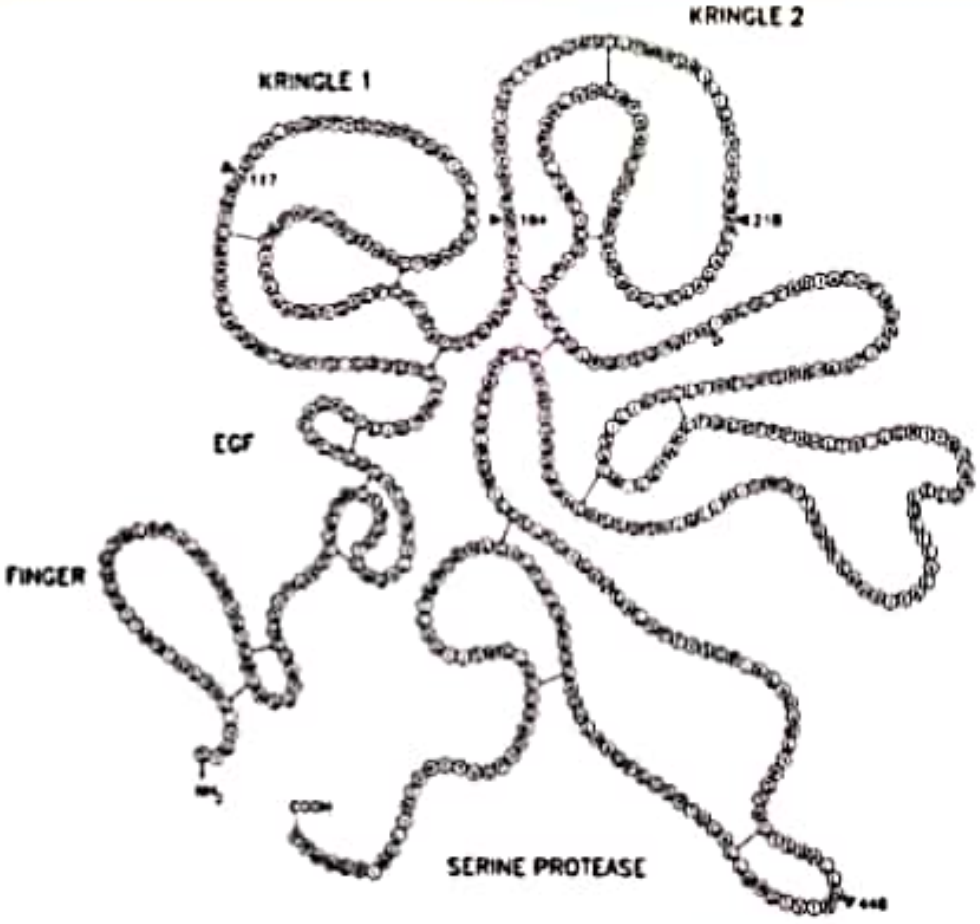
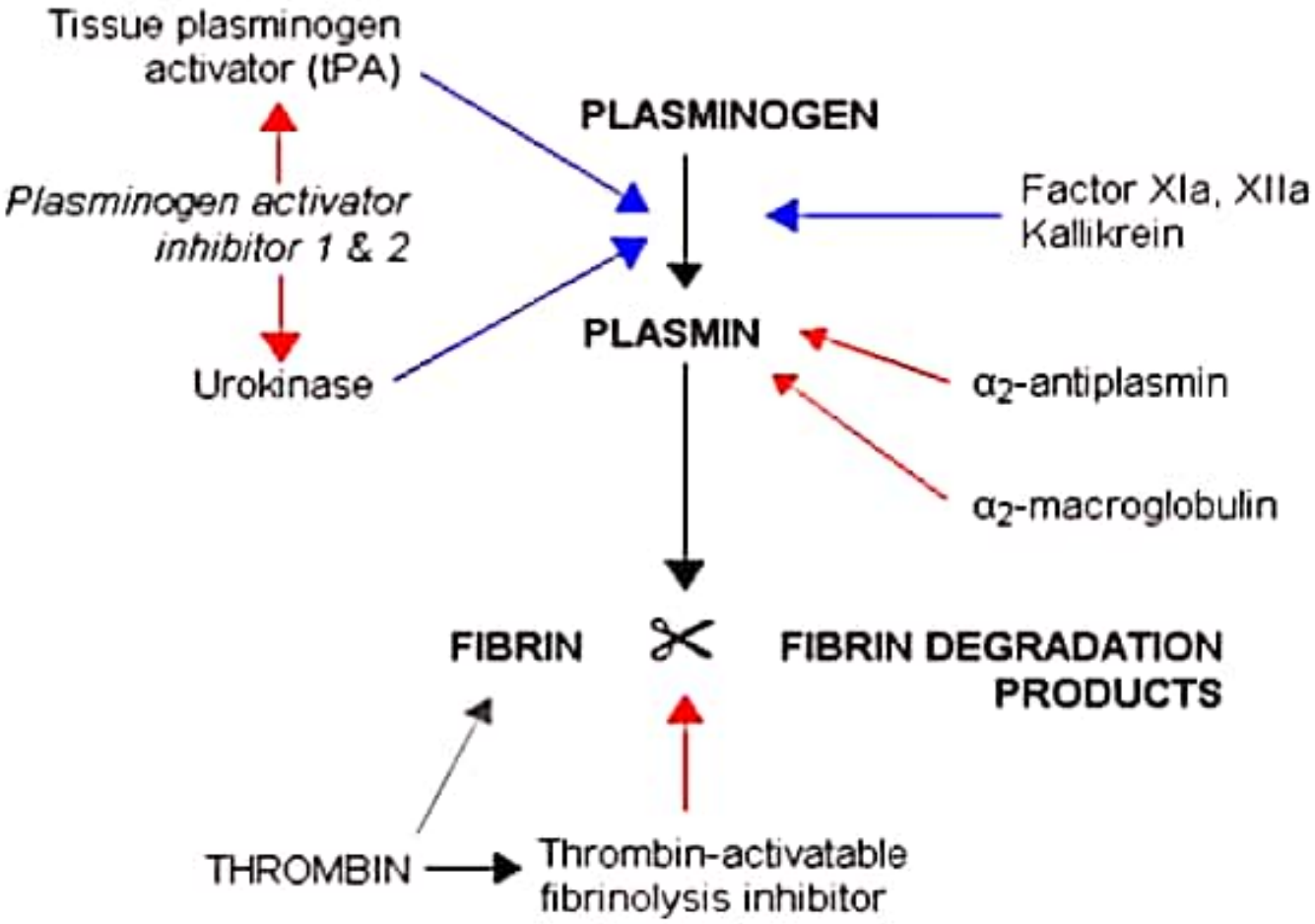
Tissue Plasminogen activator



Tissue plasminogen activator

- **Tissue plasminogen activator** (abbreviated **tPA** or **PLAT**) is a protein involved in the breakdown of blood clots.
- It is a serine protease (EC 3.4.21.68) found on endothelial cells, the cells that line the blood vessels.
- As an enzyme, it catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for clot breakdown.
- Because it works on the clotting system, tPA (such as alteplase, reteplase, and tenecteplase) is used in clinical medicine to treat embolic or thrombotic stroke.
- Use is contraindicated in hemorrhagic stroke and head trauma.

Overview of action of tpa



1.1 Production of Tissue plasminogen activator

- Therapeutic potential of tPA as a fibirinolytic agent drove the development of its large scale production.
- The host cells used in production of tPA are decribed below.....

i. **Mammalian cells**

Further two types:-

a) Non recombinant producers.

b) Recombinant producers.

ii. **Bacteria**

iii. **Yeast and fungi.**

a) Non recombinant producers.

Table 1. Plasminogen activator activity in extracts of human tissue [9]

Tissue	Activity (units/g fresh tissue)	Tissue	Activity (units/g fresh tissue)
Uterus	720	Pituitary	140
Adrenal	410	Kidney	119
Lymph node	378	Muscle	110
Prostate	334	Heart	82
Thyroid	325	Brain	35
Lung	223	Testis	25
Ovary	210	Liver	0

b) Recombinant producers

- Mammalian cells inserted with copies of human tPA gene have generally been better producers then other cell line.

- Suitable engineered mammalian cells will produce human tPA . Cloning and expression in **mouse fibroblast,**

  rat myeloma, and **CHO** cells as in recombinant human mylanoma cells containig additional copies of tPA gene.

- **CHO** cells are generally superior producers then most of cells studed

b)Recombinant producers

Table 3. Reported yields of recombinant tPA in animal cells [13].

Cell type	System	Peak titer (mg/L)	Specific productivity (mg/10^9 cells·day)
Rat	Fed batch airlift	40	4[a]
Myeloma	Fed batch airlift	52	4[a]
CHO/SV_{40}	Petri dish	–	26–49
CHO/SV_{40}	Perfused matrix	65	20
CHO/Ad2	Petri dish	–	10
Mouse	Petri dish	0.3	20
C 127	Perfused microcarrier fermentor	55	25[a]
Human melanoma	25 cm^2 flask	8	3.1
Bowes melanoma[b]	25 cm^2 flask	1	0.3

2. Bacteria

- Tha bacterium *E.coli* has been used for tPA production from tPA gene from Bowes melanoma.

- The expretion in bacteria eliminated the risk of expressing tumor associated proteins and the risk of animal viruses.

- tPA from bacterial origin is a non-gycosilated single chain polypeptide within the cell as insoluble denatrured inclution bodies consitituting 5-10%of total protein.

2.Yeast and Fungi

- Production of tPA in *Saccharomyces cereviase* has been proven possible but the yield has been low .

- Production of tPA from *Aspergillus nidulans* has been reported.
- yield has been obtained upto 1 mg / ltr .

❖ Large scale cultivation of host cells.

- The science of growing cell and microbes to produce large quantities of pharmaceuticals and chemical compound underwell specified condition is called fermention.

- The genetically engineered host cells for production of recombinant protiens must be cultured in large quantities (up to kilograms).

- Fermentation is applied on cells grows in suspension .

- These include most prokaryotes – ***E.coli***.
- Lower Eukaryotes – **Yeast.**
- Sometimes mammalian cells approved by FDA - **CHO**

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The batch size cell culture and estimated time required for fermentation

Description	Batch size (litres)	Time
Laboratory shake flask	0.1	1-2
Large flasks	1-2	2-4
Batch fermenter	50	4-6
Batch fermenter	2500	6-8
Batch fermenter	25000-100000	10-16

- **Fermentation and alternative production techniques are carried out in four different ways.**

1. Batch process.
2. Fed batch process.
3. Chemostate process
4. Perfusion configration.

1.2 Downstream processing of Tissue pa

1. Regardless of the type and configuration of the cell and bioreactor combination used to prepare recombinant product, cells are harvested at their optimal growth and viability to ensure the highest yield per unit of cell culture.
2. The downstream processing starting with the biomass cells and medium harvested from the fermentation and mass cultivation process. Can be devided in to 4 stages.

(i) Solid liquid sepration or clarification
(ii) Concentration .
(iii) Purification and
(iv) Quality control & assurance analyses.

Recombinant product must meet purity and sterlity standerds and must below acceptable cellular or microbial contamination(i.e. Less then 0.5 endotoxin)

i. Solid-liquid sepration or clarification.

1. Sepration of cells from culture media or broth is the primary step in collecting the product from cells (solid) or medium (liquid).

2. If the protein is interacellular in form of inclusion bodies then the cells are isolates and then distrupted to collect the recombnant protein fraction.

Some techniques are used for the sepration of cells.

A. **Centrifugation.**
B. **Filteration**
C. **Floculation**

i.Solid-liquid sepration or clarification.

2. Animal cell culture.

a. Sepration of cells from broth is done using microfilteration . With addition of aprotinin to supress the process of two chain molecule.
b. Cross flow Ultra filteration is used to concentrate the broth. Zinc chelate chromatography can also done.

3. Microbial culture...

a. From fungus e.g. ***Aspergillus nidulans.***
- There is the product is secreted out in the broth and recovery from culture broth is similar to the animal celll culture.

b. From yeast and bacteria e.g. ***Saccharomyces cerevisiae*** and ***E.coli*** .
- The product is intra cellular so cell disrupsion and debris removal are required prior to purification.

(ii) Concentration of recombinant protein

- Concentration is required for the proceeding of next step i.e. Purification using chromatographic conditions.

- in this step we have to reduce the volume and thereby increase the recombinant protein concentration.

Some strategies are followed.....

(i) Heat assisted evaporation stratgies
(ii) Precipitation.
(iii) Membrane sepration using ultrafiltraton technologies.

(iii) Purification

The purification process of a protein includes the following steps.

1. Intermidiate purification

- In this stage, increased purity is achived through removal of most contaminating proteins, nucleic acid, endotoxins and viruses.
- This is acomplished by chromatography.

- In this technique proteins binds to solid matrix support with various functional group to provide hydrophobic, ion exchange, and affinity interactions.
- The purified product after processing is composed of more then 90% recombinant proteins.