Purification of Early Pregnancy Factor from Cattle Sera

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(Received August 2, 1991)

Key words: EPF, RIT, bovine

Early Pregnancy Factor (EPF) is specific substances to the gestation period, which was found by MORTON *et al.*⁵⁾. It has been detected in many animal species such as human, cattle, sheep, swine, and rat since then. This EPF, which appears from immediately after fertilization to the second trimester, plays an important role in the immunological relationship between the fetus and its mother in early gestation in particular.

At present, EPF is detected with the Rosette Inhibition Test. However, it requires much time and only a small number of samples can be tested at one time. Therefore, a modification of Rosette Inhibition Test is needed. In addition, WILSON *et al.*⁷⁾ isolated EPF from pregnant ewe's sera and reported that the fractions which had high EPF activity had molecular weights of approximately 20 KD and 67 KD. CLARKE *et al.*²⁾ isolated the materials of molecular weight 12 KD and 25 KD from ovine placental extracts. They reported that the main component of the preparation was a 12 KD polypeptide which contained a highly reactive thiol group, and oxidation might occur during isolation with the result that the final

preparation was a mixture of the 12 KD polypeptide and a 25 KD disulphide linked dimer. MEHTA *et al.*³⁾ purified EPF from human pregnancy sera and confirmed that the purified protein had an apparent molecular weight of 21.5 KD as analyzed by SDS-PAG electrophoresis.

Therefore, in this study, we attempted the purification of EPF from bovine pregnancy sera according to the methods of WILSON *et al.*⁷⁾. We used Ion-exchange chromatography and FPLC-gel permeation chromatography, and the EPF active fraction obtained was immunized to Rabbit for the study of antigenicity.

Materials and Methods

The serum of 5 l was collected from pregnant bovines (n = 11). The gestation period of these bovines was 5 days ~ 6 weeks after artificial insemination. The gestation of each bovine was confirmed with the Rosette Inhibition Test according to the methods of MORIYAMA *et al.*⁴⁾ or rectal palpation.

The procedure of purification was performed according to the method of WILSON *et al.*⁷⁾

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(Fig. 1), and was advanced according to the Rossette Inhibition Titer (RIT). When the value of RIT was above 4 and below 3, we regarded that the EPF activity was positive and negative, respectively. This 5 l of serum was dialyzed with 20 mM phosphate buffer (pH 8.0) and applied to the DEAE-Sepharose Fast Flow (Pharmacia LKB Co., Ltd.) column.

Next, the unbound fraction was dialyzed with 50 mM ammonium acetate buffer (pH 5.0) and applied to the CM-Sepharose Fast Flow (Pharmacia LKB Co., Ltd.) column. Then, the fraction which was eluted by 50 mM NaCl in 50 mM ammonium acetate buffer (pH 5.0) was applied again to the DEAE-Sepharose Fast Flow column for advanced purification. The

Fig. 1. Purification proceduer and results of RIT

obtained unbound fraction was immunized with two rabbits to obtain antiserum, and the remaining unbound fraction was applied to the FPLC-gel permeation column (Pharmacia LKB Co., Ltd.) to determine the molecular weight of EPF.

On the other hand, the EPF active fraction (DEAE-1C) was immunized with two rabbits. The obtained antiserum was absorbed according to the method of CAVANAGH¹⁾. Namely, the IgG of antiserum was isolated by the ammonium sulphate precipitation method. Next, this IgG was absorbed by CNBr-activated Sepharose 4B (Pharmacia LKB Co., Ltd.) which bound non-pregnancy bovine serum protein according to the method of CAVANAGH¹⁾. Using this absorbed IgG, the neutralization test and Immunoblotting method were performed for the biological study of EPF.

Results and Discussion

The present study described the purification of EPF from pregnant bovine sera of 5 days ~ 6 weeks after artificial insemination. As shown in Fig. 1, the pooled serum had a RIT of 5. EPF activity of the DEAE-Sepharose Fast Flow column was recognized to only the unbound fraction and the value of RIT was 5. On the other hand, the EPF active fraction was eluted with 50 mM NaCl in 50 mM Ammonium Acetate buffer (pH 5.0) on the CM-Sepharose Fast Flow column and it had a RIT of 4. Therefore, EPF did not bind to the cation exchange gel but bound to the anion exchange gel. This result agreed with the reports of many researchers^{1,3,5,7)}.

Next, this EPF active fraction was applied on the DEAE-Sepharose Fast Flow column again. Only the unbound fraction (DEAE-1C) indicated EPF activity (RIT = 5).

This unbound fraction was applied on the FPLC-gel permeation column to determine the molecular weight of EPF. As a result, the EPF activity was found for a molecular weight between 15 ~ 30 KD by FPLC-gel permeation,

but the particular molecular weight of EPF could not be determined. WILSON *et al.*⁷⁾ reported that the molecular weight of EPF was 22 KD by HPLC of pregnant ewe's sera. In addition, MEHTA *et al.*³⁾ purified EPF from human pregnancy sera and obtained the EPF active moiety of molecular weight 21.5 KD by SDS-PAGE analysis. Therefore, we also thought that the EPF active moiety of bovine pregnancy serum existed in a molecular weight near 20KD.

The absorbed antiserum IgG was tested by Immunoblotting, but the difference between the non-pregnancy and pregnancy conditions was not confirmed. CAVANAGH *et al.*¹⁾, ROBERTS *et al.*⁶⁾, and CLARKE *et al.*²⁾ reported that EPF specific polyclonal antibody to the culture suspension of mouse oviduct and ovary, human urine, and ewe's placenta extraction prevented EPF activity by the neutralization test. In this study, we attempted the neutralization test, too. As a result, the RIT of pregnancy serum (≥ 4) decreased below 3 by the obtained antiserum IgG. Therefore, it was thought that the antiserum IgG contained a specific antibody to bovine EPF. However, we could not detect the specificity of anti-EPF antibody by the Ouchterlony method and Immunoblotting method. This point is a subject for future study.

Acknowledgments

The present study was supported in part by a Grant-in-Aid of Scientific Research (No. 01560282) from the Ministry of Education, Science, and Culture, Japan. The authors wish to thank to Mr. T. GOTO, Technical Research Center of Koiwai Farm Co. LTD., Japan, for providing pregnancy bovine sera, and Mr. S. YAMAGUCHI and Mr. H. TAKEUCHI, Iwate Technical Center of Daiichi Pure Chemicals Co. LTD., Japan, for help with purification of EPF.

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