ORIGINAL RESEARCH PAPER

Biochemistry

EXAMINATION OF TESTIS FOR RNASEA THROUGH COLUMN CHROMATOGRAPHY AND IMMUNOFLUORESCENCE

KEY WORDS: Pseudoaneurysm, Uterine artery embolization, Secondary PPH

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ABSTRACT

Column chromatography and immunofluorescence serve as major techniques for examination of RNaseA in testis, where as SDS PAGE have revealed that f3 have RNase similar to RNaseA and immunofluorescence have proved that RNase A is present in testis. Column chromatography has shown that RNA is one of the major contamination which is revealed after digestion with RNase A and DNase.

Introduction:

Immunofluorescence is the technique used here to identify RNase Awith Hoechst 33342 as a fluorochrome. Direct immunofluor escence gives low fluorescence than indirect immunofluoroscence, even though it is highly used in the science field to find the respective protein by using antibodies. column chromatography especially ion exchange chromatography is used for purification of RNase A in which DEAE cellulose and carboxy methyl cellulose chromatography served as the major tools to that of other techniques.

Cation chromatography consists of positive counter ions which are exchanged with the proteins in the sample where as anion chromatography exchanges counter ions with anionic proteins and finally eluted from the column by either changing the ionic strength or PH, known as isocratic or gradient elution. Here gradient elution is used with different ionic strength. It is important to find which RNase is present in the testis as some of the drugs target the RNase which maintains the transcriptome pool, cleave the RNA- DNA hybrids and also primers after DNA replication.

SDS PAGE is one of the technique that identify the proteins based on their molecular weight as, all the proteins gets negative charge after the treatment with SDS .So, all the proteins migrate to the positive end itself where as UV Visible spectrophotometry identify bio molecules through absorbance. Pure RNase A gives absorbance peak at 280nm.

RNase A is the ribonuclease present in pancreas but it is necessary to find its presence in testis. RNase A cleaves RNA in to ribonucleotides and it is stable to heat also.

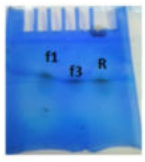
In the present study the techniques used are SDS Page, ion exchange chromatography, UV Visible spectrophotometry, Immunofluoroscence .

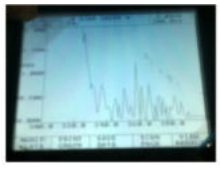
Results:

From SDS PAGE it was clear that F3 contains RNase A and F1 fraction contain protein different to that of RNaseA. Normally, the pure RNase A gives peak at 280nm which was clear from figure 5 and UV Visible Spectrophotometric analysis has shown that RNase A is present in fraction 3,after treatment with the RNase A and DNase treated samples represented by figure 3 and 4.

Incase of UV absorption F3 has the highest activity in DEAE cellulose and also in carboxymethyl cellulose but we should not come to conclusion by DEAE column chromatography because it has given negative values. So, other techniques like immunofluoroscence , UV Visible spectrophotometry and SDS PAGE are used for checking the purity and presence of the enzyme or not.

Figure: 1 **Figure: 2 F1:** **Figure: 3 F2:**





F3: **F4:**

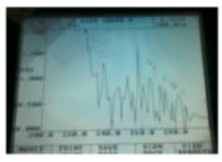
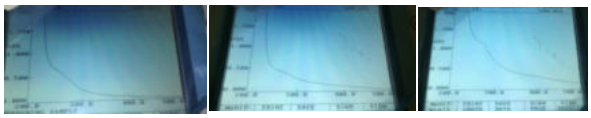


Figure 1 and figure 2 include column chromatography samples analysed through SDS page and UV- Visible spectroscopy. F1-F4 indicates various fractions eluted from carboxy methyl cellulose column and R in figure 1 indicates RNase A.

Figure: 3 (F1- F3):



Fraction 4 from the column has given absorbance above 300nm. Some amounts of contaminants have also found which is RNA that is known after digestion with RNase A and Dnase.

F4: **Figure: 4** **figure: 5**

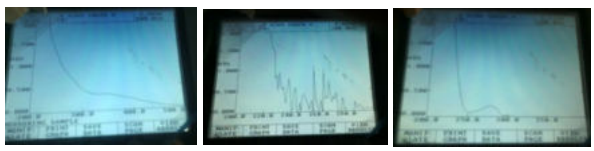


Figure 3 indicates RNase A treated samples after the elution of them and the scanned graph through UV-Visible spectroscopy, and Figure 4 indicates DNase treated sample (f3) after the elution from column and Figure 5 is the pure RNase A. F1-F4 indicates various fractions collected from carboxy methyl cellulose column.

Figure 6:

(a) **-ve control:** **(b)** **+ve control** **©**

Figure 6 includes RNase A detection in smeared samples of testis through immunofluorescence technique, –ve control represents blood and +ve control represents sample from pancreas.From the figure 6 (a) it was clearly known that blood doesn't contain RNase A as it does not given the fluorescence so it serves as negative control and figure 6(b) serves as positive control and (c) is the testis sample which has given fluorescence similar to positive control.

Figure: 7

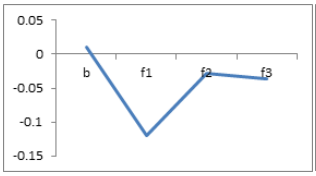
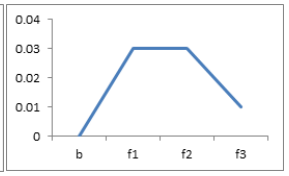


Figure: 8



From DEAE cellulose column and gradient chromatography indicates that F1 has the maximum activity which is known from absorbance values, Where as carboxy methyl cellulose fractions given 2 peaks at f1 and f3. F3 fraction contains RNase A which is clear from SDS page and also the fractions are pure. Figure 10 shows the protein estimation graph which indicates that the fractions has less amount of protein to that of earlier fraction.

Figure: 9

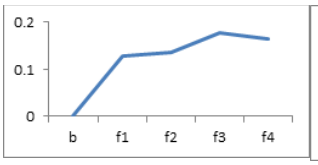


Figure: 10

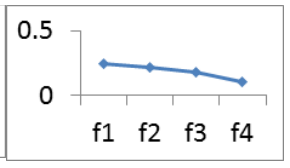


Figure 7, 8, 9 include graphs of eluted fractions of column chromatography with different ionic strength and figure 10 is the protein estimation data of various fractions.

Discussion:

Even though each technique contributes equally to the science field , column chromatography ,and above mentioned techniques can be handled easily to find whether RNase A was present in testis or not. As it is known that RNase A is inhibited by metosartan (4) so it is important to carry the studies to find which RNase is present in testis and remaining tissues. In testis RNaseA was known to be present so it is important to find whether it is inhibited in invivo condition or not. SDS PAGE has shown that f3 is similar to that of commercial available RNaseA of pancreas. Previous work has found that RNase A has PI of 9.6 and isolated from testis through chromatofocussing (4). Protein estimation clearly indicates that f1 has high protein content and f4 has the minimum protein content where as carboxy methyl cellulose column results have shown that f1 and f3 has maximum activity.

Material and methods:

Immunofluoresence:

Rat was anesthised from which testis was dissected and minced with scissors and thin smear was prepared and air dried. Antibodies of RNaseA having specifity with humans containing fluorochrome Hoechst 33342 was purchased from thermo fisher scientific Hyderabad. The air dried smear was fixed in acetone: methanol: water (2:2:1) for 15 min and incubated in antibody solution with dilution of 1:500 in 5% BSA in PBS to block the unwanted sites for 1hr. The samples were observed in the Olympus fluorescent microscope after 1 week.

Sample preparation for column: [1]

The sample was prepared as that of Larry etal(1981).

DEAE cellulose column chromatography: [1]

The samples were processed as Larry etal (1981).

DEAE cellulose gradient column chromatography: [1]

The samples were processed as Larry etal (1981).

Carboxy methyl cellulose column chromatography: [7]

The samples that have been eluted from the DEAE are loaded on to the carboxy methyl cellulose which was equilibrated with HEPES buffer containing 0.05M Kcl. The RNase sample was eluted with 300ml HEPES buffer of linear gradient from 0.05-0.22M Kcl. The elution profile was monitored by U.V Visible spectroscopy and SDS PAGE.

U.V visible spectroscopy:

The samples that were eluted from the column chromatography was scanned using UV- Visible spectroscopy (Thermo fisher scientific) of wavelength 200-500nm.

Protein estimation:

Protein is estimated by the Folin Lowry method [6]

SDS PAGE:

SDS PAGE protocol was of laemmli [5] was used.

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