A Hemostat Prepared with Fibrinogen, Factor XIII from Pig Blood and Microbial Transglutaminase

Ming-Tsao Chen, Tai-Li Hsueh and Deng-Cheng Liu

Department of Animal Science, National Chung-Hsing University, 250, Kuokung Rd.,
Taichung, Taiwan. E-mail:michen@dragon.nchu.edu.tw

Key words: fibrinogen, factor XIII, transglutaminase, hemostat, pig blood

Background

Animal blood contains many valuable materials such as proteins, iron, lipids and hormones. It mainly consists of plasma and blood cells. The plasma contains fibrinogen, thrombin and factor XIII which are associated with blood coagulation. Our lab is conducting research on the functional properties of plasma, globin and their influencing factors (Chen et al., 1984; 1987). We are also studying the characteristics of fibrinogen and thrombin which are isolated and purified from pig blood (Huang et al., 1992). Also being evaluated is the composition and storage quality of blood rice cake (Liu et al., 1993), purification and activity of transglutaminase (TGase) from pig blood (Tseng et al., 1999a, b; 2000)...etc. since there are many valuable biomedical materials found in blood. This research could increase the value of blood and this would reduce environmental pollution.. In this study, we are attempting to prepare a hemostat with fibrinogen, factor XIII and microbial transglutaminase.

Objective

The purpose of this study was to evaluate the hemostatic function of transglutaminase (factor XIII) from pig plasma and microbial TGase, and when mixed with fibrinogen as a hemostat.

Methods

Pig blood was collected from the local slaughterhouse and combined with anticoagulant for preparation of plasma by centrifugation. Porcine TGase (factor XII) (PTG) was prepared according to the procedures described by Folk and Chung (1985). In brief, the fresh pig plasma was treated with $BaCl_2$ and precipitated by glycine, then purified via gel filtration liquid chromatography, and elutes containing TGase were collected, freeze-dried. Microbial TGAse was prepared according to the methods described by Yeh et al. (2000). Streptoverticillium kentuckenseCCRC12429 was used for culturing to produce MTGase (MTG). The MTGase was prepared from the culture of S. kentuckense, and purified by precipitating with 55-75% ammonium sulfate and purified by CM-cellulose cation-exchange chromatography, followed by the same procedures used for PTGase. Fibrinogen was prepared from pig plasma according to the procedures described by Dirvakaran (1982). The hemostats were prepared with PTGase, MTGase, and mixed with fibrinogen (F), separately.

Testing animal : New Zealand white rabbits (female, 30months of age, 3.5kg of live weight) were used as testing animals to study hemostatic function of TGases and fibrinogen. The veins of both ears of testing animals were cut surgically for bleeding and then sprayed with PTGase, MTGase, FPTGase and FMTGase on the wounds, separately, and their coagulating time was recorded which was used to express the hemostatic function (Fig. 1).

The microstructures of clots were also examined by using scanning electron microscopy (Ashie et al., 1997).

Results and Discussion

Fig. 2 showed the SDS-PAGeltectrophoretogram of PTGase prepared from pig plasma. As shown in Table 1, it was observed that the yield of PTGase was higher (50%) by precipitated with 40% ammonium sulfate. Fig. 3 showed the SDS-PAGE of MTGase prepared from the culture of S. kentuckense, and table 2 showed the yield, and activity of MTGase. The yield and activity of PTGase were higher when compared with those of MTGase. SDS-PAGElectrophoretogram is shown in Fig. 4 for fibrinogen precipitated at different pHs. The higher yield of fibrinogen was obtained at pH 5.4 to 5.5 of plasma.

To compare the hemostatic function, the powder of PTGase, MTGase, fibrinogen and the mixtures both of theTgases with fibrinogen were sprayed on the wounds of the ears and their coagulating time was recorded, separately. The results indicated that fibrinogen had the shortest

coagulating time, followed by FMTGase, PTGase, FPTGase and MTGase was the longest time for clotting (Table 3). This means that fibrinogen, PTGase and FMTGase were very active on clotting on bleeding as caused by wounding. The microstructures of clots on wounds were observed the firmer clots caused by fibrinogen and both Tgases than that of the control (Fig. 5, 6 and 7). The observation of microstructure of clots on the wounds with PTGase and FMTGase individually had smaller holes and firmer network than that of the control.This result suggested that fibrinogen, PTGase and FMTGase were more efficiency on hemostatic function than that of FPTGase or MTGase. In this study, the similar microstructures of clots were also found with MTGase and FMTGase treatment, but the loose network was observed and these results indicated inferior clotting caused by only MTGase or FMTGase. The picture of Fig. 8 showed the healed status of wound after 7 days of treatment. However, no any organisms were found in the hemostats prepared from plasma and microorganism, but the aseptic process has to be done for medical clinic research in the future.

Conclusions

It is possible to prepare a hemostat from fibrinogen, porcine and microbial transglutaminases for medical clinic uses such as wound and bleeding caused by tooth extraction. However, an aseptic process has to be developed to produce a germ-free hemostat.

References

Ashie, I. N. A., B. K. Simpson and H. S. Ramaswamy. 1997. Changes in texture and microstructure of pressure-treated fish muscle tissue during chilled storage. J. Muscle Food. 8:13-32.

Chen, M. T., D. C. Liu and S. L. Guo. 1984. Utilization of Animal blood process in processed meat products. 1. The determination of functional properties of blood proteins and their affecting factors. J. Chinese Agri. Chem. Society. 22:201-206.

Chen, M. T., D. C. Liu and S. L. Guo. 1987. 2. The functional properties of porcine plasma proteins modified by phosphates and lactose. J. Agri. and Forestry. 36:93-102.

Divakaran, S. 1982.Animal blood processing and utilization, Central Leather Research Institute, Madras, India.

Folk, J. E. and S. I. Chung.1985.Transglutaminases. Methods in Enzymology. 113:3358-375.

Huang, H. J., D. C. Liu and S. L. Guo. 1992. Preparation of hemostat from porcine blood-study on properties of fibrinogen and thrombin. J. Chinese Society of Anim. Sci. 21:203-211.

Liu, D. C., M. T. Chen and S. L. Guo.1993. Studies on the composition and storage quality of blood cake. J. Chinese Society of Anim. Sci. 22:44463-468.

Tseng, T. F., M. T. Chen and D. C. Liu. 1998. Comparison of the transglutaminase activity, rheological properties and microstructure of pig and poultry blood. Fleischwirschaft. 78:1211-1213.

Tseng, T. F., D. C. Liu and M. T. Chen. 2000. Effects of TGase on the quality of low-salt restructured pork. J. Agri. Asso. of China. 1:229-236.

Tseng, T. F., D. C. Liu and M. T. Chen. 2000. Characteristics of TGase derived from pig plasma. J. Agri. Asso. of China. 1:452-458.

Tseng, T. F., D. C. Liu and M. T. Chen.2000. Evaluation of TGase on the quality of low-salt chicken meat balls. Meat Science. 55:427-431.

Yeh, Y. W. 2000. Purification, biochemical characteristics and application of TGase from Streptovercillium kentuckense CCRC12429, Master thesis, Department of Anim. Sci. National Chung-Hsing University, Taiwan.

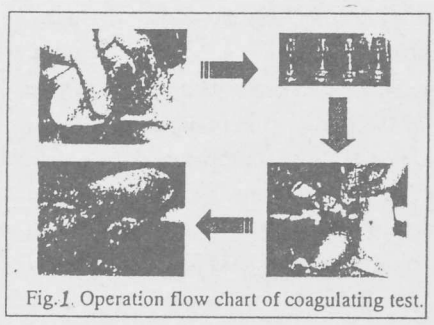


Fig. 1. Operation flow chart of coagulating test.

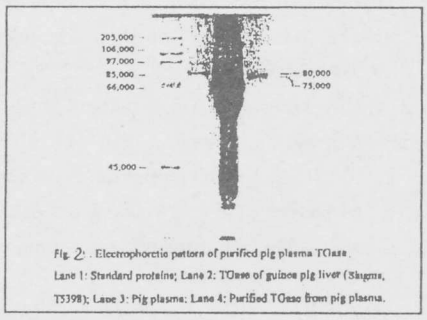


Fig. 2. Electrophoretic pattern of purified pig plasma TGase.
Lane 1: Standard proteins; Lane 2: TGase of guinea pig liver (Sigma, T5398); Lane 3: Pig plasma; Lane 4: Purified TGase from pig plasma.

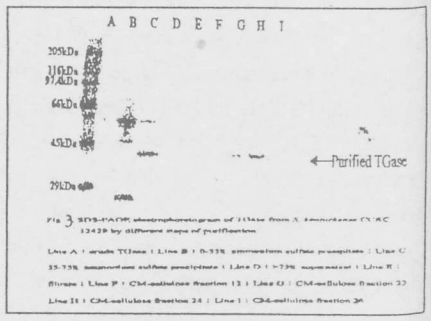


Fig. 3 SDS-PAGE electrophoretogram of TGase from S. hygroscopicus CGMCC 1242D by different steps of purification

Lane A : crude TGase ; Line B : 0-33% ammonium sulfate precipitate ; Line C : 33-75% ammonium sulfate precipitate ; Line D : >75% supernatant ; Line E : filtrate ; Line F : CM-cellulose fraction 12 ; Line G : CM-cellulose fraction 22 ; Line H : CM-cellulose fraction 24 ; Line I : CM-cellulose fraction 26

Table 1. Activity and protein concentration of TGase from the culture of *S.kentuckense*

Procedure	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Crude TGase filtrate	143.92	70.11	0.48	1.00	100
Ammonium sulphate precipitate (55-75%)	21.33	30.31	1.42	2.96	43.23
Dialysis	20.16	22.17	1.10	2.29	31.62

Table 2. Activity and protein concentration of TGase from pig plasma.

Procedure	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Plasma	3340	965.41	0.29	1.00	100
Ammonium sulphate precipitate (40 %)	224.53	481.15	2.14	7.38	49.84

Table 3. Comparisons on function of hemostats on testing animals.

Treatments	Control	Fg	PTG	MTG	PTGF	MTGF
Coagulating time (min - sec)	4'07"	2'28"	2'53"	3'46"	3'20"	2'40"

Fg : Fibrinogen ; PTG : transglutaminase (TGase) form pig plasma ; MTG : microorganism TGase ; PTGF : TGase form pig plasma mixed with fibrinogen ; MTGF : microorganism TGase mixed with fibrinogen.There are six rabbits of each batch.

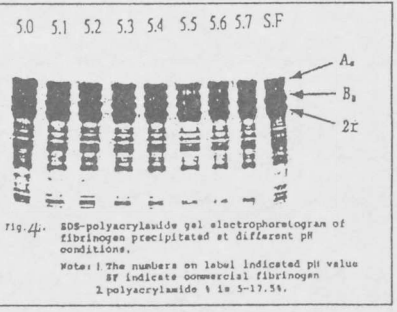


Fig. 4. SDS-polyacrylamide gel electrophoretogram of fibrinogen precipitated at different pH conditions.
Note: 1 The numbers on label indicated pH value
Sf indicate commercial fibrinogen
2 polyacrylamide % is 5-17.5%.

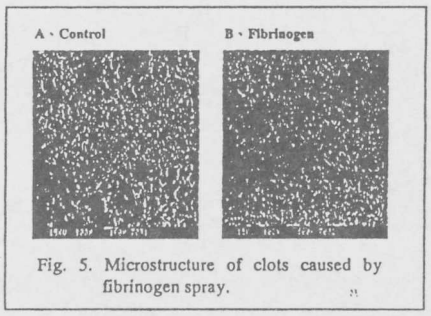
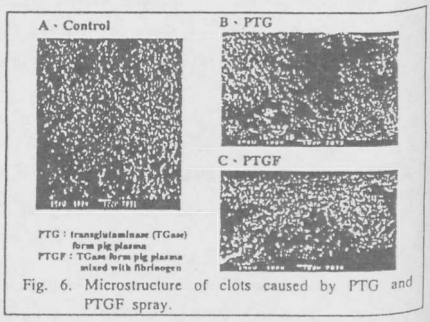
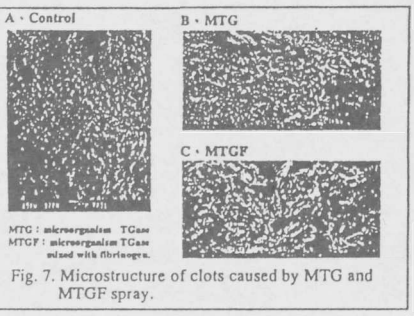


Fig. 5. Microstructure of clots caused by fibrinogen spray.



PTG : transglutaminase (TGase) form pig plasma
PTGF : TGase form pig plasma mixed with fibrinogen

Fig. 6. Microstructure of clots caused by PTG and PTGF spray.



MTG : microorganism TGase
MTGF : microorganism TGase mixed with fibrinogen.

Fig. 7. Microstructure of clots caused by MTG and MTGF spray.

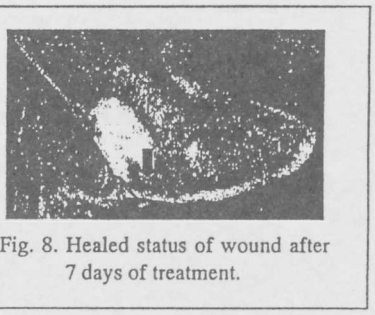


Fig. 8. Healed status of wound after 7 days of treatment.