DETERMINATION OF THE MOLECULAR WEIGHT OF PARTIALLY PURIFIED THERMOSTABLE MUSCLE ANTIGEN USING GEL FILTRATION TECHNIQUE

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SUMMARY

Thermostable muscle antigens have proved useful in meat species identification of fresh, heat-treated meat and meat products using various immunological techniques. The molecular weight of the partially purified thermostable muscle antigen was determined using gel filtration. Using a molecular weight standard curve the thermostable muscle antigen was found to have a molecular weight of 210kd.

INTRODUCTION

Species identification of cooked, autoclaved meat or meat products requires isolation and use of a meat component that is (i) robbust to processing temperatures (ii) characteristic of meat and (iii) incapable of being added per se to enhance the apparent meat content. Thermostable muscle antigen(s) have been shown to fulfil the above three criteria. Thermostable muscle antigens have been used in species identification of fresh meat,(Kang'ethe *et al.* 1985) heat-treated meats (Kang'ethe and Lindqvist 1987a) and internal organs (Kang'ethe and Lindqvist 1987b) using various immuno assay techniques (Kang'ethe *et al.* 1985; Kang'ethe and Gathuma 1987)

Despite the usefulness of antisera to TMA for species identification of fresh cooked and or heat-treated meat and meat products, the physico-chemical characteristics of the TMA have not been elucidated. This paper describes the molecular weight determination of the TMA using gel filtration.

MATERIALS AND METHODS

Preparation of partially purified TMA (PTMA)

Thermostable muscle antigens were extracted from meat samples using a modification of the original method of Milgrom and Witebsky (1962) as follows:

The meat samples were trimed off external fat and connective tissue, minced and homogenised in saline. The homogenate was sonicated at 300W using a Braunsonic 1510 instrument (B.Braun Melsungen AG, W. Germany) for 10 min at in 1 min bursts. The sonicated homogenate was centrifuged a 2000 x g and the supernatant filtered through a Whatman filter paper No 3. The filtrate was centrifuged at 86000 x g for 30 min and the supernatant autoclaved at 121°C for 30 min. The autoclaved material was centrifuged at 2000 x g for 15 min and the antigens precipitated from the supernatant by the addition of 3 volumes absolute ethanol. The mixture was left at room temperature overnight, the precipitate separated by centrifuging at 2000 x g for 15 min, and excess ethanol removed by drying in a Rotavapor (R) (Bauchi, Flawil Switzerland). The dried precipitate was dissolved in a small volume of saline and concentrated by ultrafiltration in an Amicon cell with a PM 30 membrane having a molecular weight cut-off of 30 000 daltons. The concentrated fraction was then applied to a Sephadex G-200 Column (height 95.5cm), eluted using phosphate buffered saline (PBS) and fractions of 7ml/tube collected.

Detection of the antigen in the fractions using ELISA

The G-200 fractions were diluted 1:2 in the coating buffer (0.05M carbonate-bicarbonate buffer pH 9.6), and 100µl/well of each antigen

dilution was used to coat the microtitre plates (Dynatech M 129A Micro ELISA). Coating was carried out at room temperature, overnight in a humid chamber. The plates were washed five times using 0.15M phosphate buffered saline pH 7.2 containing 0.05% Tween 80 (PBS-T) and 100µl/well of the aborbed goat anti-TMA diluted 1:80 in the serum diluent (0.05M phosphate buffer pH 8.0 + 75g KCL + 2.5g benzoic acid + 1g EDTA + 0.1% Tween 80 + 5% normal rabbit serum per litre and pH final adjusted to 7.5) was added to the plates and incubated for 1 hour at 37°C. The plates were washed 5 times with the PBS-T and 100µl/well of the rabbit anti-goat- IgG - Glucose Oxidase conjugate prepared according to the method of Wilson and Nakane (1978) diluted 1/1000 in the conjugate buffer added. The plates were incubated for 1 hr at 37°C. The conjugate diluent was similar to the serum diluent except that Tween 80 was used at 0.5% instead of 0.1%. The plates were washed five times using PBS-T and the substrate added 100µl/well. For one microtitre plate, the substrate solution was prepared by mixing 10ml of 0.05M ammonium citrate buffer pH 5.0 + 1ml of 20% glucose + 0.1ml of ABTS 25mg/ml in distilled water, and 0.1ml of peroxidase type II (sigma) 1mg/ml in ammonium citrate buffer pH 5.0. The plates were incubated for 1 hour at room temperature and the absorbance read at 410nm using ELISA mini reader (Dnynatech,Santa Monica CA). The fractions containing the PTMA were pooled and concentrated by ultrafiltration.

Calibration of Sephadex G-200 Column

A chromatographic column 94.2cm in length and 2.6cm internal diameter was packed with swollen Sephadex G-200 (Pharmacia) as

described by Hudson and Hay (1980). After equilibration with PBS, the column was calibrated using the following protein markers:

Blue dextran	72,000,000	daltons
Purified IgG	150,000	"
Bovine serum Albumin	67,000	"

The calibration was repeated twice and the average void and elution volumes of the protein standards determined. The Kav constants for each marker was calculated using the following formula:

(Gel filtration : Theory and practice pharmaine fine chemicals, Uppsala Sweden)

$$Kav = \frac{Ve - Vo}{Vt - Vo}$$ where

Ve = void volume (elution volume of blue dextran)

Vo = elution volume of the other protein marker

Vt = Total bed volume

Kav = The fraction of the stationery gel volume which is available for diffusion of a given solution.

Partially purified TMA was mixed with 1mg of blue dextran 20mg of human IgG and 40mg of bovine serum albumin in PBS to total volume of 3ml and were applied to the calibrated column. The PTMA was detected in the eluted fractions by EIA as described above.

The Kavs of the protein markers were plotted against their corresponding molecular weights on a semilogarithimic graph paper . The best line which joins the points on the graph paper was drawn to give the molecular weight standard curve using the least square method.

The elution volume and the Kav of the PTMA was calculated. The molecular weight of the PTMA was read off the standard curve.

RESULTS AND DISCUSSION

The results of the fractionation of the TMA on Sephadex G-200 and antigen detection using EIA, are shown in Fig 1. The absorbance values were measured at 280nm and the antigen activity determined by the EIA at 410nm. The major portion of the antigen was eluted just after the void volume. Those fractions from the Sphadex G-200 column containing the PTMA showed low absorbances at 280nm indicating low protein content. The apparent low absorbance at 280nm of the Sphadex G-200 fractions containing the PTMA may be due to the dilution factor of the specific antigen. Fractions that showed high absorbance at 280nm contained no PTMA activity as demonstrated by EIA.

Figure 2, shows the elution profile from the Sephadex G-200 of the PTMA and the molecular weight markers. This shows that the PTMA was eluted just after the void volume and before the elution peak of the purified IgG. Figure 3 shows the molecular weight standard curve and the extrapolated reading of the molecular weight of the PTMA calculated to be 210,000 daltons.

Whether PTMA is a single protein molecule or an aggregate of many due to denaturation and renaturation, is still unknown and investigations to accertain the nature of PTMA are in progress. This will form the basis of our future communication.

ACKNOWLEDGEMENTS:

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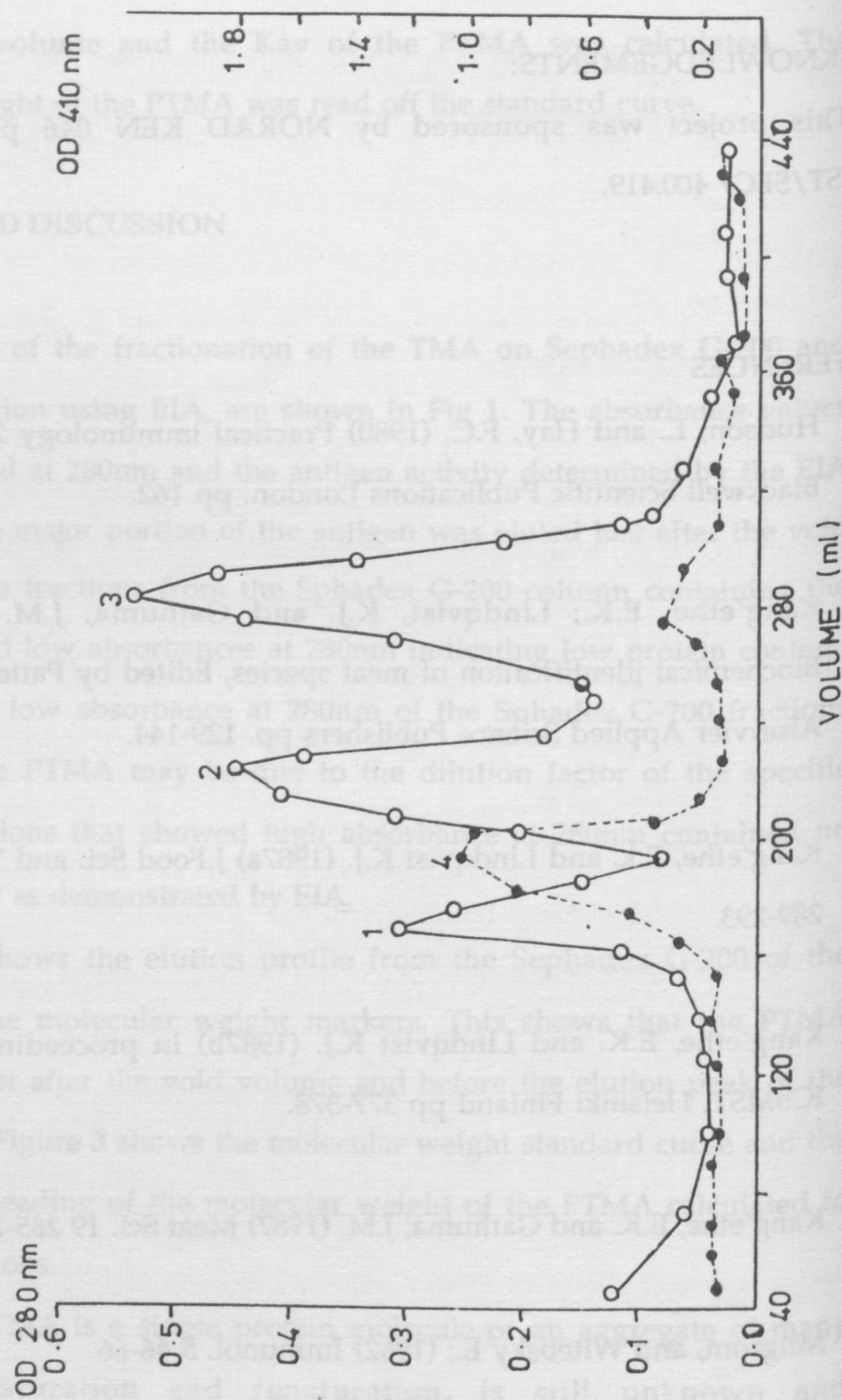

Figure 1: Fractionation of the TMA on a Sephadex G-200 column —O——O——O— OD 280nm and detection of the PTMA by EIA,-•---•---•---•--- OD 410nm.

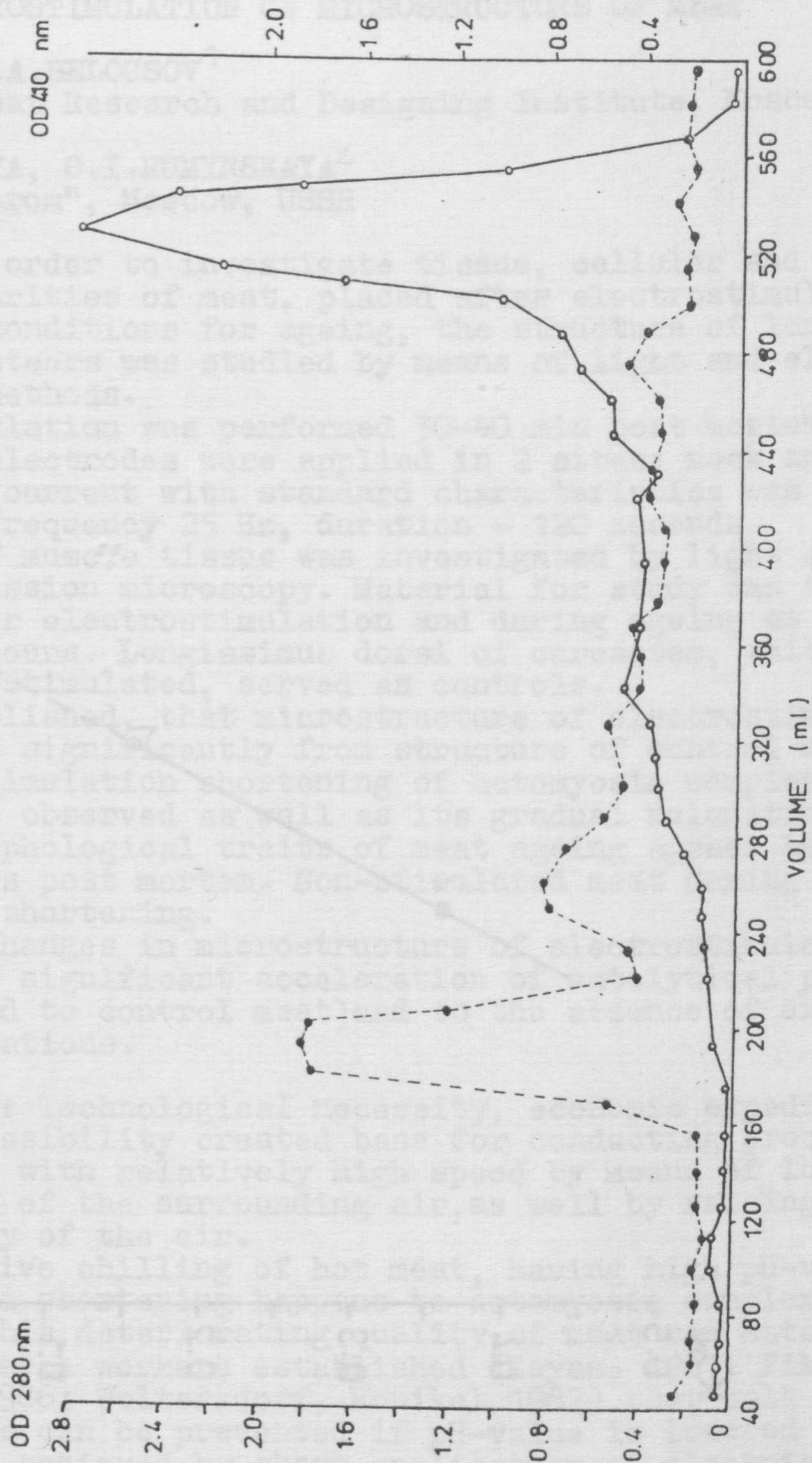

Figure 2:

Elution profile of the PTMA --•--•--•-- OD 410nm and protein makers -o-o-o-o— OD 280nm through a Sephadex G-200 column 1= blue dextran, 2= human lg G 3= bovine serum albumin.

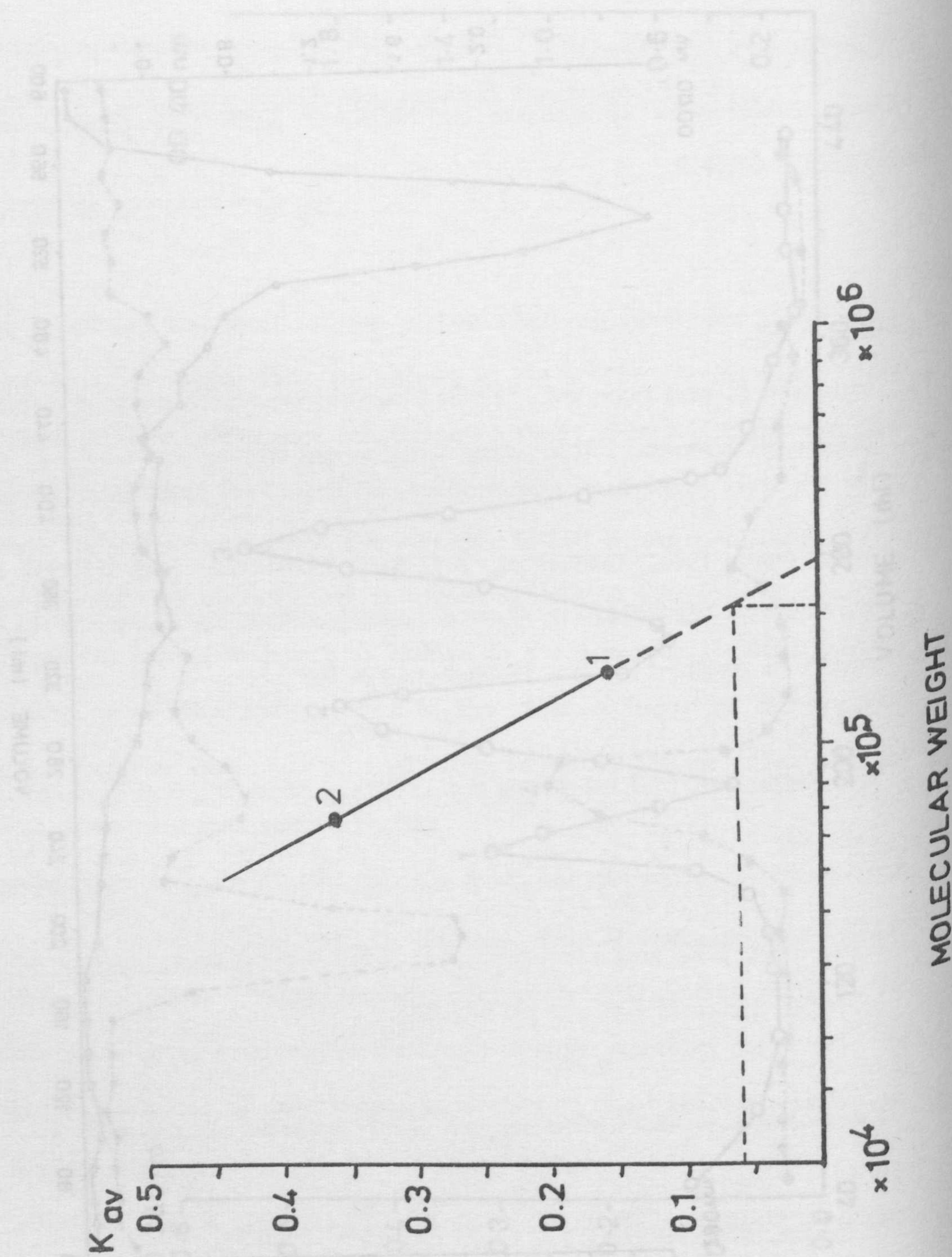

Figure 3: Standard curve for the molecular weight determination using Sphadex G-200 column. (1 = human Ig G,150kd; Kav = 0.15 and 2= BSA 67 kd; kav = 0.36).