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Technical Note

A Preliminary Note on the Detection and Partial Characterization of Chicken Muscle Soluble Proteins by Immunoelectrophoresis in Agarose Gels

ABSTRACT

Immunoelectrophoresis in agarose gels has been used to detect and partially characterize specific protein precipitin bands of chicken muscle soluble proteins (CHSP), free of crossreactions with muscle soluble proteins of cow (CSP), pig (PSP) and horse (HSP). Six precipitin bands were obtained by reacting CHSP against an anti-CHSP antiserum produced by a rabbit. These precipitin bands did not appear when the protein extracts from cow, pig and horse were analyzed against the same anti-CHSP antiserum. The six precipitin bands were specific of the chicken muscle soluble proteins. This technique may have the potential to detect the presence of chicken meat in unheated ground meat products.

INTRODUCTION

The possibility that unheated ground-meat products may contain the flesh of species not indicated by the product description, whether by accident or intention, is certainly not a new problem. Some sausages, burgers and many delicatessen products contain the flesh of more than one species (Deschreider & Meaux, 1974) or vegetable proteins (Parsons & Lawrie, 1972). A simple, rapid test would make it possible to determine the addition of undeclared meat in a product and give the consumers greater protection.

Methods of determining species origin are commonly based on immunological antibody–antigen reactions using various forms of precipitin test. Antisera to muscle proteins (Warnecke & Saffle, 1968) or

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to blood serum (Karpas *et al.*, 1970) have been used in agar gel immunodiffusion (Hayden, 1981), isoelectric focusing (Sinclair & Slattery, 1982), radioimmunoassay (Johnston *et al.*, 1982) and ELISA procedures (Kangethe *et al.*, 1982; Whittaker *et al.*, 1983).

In an effort to detect specific protein bands of chicken muscle soluble proteins, free of crossreactions with muscle soluble proteins of cow, pig and horse, the technique of immunoelectrophoresis in agarose gels has been used. These specific protein bands may indicate the presence of chicken meat in fresh meat products.

MATERIALS AND METHODS

Preparation of the antigenic extracts

Skeletal muscle tissue from chicken (*Ms. pectoralis* and *Ms. supracoracoideus*), cow (*Ms. rectus femoris*, *Ms. vastus medialis* and *Ms. vastus lateralis*), pork (*Ms. intercostalis externi* and *Ms. trapezius*) and horse (*Ms. glutaeus superficialis* and *Ms. biceps femoris*) in a total weight of 250 g were finely triturated, minced and homogenized in 500 ml of a 0·85% saline solution. The soluble proteins were extracted by constant agitation of these homogenates for 1 h at 1 °C. The protein extracts were filtered through a Whatman No. 1 filter paper, lyophilised, and the dried extracts placed in an airtight container and stored at −20 °C until use.

Preparation of the antiserum

Serum containing suitable CHSP antibodies was obtained by injecting, subcutaneously, New Zealand male rabbits with single doses of lyophilised chicken protein extracts (48 mg) in 2 ml of deionized and distilled water emulsified in 0·5 ml of Freund complete adjuvant (Difco). Fifteen booster doses were applied subcutaneously every 4 days for 62 days. The rabbits were periodically bled from the marginal ear vein and the blood was allowed to clot at room temperature and centrifuged at 1000 g for 10 min to remove any remaining blood cells present.

Agarose gel immunoelectrophoresis

The basic technique was that of Grabar & Williams (1953) modified by

Scheidegger (1955). Consequently, only the details relevant to this application are given here. 1 % agarose gel in veronal buffer, pH 8·6, was used. The gels were punched with a well cutter giving a hole of 1 mm in diameter and a band of 0·2 × 5 cm. 1 μl (24 μg) of the antigenic extracts were deposited in the hole. The plate was subjected to electrophoresis for 4 h at 130 V. When the electrophoresis was concluded, the plate band was filled with 0·3 ml of the corresponding antiserum. Immunodiffusion was performed for 18–24 h at 37 °C. The protein precipitin bands were visualized with Amido black (Clausen, 1981*a*). The immunochemical partial characterization of these bands comprised the detection of glycoprotein fractions according to Nadi's method (Clausen, 1981*b*) and lipoprotein fractions with Sudan black (Uriel *et al.*, 1963). The degree of displacement of each band relative to bovine serum albumin (Fraction V Cohen, Sigma Chemical Co.), used as standard, was defined as follows:

$$\text{absolute mobility} = \frac{\text{band displacement (mm)}}{\text{V mm}^{-1}\,\text{s}}$$

$$\text{relative mobility} = \frac{\text{band displacement (mm)}}{\text{bovine serum albumin displacement (mm)}}$$

$$\text{per cent mobility} = \text{relative mobility} \times 100$$

RESULTS AND DISCUSSION

To detect specific protein bands of chicken muscle soluble proteins (CHSP), free of crossreactions with muscle soluble proteins of cow (CSP), pig (PSP) and horse (HSP), lyophilised soluble protein extracts of each animal species were analyzed by immunoelectrophoresis in agarose gels against an anti-CHSP antiserum, produced by a rabbit.

The lyophilised chicken muscle soluble protein extracts (CHSP) analyzed against an homologous antiserum (anti-CHSP) allowed the identification of six protein precipitin bands of per cent mobilities between 17 ± 0·27 and 85 ± 1·21 % (Table 1 and Fig. 1). Three of the six observed protein precipitin bands were arbitrarily defined as major bands (bands No. 1, 3 and 6) by their strong staining with Amido black. The remaining three bands were defined as minor bands by their weak staining with the same dye. The partial immunochemical characterization of these

TABLE 1

Absolute, Relative and Per cent Mobilities of Protein Precipitin Bands of Chicken Muscle Soluble Proteins (CHSP) Against a Rabbit Homologous Antiserum (Anti-CHSP)[a]

Precipitin band (number)	*Band displacement (mm)*	*Absolute mobility*	*Relative mobility*	*Per cent mobility*
1	6·5	0·75 ± 0·06	0·17	17 ± 0·27
2	11	1·27 ± 0·11	0·29	29 ± 0·29
3	16·5	1·90 ± 0·18	0·44	44 ± 0·44
4	23	2·66 ± 0·23	0·62	62 ± 0·37
5	29·5	3·41 ± 0·35	0·79	79 ± 1·66
6	31·5	3·64 ± 0·35	0·85	85 ± 1·21
B. Albumin	37	4·28 ± 0·34	1·00	100
Dextran	0·0	0·0	0·0	0·0

[a] The degree of displacement of each band relative to bovine serum albumin used as standard was defined as stated in Materials and Methods. The numbers refer to mean value plus standard deviations from 14 independent assays.

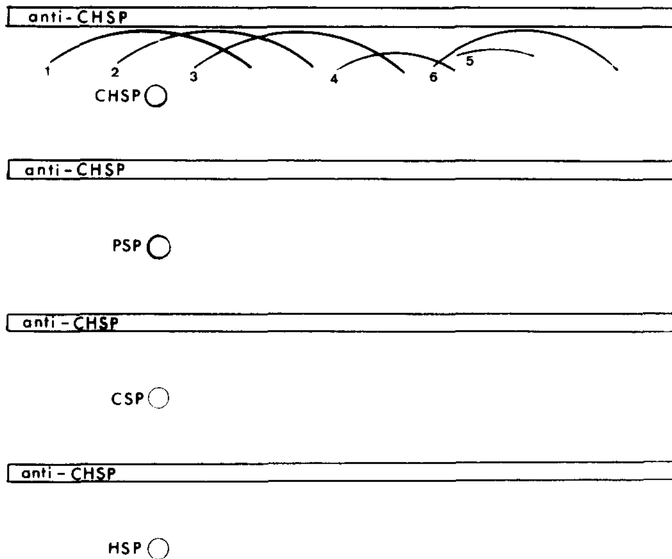

Fig. 1. Protein precipitin bands from CHSP.

TABLE 2
Detection and Immunochemical Characterization of Protein Precipitin Bands of Chicken Muscle Soluble Proteins (CHSP) Against a Rabbit Homologous Antiserum (Anti-CHSP)

Immunization period		*Precipitin bands (band number)*		*Immunochemical characterization (band number)*	
Bleeding	*Days*	*Major*	*Minor*	*Glycoprotein*	*Lipoprotein*
B_1	12	1, 3, 6	—	—	3
B_2	28	1, 3, 6	—	—	3
B_3	48	1, 3, 6	2, 4	2	3
B_4	62	1, 3, 6	2, 4, 5	2	3

bands (Table 2) to detect the presence of glycoprotein and lipoprotein fractions in their structure resulted in one glycoprotein band (band No. 2) and one lipoprotein band (band No. 3).

When the lyophilised muscle soluble protein extracts of cow (CSP), pig (PSP) and horse (HSP) were analyzed against an anti-CHSP antiserum (Fig. 1), no precipitin band was observed. Therefore, the six protein precipitin bands obtained when we analyzed the chicken muscle soluble proteins (CHSP) against a rabbit homologous antiserum (anti-CHSP) are specific chicken protein precipitin bands. These specific chicken protein bands may indicate the presence of chicken meat in meat products, when meat soluble protein extracts are analyzed against an anti-CHSP antiserum.

Immunoelectrophoresis in agarose gels to differentiate proteins from different animal species has been used with antisera against serum proteins as reference patterns (Karpas *et al.*, 1970). We believe that the use of muscle soluble protein antisera instead of antisera against serum protein may improve this assay, due to the fact that it is more rigorous to analyze muscle soluble proteins against anti-muscle soluble proteins than muscle soluble proteins against anti-blood serum proteins. On the other hand, the sensitivity of immunoelectrophoresis in agarose gels to detect the presence of species-specific protein bands may be exploited in future studies to isolate those proteins and to use them as diagnosis agents for testing unprocessed meat for contamination by extraneous animal species.

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Carmen Casas, Jose Tormo, Pablo E. Hernandez & Bernabe Sanz
Departamento de Higiene y Microbiología de los Alimentos,
Facultad de Veterinaria,
Universidad Complutense, 28040 Madrid,
Spain

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