Exploring The Use of Enzymes To Dissolve Blood Clots

Blake Randall and Sundararajan V. Madihally

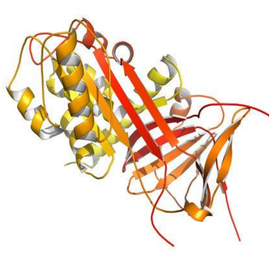
School of Chemical Engineering, Oklahoma State University, Stillwater, OK 74078

Abstract

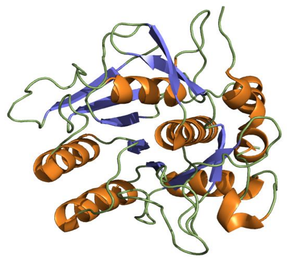
Coronary artery disease (CAD) is a condition caused by the buildup of plaque in the coronary artery. This plaque is mostly composed of fibrin and other components of blood clots. Coronary heart disease is caused by blood clots in the coronary artery and accounts for about 25% of deaths in the USA. Providing treatment or a way to lower the effects of CAD is very important. Finding a way to fight the formation of blood clots before they become hazardous would be a great step in the right direction.

The objective of this research is to explore possible ways to dissolve blood clots as an alternative to traditional methods for removing blockages in arteries. Nattokinase is an enzyme that shows potential to have the ability to dissolve blood clots or prevent blood from clotting. In this project, the effect of Nattokinase during the clotting process was explored in fresh horse blood. Using thromboelastography, properties such as clot strength and clot lysis were analyzed during the clotting process with varying concentrations of a Nattokinase solution. This study shows potential applications in situations where clot dissolution is necessary.

Nattokinase



Small Section of Fibrin Protein



Possible Structure of Nattokinase

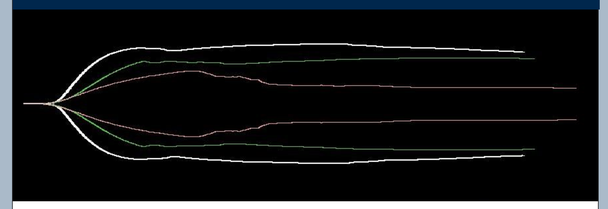
Acknowledgment

We thank the Lew Wentz Foundation for funding and Dr. Lyndi Gilliam for providing access to the TEG and donor animals.

Methodology

- Nattokinase (20000 Units/g) was obtained from Creative Enzymes (Shirley, NY). A stock solution of 1,000 Functional Units/milliliter (FU/mL) was made by mixing Nattokinase enzyme with phosphate buffer solution (PBS).
- From a donor horse, 3 mL of blood was drawn into a buffered sodium citrate collection tube. The blood was drawn. The blood was transferred to the lab and allowed to rest for 20 minutes from the time it was drawn.
- After 20 minutes various amounts of the enzyme were added to create a concentration of 80, 120, and 150 FU/mL within the sodium citrate collection tube. In addition, 100 µL of 40 µg/mL of kaolin solution was added to the blood to initiate the clotting process and the sample was gently inverted 5 times. For a control, no enzyme was added, but all other procedures were kept constant.
- From that mixture, 320 µL of blood with enzyme solution and 20 µL of 0.2 M calcium chloride solution was added (to reverse the effect of citrated buffer). The sample was placed into the cup of the TEG 5000 Thrombelastograph Hemostasis Analyzer (TEG) by Haemonetics. It is important to keep the agitation of the sample to a minimum when placing it in the TEG for consistency.
- The TEG was run for 60 min and viscoelastic property data were collected to understand the effect of Nattokinase on blood clotting.

Clot strength V. time



The outer white curve corresponds to 80 FU/mL, the green curve is the 120 FU/mL, and the pink curve corresponds to 150 FU/mL.

Homogenization From Enzyme

The photograph is an example of how there were some homogenizing affects observed. The sample on the left contains enzyme. This was taken 1 hour after mixing the enzyme with the blood and the sample was allowed to rest with no agitation. This was observed at a very high concentration and this sample had a concentration of 1450 FU/mL. No clotting was observed at this concentration.

Clot Characteristics

FU/mL	R(min)	K (min)	Angle (°)	MA (mm)	LY30 (%)	LY60 (%)
0	12.9	3.4	51.1	66.7	0.2	62.8
80	5.1	3.2	49.2	45.3	0.7	1
120	5.6	6.4	31.5	34.9	2.6	1.3
150	5.2	11.6	21.1	26.8	34.6	42.7

The table above shows clot characteristics by the TEG. Terminologies are defined below. In the presence of the enzyme, the clots had a much higher percentage of lysis compared to little or no lysis when the enzyme is absent.

Clotting Process	Variable	Definition	Normal Value	Value LOWER than normal	Value HIGHER than normal
Clotting Time or "Reaction" Time	R	Time until first fibrin strand development	4-8 minutes	Over stimulation of enzymatic pathway	Factor deficiency and/or residual heparin
				Give anticoagulant	Give FFP or protamine
Clot Kinetics	Angle deg or K	The rate of thrombin formation	47-74 degrees	Fibrinogen and factor VIII deficiency	Platelet hypercoagulabilty
				Give cryoprecipitate or FFP	No treatment needed
Clot Strength	MA	Maximum Amplitude	55-73 mm	Low platelet count, low fibrinogen, poor platelet function	Platelet hypercoagulabilty
	G	Shear elastic modulus strength (dyn/cm²)	6.0k-13.2k d/cm²	Give platelet transfusion	Start Antiplatelet therapy; add platelet mapping
Clot Stability	EPL	Estimated percent lysis overall after MA achieved	0-15 %		Primary fibrinolysis–high tPA levels
					Antifibrinolytic agents
	LY30	Amount of clot lysis 30 minutes after MA achieved	0-8 %		Primary fibrinolysis–high tPA levels
					Antifibrinolytic agents

Summary

- Clot lysis showed a strong effect of Nattokinase concentration. It can be seen that at these concentrations' clots are allowed to form, but then are degraded by Nattokinase enzyme.
- Clots were weaker, more lysed, formed at a slower rate, and fibrinolysis was beginning to be observed with higher enzyme concentration.
- It appeared that the blood was thinned due to the addition of the enzyme which could have some additional positive effects.
- Very high concentration of Nattokinase (1450 FU/mL) affected the clotting process. When high concentrations of Nattokinase were used, the clot did not form and it was found that the enzyme had a homogenizing effect when high enough concentrations were used.
- As the enzyme concentration is increased the clot strength decreases and clot lysis increases. This would be very favorable in the context of dissolving clots.
- At high concentrations of enzyme, the rate of dissolution is higher than the rate of clot formation. This is a possible reason no clot was formed and the blood was seemingly homogenized. While this is not a practice we see now as a chemical treatment for blockages due to blood clots, there may still be some applications.

Future Work

- Determine a safe concentration of enzyme in body.
- Determine if there are any undesirable reactions in the body at high concentrations of Nattokinase
- More tests are needed to understand the mechanism, but this could show promise as a preventive treatment.
- Could this be used as a replacement for blood-thinners in some situations?