the labeled antibody was separated from the free ^{125}I by Sephadex G-50 minicolumn centrifugation.

	Sample	
	A	*B*
Initial total protein (μg)	2000	2000
Initial sample volume (μl)	225	225
Final total protein (μg)	1800	1800
Final sample volume (μl)	215	210
Radioactivity recovered (%)	84.0	85.4
Specific radioactivity (cpm/μg)	$8.8 \cdot 10^4$	$8.8 \cdot 10^4$

column indicating good separation of free ^{125}I from protein bound radioactivity. To ensure the complete separation of free ^{125}I from radiolabeled antibody, the eluted fraction was recentrifuged through a 3.0-cm Sephadex G-50 minicolumn. This resulted in the recovery of 99.4% of the protein bound radioactivity added to the column.

Labeling antibody with alkaline phosphatase

Antibody labeled with ^{125}I was described above was double labeled with alkaline phosphatase. Measurement of the radioactivity permitted the monitoring of the antibody at each step throughout the enzyme conjugation and purification procedures. As shown in Table II, 99–100% of the total protein in between 195- and 200-μl volumes were recovered at each centrifugation step.

After the first centrifugation step, no $(NH_4)_2SO_4$ could be detected in the eluted fraction and as much as 70% of the original enzyme activity was preserved. Despite the loss of an additional 23% of the enzyme activity during the conjugation reaction and removal of glutaraldehyde, the specific enzyme activity was 110 U/mg.

TABLE II

RECOVERY OF ^{125}I AND ALKALINE PHOSPHATE DOUBLY LABELED ANTIBODY BY SEPHADEX MINICOLUMN CENTRIFUGATION

Radioiodinated antibody was doubly labeled with alkaline phosphatase by the glutaraldehyde linkage method. Excess $(NH_4)_2SO_4$ was removed from the enzyme–antibody mixture by Sephadex gel centrifugation. The mixture was then incubated with 0.2% glutaraldehyde for 2 h after which excess glutaraldehyde was removed by gel centrifugation. The total volume, protein, radioactivity, and enzyme activity recovered after each step were measured.

	Volume (μ)	*Total protein (μg)*	*Radioactivity (%)*	*Enzyme activity (U)**
Initial sample	200	2481	100	550.4
Removal of $(NH_4)_2SO_4$	200	2480	99.1	387.2
Removal of glutaraldehyde	195	2500	100	275.8

* One international phosphatase unit, U, will yield 1 μmol of *p*-nitrophenol per minute from *p*-nitrophenol phosphate at 37°C.

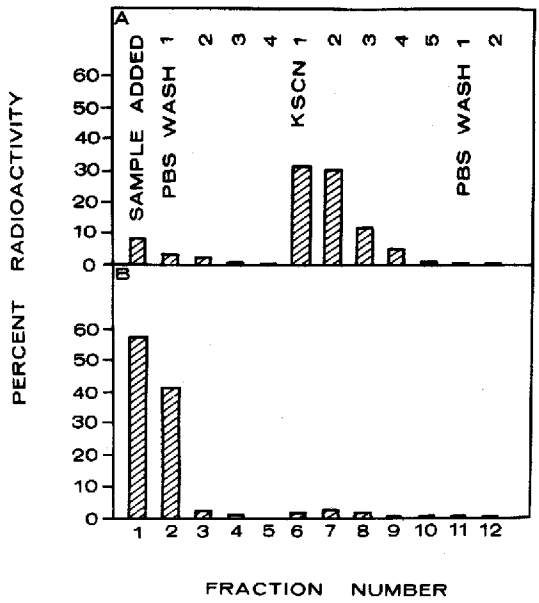

Fig. 2. The recovery of ^{125}I and [^{125}I]AP labeled antibody from a 0.3-cm Protein A Sepharose minicolumn by centrifugation. Volumes of 200 μl ^{125}I (A) and [^{125}I]AP (B) labeled antihorse IgG were applied to the column and centrifuged. Unbound proteins were removed from the gel by four 200-μl PBS washes. Protein A bound antibody was then eluted from the column by five additional 200-μl washes of 3 *M* KSCN. The percentage of the total radioactivity recovered in each 200-μl fraction was determined.

This compared well with specific activities of between 106 and 156 U/mg routinely obtained in our laboratory using conventional labeling methods[14].

Purification of labeled antibody by Protein A Sepharose minicolumn centrifugation

The high IgG binding capacity of Protein A Sepharose CL 4B (*ca.* 23 mg IgG per ml of gel) permitted the use of a small bed height (0.3 cm) and 200-μl elution volumes. This enabled the recovery of as much as 91.6% (^{125}I) of 86.6% ([^{125}I]AP) bound to the Protein A column in the first three 200-μl fractions after the addition of the 3 *M* KSCN (Fig. 2). A total of 80.6% of the ^{125}I-labeled antibody was bound to the Protein A column and could be recovered by elution, however this is in contrast to the binding and recovery of only 5.9% of [^{125}I]AP. KSCN was removed from the peak fractions by Sephadex minicolumn centrifugation.

Purification of [^{125}I]AP by Protein A Sepharose minicolumn centrifugation resulted in an almost a two-fold increase in specific enzyme activity and when the antibody activity was compared with ^{125}I by RIA, both preparations exhibited an identical sensitivity detecting 2.3 ng of horse IgG (Table III).

Sample concentration by Sephadex minicolumn centrifugation

Pooling of the first two peak fractions eluted by centrifugation from a Protein A Sepharose minicolumn results in a two-fold dilution of the original sample. The degree of hydration in a Sephadex minicolumn could be varied by increasing the time and speed of centrifugation prior to the addition of sample (pre-spin time). The

TABLE III

THE ANTIGEN DETECTION LIMIT OF PROTEIN A PURIFIED ^{125}I AND ALKALINE PHOSPHATE ^{125}I ([^{125}I]AP) LABELED ANTIBODY MEASURED BY ENZYME IMMUNOASSAY AND RADIOIMMUNOASSAY

Nanogram amounts of horse IgG and control bovine globulin were fixed to triplicate microplate wells with methanol and detected by the addition of 0.25-μg well of labeled antibody (^{125}I or [^{125}I]AP). After incubation at room temperature for 4 h unbound antibody was removed by washing the plates in PBS. Bound labeled antibody was detected by either gamma scintillation counting (RIA) or by the addition of substrate and the measurement of colour development spectrophotometrically (EIA). The antigen detection limit of the labeled antibody was determined by calculating a specific binding ratio. The point at which the specific binding ratio equalled 1.0 was the antigen detection limit of the antibody preparation.

Antibody	*Assay*	*Antigen concentration (ng)*						
		37.5	*18.75*	*9.37*	*4.68*	*2.3*	*1.17*	*0.5*
^{125}I	RIA	8.11*	7.60	3.05	2.71	1.83	0.79	0.58
[^{125}I]AP	RIA	4.50	4.56	2.21	1.75	1.05	0.78	0.86
	EIA	5.84	6.36	4.17	2.26	1.65	0.31	0.21

* The specific binding ratio, defined as the mean test cpm or $O.D._{405}$ divided by the mean plus 3 S.D. of the triplicate control cpm or $O.D._{405}$.

application and centrifugation for 2 min at 100 *g* of a 400-μl sample, resulted in the concentration of the labeled protein fraction from between 2.1 and 5.3 times with up to 83.2% recovery of total protein.

DISCUSSION

The establishment of minicolumn gel centrifugation chromatography for the separation of low-molecular-weight solutes from protein solutions requires the use of a gel grade with good flow characteristics, which will allow the rapid removal of void space liquid without compression and channeling of the gel bed. The optimum times and centrifugation speeds must established under individual laboratory conditions. This is easily achieved by experiment using a mixture of indicator and molecular marker dyes depending on the molecular size of the reagent to be separated and the protein to be recovered. In our system the retention of phenol red (mol. wt. 354.3) in the upper portion of the column indicated the complete removal of compounds with molecular weights of less than 354.3 daltons under identical conditions of concentration, centrifugation time and speed. The concentration of the reagent and the sample volume were both found to influence the amount of gel used. A relationship between sample volume and bed height has been investigated by Neal and Florini[5] who established that the optimum sample volume to bed height ratio of between 0.17 and 0.27 was required for the removal of 0.1 *M* $(NH_4)_2SO_4$ from a 3–5 ml solution with maximum protein recovery. Similar ratios were used by Christopherson *et al.*[6] and Tuszynksi *et al.*[7], however the effects of higher concentrations of $(NH_4)_2SO_4$ were not investigated by these workers. In our system the complete removal of 3 *M* $(NH_4)_2SO_4$ and 3 *M* KSCN from a 200-μl volume required a minimum gel bed height of 2.0 cm (sample volume: bed height ratio 0.1). Any further decrease in the gel bed height failed to completely remove all the $(NH_4)_2SO_4$ and KSCN. Preadsorbing

the 3.0-cm minicolumn components with FGS prior to use, reduced the non-specific binding of the sample protein to the column components. This allowed the use of a low sample volume to gel bed height ratio (0.06) with high total protein recoveries and the complete removal of $(NH_4)_2SO_4$ and ^{125}I from the sample. Removal of free ^{125}I from labeled antibody by minicolumn centrifugation in 85.4% recovery in the eluted fraction, of the total radioactivity added. The recovery compares favorably with the trichloroacetic acid-precipitable protein recovery reported by Tuszynksi *et al.*[7] using a similar system. The complete removal of free ^{125}I from the radiolabeled antibody in this study was clearly demonstrated by recentrifugation of the eluted radiolabeled fraction through a 3.0-cm Sephadex G-50 minicolumn which resulted in the recovery of 99.4% of the added radioactivity.

The purification of enzyme labeled antibody is desirable for many EIA procedures since it reduces the level of non-specific enzymatic activity and consequently increases the sensitivity of both qualitative and quantitative immunoassays. The separation of unconjugated enzyme from antibody–enzyme conjugates has been accomplished by affinity chromatography using Protein A Sepharose CL 4B[3]. Conventional flow through column systems, possess many of the disadvantages of gel filtration. They are both time consuming, require fraction collecting devices, and result in the extensive dilution of the original sample. The excellent flow characteristics of Sepharose 4B, together with the high affinity and specificity of Protein A facilitate the rapid elution of Protein A bound antibody from the column by gel centrifugation. In contrast to the Sephadex minicolumn system where the sample is recovered without further dilution, complete removal of antibody from the Protein A Sepharose column requires the sequential application and centrifugation of the eluting agent. However the use of a small gel bed height permitted the recovery of 80–90% of the total IgG bound to the protein A column within the first three 200-μl fractions collected minimizing the dilution of the original sample.

Purification of [^{125}I]AP by Protein A Sepharose minicolumn centrifugation resulted in the binding and subsequent recovery of 5.9% of the enzyme labeled antibody (Fig. 2). The reduction in [^{125}I]AP binding to Protein A when compared to the binding of ^{125}I-labeled antibody may be due to masking of the Fc* portion of the antibody molecule by conjugated enzyme or by the formation of enzyme–antibody complexes. In a recent study comparing ^{125}I and [^{125}I]AP antibodies we demonstrated that the ^{125}I-labeled antibody bound seven times more antigen than did the antibody with enzyme[15]. This was consistent with observations made by other workers that as little as 1% of the enzyme-labeled antibody remained active after conjugation[2].

The main advantages of gel centrifugation for the preparation and purification of labeled antibodies are that the columns used are easily constructed from inexpensive components readily available in most laboratories. The columns are reusable and the use of small quantities of gel and other reagents make this system especially economical. Labeled antibodies can be prepared rapidly without further dilution eliminating additional protein concentration procedures which may contribute to further protein losses and/or inactivation of either the antibody or enzyme. Thus alkaline phosphatase labeled antibody can now be prepared and purified within 3 h.

* Crystallizable fragment obtained by papair digestion of IgG. Binding site for Protein A.

This compares with a preparation time of 72–96 h using conventional labeling methods requiring dialysis and/or gel filtration to remove low-molecular-weight reactants. Similarly the preparation of radiolabeled antibody using gel centrifugation required only a few minutes for the removal of free isotope from the labeled protein. Multiple columns can be prepared and easily handled by one person. This makes possible the purification and labeling of IgG from individual patient sera for assay by the direct immunoassay avoiding many of the problems of cross reactivity and non-specific binding frequently encountered using indirect assay systems. Since the glutaraldehyde one step reaction can be used for coupling a wide variety of enzyme–protein systems, Sephadex and Protein A Sepharose minicolumn centrifugation should have wide application for the preparation and purification of enzyme labeled antibodies and antigens commonly used in immunohistologic and enzyme immunoassays.

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REFERENCES

1 J. H. Kennedy, L. J. Kricka and P. Wilding, *Clin. Chim. Acta*, 70 (1976) 1.
2 E. Engvall and Perlmann, *Immunochemistry*, 8 (1971) 871.
3 M. Page, M. Audett and M. Caron, *Can. J. Biochem.*, 57 (1979) 286.
4 N. I. A. Emnéus, *J. Chromatogr.*, 32 (1968) 243.
5 W. M. Neal and J. R. Florini, *Anal. Biochem.*, 55 (1973) 328.
6 R. I. Christopherson, M. E. Jones and L. R. Finch, *Anal. Biochem.*, 100 (1979) 184.
7 G. P. Tuszynksi, L. Knight, J. R. Piperno and P. N. Walsh, *Anal. Biochem.*, 106 (1979) 118.
8 K. B. Andersen and M. H. Vaughan, *J. Chromatogr.*, 240 (1980) 1.
9 W. M. Hunter and F. C. Greenwood, *Nature (London)*, 194 (1962) 495.
10 J. Kalmakoff, A. J. Parkinson, A. N. Crawford and B. R. G. Williams, *J. Immunol. Methods*, 14 (1977) 73.
11 A. J. Parkinson and J. Kalmakoff, *J. Clin. Microbiol.*, 3 (1976) 637.
12 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randell, *J. Biol. Chem.*, 193 (1951) 265.
13 G. N. Bowers and R. B. McComb, *Clin. Chem.*, 12 (1966) 70.
14 E. N. Scott, F. G. Felton and H. G. Muchmore, *Mycopathologia*, 70 (1980) 55.
15 A. J. Parkinson, E. N. Scott and H. G. Muchmore, *J. Clin. Lab. Automation*, 2 (1982) 177.