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Short note

IgA PURIFICATION FROM SOW MILK

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Résumé

PURIFICATION DES IgA DU LAIT DE TRUIE. — Les IgA du lait de truie ont été facilement purifiées par des techniques simples de chromatographie d'échange d'ions (DEAE Trisacryl M) et de filtration sur gel (Ultrogel AcA 22).

The secretory IgA is the predominant immunoglobulin in external secretions. This immunoglobulin seem very important for the protection against bacterial and viral aggression (Offit, 1985; Bohl and Saif, 1975; Tabliabue *et al.*, 1983). The purification of this class of immunoglobulin is very important to make reactif. The IgA immunoglobulin have been greatly studied, and purified with cellulose matrix ion exchange chromatography (Porter, 1969; Bourne, 1969; Debuysscher *et al.*, 1975). The use of this technique is laborious. The interaction of porcine IgA with protein A gives good yield but does not eliminate the IgG class (Bennel and Watson, 1980).

Recently, synthetic matrix (Trisacryl) have been used to easily purify the IgG class of immunoglobulin (Corthier *et al.*, 1983).

This communication will describe a very simple way to prepare IgA from milk by classical precipitation and chromatographic technique.

Materials and Methods

1) Chemicals

The ultrogel AcA 22 (LKB) and the DEAE-Trisacryl M (LKB) were purchased from reactif IBF (France); the peroxidase (grade 1) from Boehringer; the human albumin (fraction V) from Sigma.

All the other chemicals and the buffer were of an analytical grade.

2) Milk

Milk samples from various sows were manually collected after ocytocine intramuscular inoculation (2 units) and immediately used or stored at -20°C .

3) Milk globulin extraction

We collected 45 ml of milk. The cream was separated from the skimmed milk by low-speed centrifugation (2500 g, 10 min, 5 °C, Heraeus Christ). The caseins were removed by high-speed centrifugation 45000 g on Beckman Spinco L8-55 rotor R 30. The whey proteins

Table 1. — Yield of each step of IgA purification

	Volume (ml)	Proteins ^a		IgA ^b		Yield (%)	
		Concentration (mg/ml)	Quantity (mg)	Concentration (mg/ml)	Quantity (mg)	Protein	IgA
Milk	45	62	2 790	14.1	635	—	—
Whey	43	28	1 200	7.7	331	43	52
50 % precipitation	40	20	800	6.4	256	28.5	40.3
DEAE start	25	15	375	4.5	112	13.5	17.6
A.C.A. 2.2. start	10	15	150	6.8	68	5.4	10.7
IgA AcA pic	—	—	—	—	51.5 ^c	1.8	8.1

a: The protein concentration was estimated by the Lowry method.

b: The IgA concentration was estimated by the ELISA technique described in the Materials and Method.

c: After lyophilization.

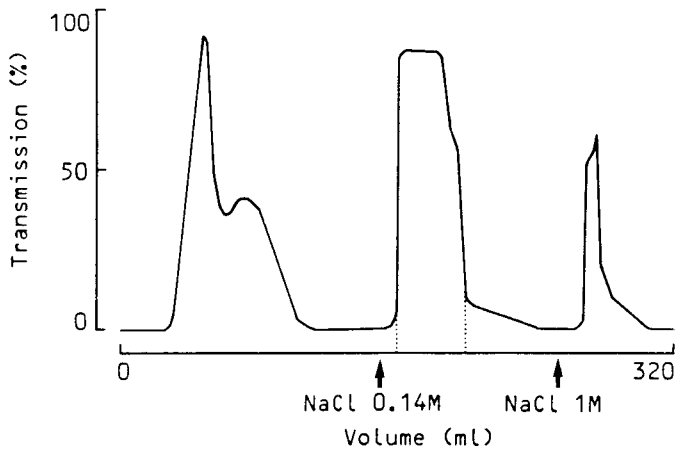


Fig. 1. — Protein elution from DEAE Trisacryl M (LKB): 25 ml of soluble material was applied to a column 1.6×10 cm in 0.025 M Tris-HCL buffer pH 8, 0.05 M NaCl, 90 ml/h. Two elution steps were made: 0.14 M and 1 M.

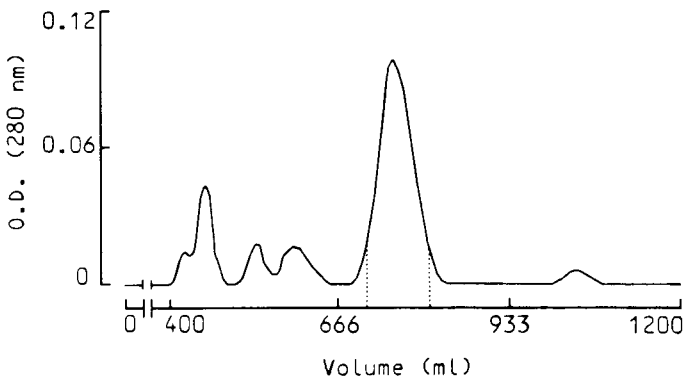


Fig. 2. — Gel filtration of the IgA DEAE eluted pic on AcA 22 Ultrogel (LKB) 10 ml of material was applied to a column (4.5×75) cm in Tris-HCL 0.025 M, pH 8, 0.05 M NaCl.

(43 ml) were used immediately. The gammaglobulin fraction was obtained by ammonium sulphate precipitation (50 % of saturation V/V) at 4 °C for 2 h. After centrifugation, the pellet was resuspended in 40 ml of 0.025 M Tris-HCl buffer, pH 8.8 and reprecipitated in 33 % of ammonium sulphate concentration. The pellet was collected in 25 ml of 0.025 M Tris-HCl, pH 8.8 containing 0.05 M NaCl and extensively dialysed at 4 °C against this same buffer.

4) IgA globulin purification

The ion exchange chromatography was carried out on a column (1.6 × 10 cm) of DEAE-Trisacryl M, equilibrated in 0.025 M Tris-HCl buffer, pH 8.8, NaCl 0.05 M. The gel filtration was performed on a column (4.5

× 75 cm) of AcA 22 utrogel in the same buffer. The flow rate was 40 ml/h.

The chromatographic experiments were continuously recorded with ISCO at 280 nm (model UA 5 absorbance monitor), and the eluate affluent collected with ISCO fraction collector.

The protein concentration was determined by the Lowry technique (Lowry *et al.*, 1953) with human albumin as reference.

5) Immunelectrophoresis (IEF)

Immunelectrophoresis in 1 % agar was carried out according to standard procedures with total anti-swine serum proteins and anti-swine skim-milk protein antibodies (rabbits).

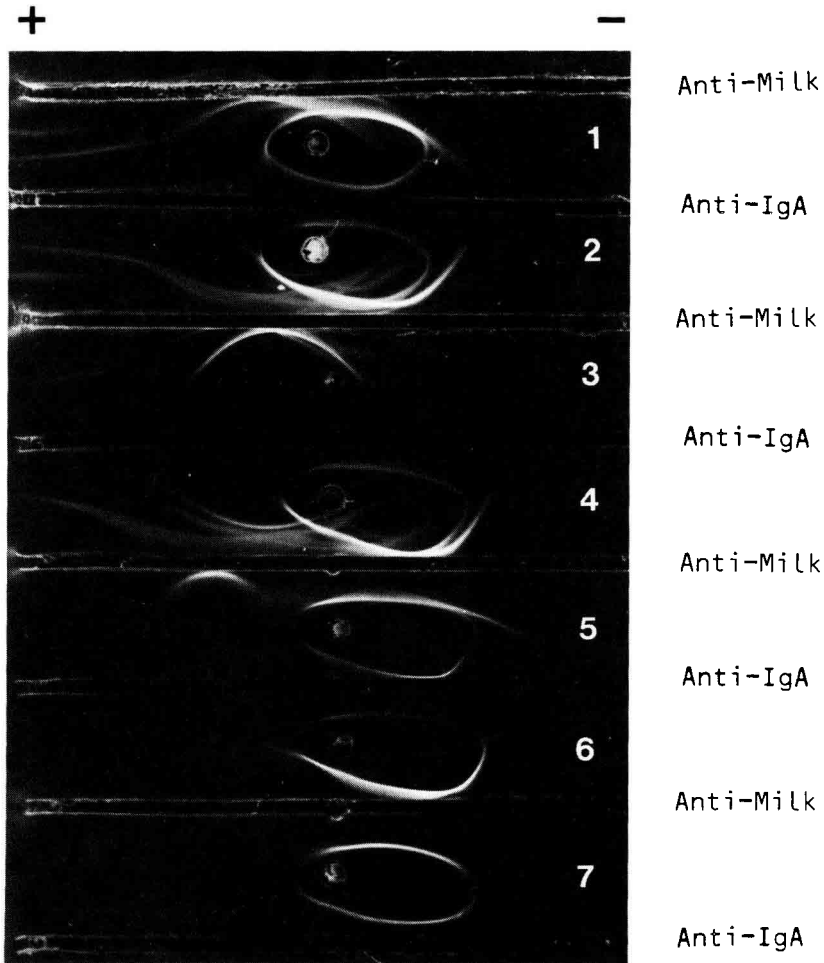


Fig. 3. — Immunelectrophoresis of the different steps of IgA purification. Anti-milk antibodies; specific anti-IgA; 1: milk; 2: whey; 3: supernatant of the 50 % ammonium sulphate precipitation; 4: 50 % ammonium sulphate precipitate; 5: DEAE starting material (33 % ammonium sulphate precipitate); 6: AcA 22 starting material; 7: IgA pic on the AcA 22.

Results and Discussion

The procedure described here allows the preparation of highly purified IgA with a good yield.

The IgA purification is subordinate to the ratio between the IgA and the other components of the starting material. The swine milk contains from 10 to 15 mg/ml of IgA, and the ratio IgA/IgG is very high (about two times as much or more).

Table 1 gives the protein yield of the different steps of IgA purification. The first purification step after skimming is to separate the globulin fraction from the other components. Half of the IgA seemed trapped in the casein pellet. After the first step of ammonium sulphate precipitation (50 % V/V) we lost half of the proteins, and 60 % of IgA. The second precipitation dramatically decreased the IgA level. A better yield of IgA purification could be obtained by avoiding the second ammonium sulphate precipitation which eliminates many proteins but also eliminates a fair quantity of IgA.

The globulin preparation was passed through the DEAE gel. The elution profile (fig. 1) shows several peaks. The unadsorbed fraction is mainly constituted of IgG (Corthier *et al.*, 1984). The second pic eluted with one step gradient of 0.14 M NaCl contains IgA. After this step the puri-

fication of this material needs an exclusion chromatography (fig. 3). This fraction was concentrated to 10 ml by ammonium sulphate precipitation and layered on the top of the ultragel column. The elution profile is shown in figure 2. The elution behaviour of the major peak ($K_{av} = 0.41$) corresponds to a molecular mass of approximately 400 000 daltons.

The proteins were concentrated by ammonium sulphate precipitation (50 % V/V). The IEP analysis gave only one precipitating arc with either the IgA specific antiserum (kindly given by J.J. Metzger) or the anti-milk antibodies (fig. 3).

The acrylamide gel electrophoresis (Dubray and Bezard, 1982) of this IgA preparation gives only three bands for the H- and L-chains and for the secretory piece (results not shown). The yield of the purification could be improved more but the purification of this IgA by this method rapidly gives IgA which only gives three bands in polyacrylamide gel.

The ELISA reactions of this immunoglobulin against specific swine anti- IgG or IgM, are very low (personal results) and the estimated contamination less than 1 %.

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Summary

IgA from swine milk may be easily purified with DEAE-Trisacryl M ion exchange chromatography (upper than 75 % of purity) and AcA 22 Ultragel gel filtration.

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