PARTIAL PURIFICATION OF CHICKEN SPECIFIC MUSCLE SOLUBLE PROTEINS BY IMMUNOADSORPTION CHROMATOGRAPHY

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INTRODUCTION

Poultry (chicken and turkey) tissue represents a major source of protein, generally less expensive than red meat being consumed and imported throughout the world. These factors, together with the regulated increasing use of mechanically separated poultry meat produces a significant potential for the substitution of red meat by poultry products.

Most of the methods currently available for meat speciation involve ELISA methodologies and the use of polyclonal antisera raised against blood proteins (Kang'ethe et al., 1982; Griffiths and Bellington, 1984; Patterson and Jones, 1985; Jones and Patterson, 1985, 1986; Patterson and Spencer, 1985) or muscle soluble proteins (Martín et al., 1987, 1988a, 1988b, 1988c). Such antisera require a purification treatment to eliminate significant cross-reactions. This obviously represent an important problem in the long term. The development of hybridoma technology (Köller and Milstein, 1975) has provided the means for continuos production of monospecific antibodies of known biological activity and consistent specificity from single cell lines, once selected by suitable stringent screening procedures and in vitro tissue culture. For meat speciation, monoclonal antibodies of required specificity would permit the use of a contrasted reactive to be universally utilised. Hybridoma technology does not neccessarily require highly purified antigens but selected antigens should produce a higher yield of monoclonal antibodies of interest. In this work, chicken-specific polyclonal antibodies were immobilised on a protein A-Sepharose CL-4B column and used to isolate chicken specific proteins.

MATERIALS AND METHODS

Preparation of chicken muscle soluble proteins

Chicken muscle soluble proteins (CHMSP) were prepared from 1 Kg of trimmed, well-mixed, hand deffated chicken meat. Representative 100 g samples were thoroughly homogenised in 300 ml of a saline (0.85% NaCl) solution and the soluble proteins were extracted by gentle agitation for 24 h at 4ºC. Insoluble material was removed by centrifugation at 1500 g for 5 min at 4ºC and the supernatants filtered through a Whatman No.1 filter paper and lyophilised. The dried CHMSP were stored in an airtight container at -20ºC until required for use.

Preparation of chicken specific proteins

Chicken muscle soluble proteins with species-specific epitopes were isolated by immunoadsorption chromatography. The chicken-specific fraction was obtained by passing 30 mg of the freeze-dried CHMSP diluted in 10 ml of phosphate buffered saline (PBS), pH 7.2, through a Protein A-Sepharose CL-4B column (Pharmacia Fine Chemicals, Uppsala, Sweden) containing 571 mg of Sepharose coupled to 40 mg of chicken-specific antibodies previously produced against chicken muscle soluble proteins and rendered species-specific by affinity chromatography (Martín et al., 1988c). The adsorbed chicken specific proteins (CHSP) were released from the column by elution with 0.05 M diethylamine buffer, pH 11.5, and the eluted fractions showing an absorbance at 280 nm higher than 0.1 were pooled, adjusted to pH 7.2 with 0.5 M sodium phosphate buffer, dialysed overnigth against PBS and liophilised.

Electrophoretic separation

Chicken muscle soluble proteins (CHMSP) and chicken-specific proteins (CHSP) were separated electrophoretically in sodium dodecyl sulphate-polyacrilamide gels (SDS-PAGE), essentially according to the method described by Laemmli (1970).

SDS-PAGE of the CHMSP (50 μg) and the CHSP (50 μg) fractions was perfor-

med in tubes containing a 3% stacking gel and a 12.5% separating gel. Electrophoresis was performed at 1.5 mA tube for 4 h. Gels were stained with 0,25% Coomassie Blue G-250 in 45% methanol plus 9% acetic acid at 37ºC for 2 h and destained in 5% methanol plus 7% acetic acid in distilled water. The electropherograms were obtained following a spectrofotometric scanning (580 nm) of the resulting Coomassie Blue stained gels. The protein markers were from a low molecular weight protein standard (Bio-Rad Laboratories, Richmond, CA 94804, USA).

RESULTS

SDS-PAGE of the chicken muscle soluble proteins (CHMSP)revealed the existence in the gel of 15 protein subunits (Figure 1A), of an apparent MW between 17 KDa and 150 KDa. SDS-PAGE results of the affinity chromatography recovered (CHSP), showed the presence in the gel of 12 protein subunits (Figure 1B); three of the subunits of an apparent MW of 30 KDa, 58 KDa and 94 KDa were found strongly enriched after the immunopurification step.

CONCLUSIONS

Hybridoma technology does not required highly purified antigens as a prerequisite to a succesful fusion. However, as muscle soluble proteins are highly conserved between species, it was considered of interest to use specific antigens to obtain chicken-specific monoclonal antibodies. The three protein subunits enriched by immunoadsorption chromatography should have the highest potential to induce the formation of monoclonal antibodies of required specificity. Further work using these enriched chicken-specific proteins has resulted in the rapid isolation of monoclonal antibodies specific to chicken proteins. One of the monoclonal antibodies strongly reacts with a protein subunit of an apparent MW of 58 KDa (Martín et al., 1989), identified as pyruvate kinase using a commercial preparation of the enzyme im immunobloting experiments.

ACKNOWLEDGMENTS

Tis work is supported by a grant No. 0750-84 from the Comisión Asesora de

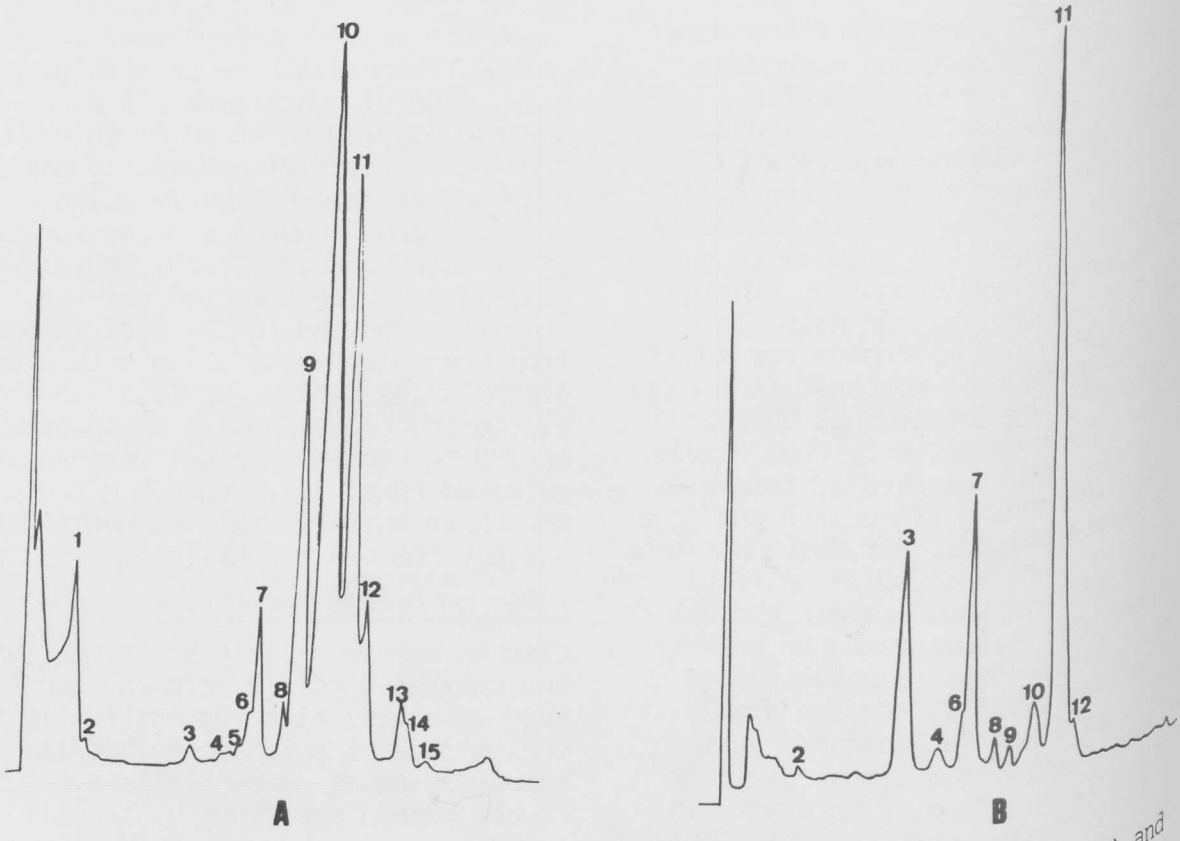

Figure 1. SDS-PAGE resolution of the chicken muscle soluble proteins (A) and chicken-specific proteins (B) after the immunopurification step.

Investigación Científica y Técnica (CAICYT), SPAIN.

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