加熱変性されたマグロ血清蛋白より種特異的抗原性の回復

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Short Paper

Recovery of Species-specific Antigenecity from Heat Denatured Serum Protein of Tuna*[1]

To identify the original fish species in heat processed fish products, electrophoretic and/or chemical methods have been proposed recently by some investigators.[1-5] These methods, however, still leave much problems to be solved. Serological method has been known to be excellent to identify the fish species if the sample is raw, but for a heated sample, especially when heated over 80°C, the method has been considered impossible to apply, because most of the proteins become insoluble and the native antigenecity of the proteins are lost by heat denaturation. The present preliminary experiments described below, however, indicate that the protein fragments recovered from boiled serum of tuna still show the species-specific antigenecity.

Serum samples were obtained from living specimens of yellowfin tuna *Neothunnus macropterus*, carp *Cyrinus carpio*, eel *Anguilla japonica*, cattle, and pig. Antisera were made by immunizing rabbit with intravenous injection of yellowfin serum.

Heat denaturation and recovery of active preparation: Aliquots of intact serum of fish and animal were heated on a steam bath for 30 min in covered test tubes. The content of tube was homogenized and the aliquot of homogenate was taken into another small test tube. After the addition of SDS-urea solution (1% each of sodium dodecyl sulfate and 2-mercaptoethanol in 8 M urea), the tube was sealed and incubated at 37°C for about 24 hr with occasional shaking by a stirring machine. The heat denatured serum was observed to resolve almost completely to give a transparent solution. The aliquot of this solution was passed through a column of Bio-Rad AGX210 and eluted with 0.05 M phosphate buffer (pH 7.0) to eliminate the SDS from SDS-protein compound according to LENARD's method.[6] The effluent was dialyzed against 0.9% saline, followed by condensation by way of dialysis against 50–60% polyethyleneglycol 4000 at 35°–40°C.

Micro-gel diffusion analysis: Hot solution of 1.5% agarose was poured on a microscope slide to prepare thin layer of agarose gel, and the small wells were punched in the gel. The micro-volumes of native sera or the preparations recovered from heat denatured sera were put in the wells of gel, then the precipitating reaction with anti-yellowfin serum were observed after incubation in a wet chamber. Results are given in **Fig. I.** As shown in **Fig. 1-1**, species-specific bands of precipitation were detected between the native yellowfin sera and the anti-yellowfin serum; besides the recovered

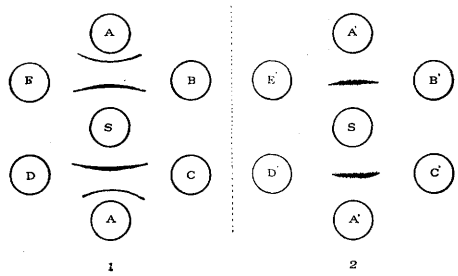

Fig. I. Results of micro-gel diffusion analysis. Central wells **S** contained the anti-yellowfin serum. Surrounding wells in the left figure-I contained native sera, and those on the right figure-2 contained the preparations recovered from heat denatured sera.
A, A′ . . . yellowfin tuna, **B, B′** . . . eel, **C, C′** . . . carp, **D, D′** . . . cattle **E, E′** . . . pig.

preparation from heat denatured serum of yellowfin still showed specific antigenecity as given in **Fig. I-2.** However, the parallel experiment proved that the supernatant solution which was separated centrifugally from heat denatured serum did not show any specific antigenecity. On the basis of these results, it may be concluded that the principal parts of primary conformation which concern to the species-specific antigenecity of serum protein are not destroyed by heat denaturation under the conditions of present experiment, thus the polypeptides recovered from the denatured serum still retained their specific antigenecity. The author believes the above described method will serve not only to identification of the fish species in the processed products but also to investigation of the structures of proteins.

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