Short Communications

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The use of antigen–cellulose suspensions for the isolation of specific antibodies

Repeated attempts have been made to develop general methods for the isolation of antibodies in sufficient quantity for chemical and physical studies. Most have depended on the preparation of the antigen in an insoluble form for use as a specific adsorbent and difficulty has arisen because of low capacity, non-specific adsorption and failure to achieve full desorption under conditions which do not destroy antibody activity (see, *e.g.*, ISLIKER[1] and PORTER AND PRESS[2]). The first two difficulties have been overcome to a considerable extent by a recent modification[3] of the cellulose-linked antigen method of CAMPBELL[4] and we wish to report a more detailed study of this method.

N-(*m*-nitrobenzyloxy)Methylpyridinium chloride was synthesised[5] and coupled to cellulose powder (Munktell, Sweden) as described by GURVITCH *et al.*[6] using a 6 % solution of pyridinium salt in 2.5 % sodium acetate (see also KABAT AND MAYER[7]). The product, referred to as aminocellulose, could be stored dry at room temperature over months.

Aminocellulose suspension was prepared by dissolving 1 g of the powder in 33 ml ammoniacal copper solution (13 ml water, 20 ml NH_3 solution (sp. gr. 0.88), 1.5 g $Cu(OH)_2$ and 0.33 g sucrose). A further 40 ml NH_3 solution was added followed by 40 ml of warm water (70°). Precipitation of the cellulose commenced as the water was added and was completed by cautious addition of 10 % H_2SO_4 until the solution just lost its blue colour. The cellulose was centrifuged and washed 6 times with water. It was stored as a well-dispersed suspension in water at 2°.

Protein antigens were coupled to this aminocellulose suspension as follows. To 25 ml of suspension containing 250 mg aminocellulose was added 42.5 ml water and 7.5 ml HCl (36 %, w/v). The mixture was cooled in an ice bath, 1.9 g $NaNO_2$ added and stirred for 30 min. The diazocellulose was centrifuged and washed twice with cold water and then twice with cold 0.2 M borate buffer (pH 8.7). 250 mg protein antigen dissolved in 25 ml cold 0.2 M borate buffer (pH 8.7) was added to the diazocellulose and the mixture stirred at 2° for 24 h. The cellulose-coupled antigen was then washed with cold 1 % NaCl once at neutral pH, 3 times at pH 3.2 and again 3 times at neutral pH. This extensive washing was necessary to remove all the free antigen. The protein in the supernatants was estimated by the method of LOWRY *et al.*[8] and the difference between this and the 250 mg protein added gave the amount of antigen bound to the cellulose. This antigen–cellulose preparation appears to be stable at 2° over months.

The maximum antibody-binding capacity of an antigen–cellulose preparation was determined by adding increasing volumes of specific antiserum to a suspension of 50 mg of antigen cellulose, incubating 1 h at room temperature and overnight at 2°. 950 mg cellulose (Solka Floc) were then added and mixed well and the suspension filtered at 2° on a sintered-glass funnel. If cellulose was not added filtration was

TABLE I

DISSOCIATION AND ELUTION OF ANTIBODY

No.	System	Antigen bound* (mg/g of antigen-cellulose)	Antibody bound* (mg/g of antigen-cellulose)	Dissociating agents	Yield of dissociated protein soluble at neutral pH (mg/g cell)		Dissociated protein precipitated by the specific antigen antibody/protein × 100 (%)	
					Batchwise operation	Column operation	Batchwise	Column
1	Human serum albumin and rabbit antiserum	375 (300; 400; 425)	125 (100; 125; 150)	1% NaCl (pH 3.2) at 15–25°	70	75	100	100
				1% NaCl (pH 2.0) at 15–25°	80	75	90	95
				1% NaCl (pH 2.0) at 37°.	60	65	90	85
				0.5% sodium dodecyl sulphate at 15–25°	—	10	—	50
2	Human γ-globulin and rabbit antiserum	271 (240; 273; 300)	103 (80; 130; 130)	1% NaCl (pH 3.2) at 15–25°	40	30	100	90
				1% NaCl (pH 2.0) at 37°	50	45	80	90
				0.05 M glycine–HCl buffer (pH 2.9) at 15–25°	40	—	50	—
				0.5% sodium dodecyl sulphate at 15–25°	60	—	0	—
3	Rabbit γ-globulin and horse antiserum	450 (300; 450; 600)	150 (100; 170; 180)	1% NaCl (pH 3.2) at 15–25°	65	50	100	90
				1% NaCl (pH 2.0) at 37°	75	65	90	90
4	Hen-egg albumin and rabbit antiserum	450 (350; 500; 500)	100 (75; 100 ;125)	1% NaCl (pH 3.2) at 15–25°	—	20	—	100
				1% NaCl (pH 2.0) at 37°	25	23	100	100
				0.05 M glycine–HCl buffer (pH 2.9) at 15–25°	10	—	—	—
				0.035 M aq. CO_2 (pH 5.0) at 15–25°	10	—	—	—
5	Turkey-egg albumin and rabbit anti-hen ovalbumin	320 (275; 350)	60 (50; 80)	1% NaCl (pH 2.0) at 37°	40	30	80	75

* Average values are given for antigen- and antibody-binding capacity, the individual values being in parentheses.

impossible. The suspension was washed on the funnel with cold 1 % NaCl until the washings contained no protein as judged by the absorption at 280 mμ. The washings were combined and antibody estimated by precipitation[9].

Dissociation of antibody from the antigen cellulose gave the greatest difficulty and a variety of eluting agents were investigated. These included 1 % NaCl adjusted with HCl to pH 3.2 or 2.0, 0.02 M glycine–HCl buffer (pH 2.9), 0.035 M aq. CO_2, 0.2–0.5 % sodium dodecyl sulphate.

Elution of the antibody was carried out either batchwise or by washing through a column prepared with the antigen–antibody suspension. In the column elution a slow rate (1 ml/h) of flow was essential. For batchwise elution, the suspension was incubated at 37° or room temperature overnight in the eluting solvent, 5 ml/50 mg suspension. The suspension was centrifuged and washed several times with the same volume of eluting solvent.

In both techniques some protein precipitated on neutralisation of the eluant and was centrifuged off before protein and antibody estimations were made.

Antigen- and antibody-binding capacities are given in Table I. Both are high for the various systems tried though not as high as found by GURVITCH *et al.*[3] using the human serum albumin–rabbit antiserum system. Non-specific adsorption of protein was less than 1 mg/g adsorbent.

The results of dissociation of antibody in the different systems by a variety of solvents are listed in Table I. No significant difference in yield was found between column and batchwise elution and the column method was found to be more rapid and convenient. 1 % NaCl (pH 2.0) at room temperature gives as good a recovery of 90–100 % precipitable antibody as was obtainable and this is recommended for routine uses. The weight of antibody bound per gram of antigen cellulose is relatively constant at 100–120 mg when using homologous antibody but the recovery on dissociation ranges from 25 % for ovalbumin–rabbit antiovalbumin to 60 % for serum albumin–rabbit antiserum albumin.

This technique therefore offers a practicable method of preparing in reasonable yield, on a 100–200-mg scale, antibodies which are free of inactive protein. It is not entirely satisfactory in that there is presumably a selection of the most easily dissociated antibodies and this has to be considered in any further studies of antibodies prepared with this method.

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