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SONGJUN PUTHONG : PURIFICATION OF HUMAN ALPHA-FETOPROTEIN FROM SERUM OF HEPATOMA PATIENT. THESIS ADVISOR : ASST. PROF. KINGKARN LAOHATHAI, M.D., Ph.D. THESIS CO-ADVISOR : ASSOC. PROF. PAIROH PINPHANICHAKARN, Ph.D. 119 pp. ISBN 974-634-927-9

Alpha-fetoprotein (AFP) is a glycoprotein containing about 4% carbohydrate. In normal condition it is mainly produced by liver cell and yolk sac. AFP is secreted and accumulated into serum and amniotic fluid at significant volume during fetus stage until 6 months, after birth. The production is then gradually fall to insignificant level in normal adult serum. AFP became significant again when one has gotten hepatoma or related cancers. Therefore, AFP is used as a tumour marker which is more popular by using immunodiagnostic form. However, anti-AFP, which is commonly used in diagnostic kits, has a limitation in providing an early diagnosis for hepatoma. Since AFP are classifiable into fetal, yolk sac, and hepatoma AFP, according to the sources and its carbohydrate compartment, the specificity of antibody, especially the monoclonal antibody type, is controllable by the purity of immunogen. By these reasons, the hepatoma AFP will provide the specificity which overcome the above-mentioned limitation.

The work done in this thesis puts the main effort on separating the hepatoma AFP from patients who carry hepatoma. The techniques were firstly tried to get rid of undesired proteins, especially the albumin and those are large molecules. From the first step about 95% of nonspecific proteins were cleaned-up. At this step the purity of AFP were of 31% yield which was countable with 8.2 fold. The second step was to separate the hepatoma AFP from fetal AFP by using the Con A-sepharose column. At this process AFP occurred in three different fractions. Theoretically, the first fraction was supposed to be fetal AFP. The second peak should has lentil AFP mixed with. The hepatoma AFP occurred lately as it is Con A bound AFP. The ratio of these AFP were 3.5, 14.0 and 33.4 fold, respectively. In the attempt to subseparate the hepatoma AFP by HPLC, for an anion exchange chromatography, the Mono Q 5/5 was used. According to this anion exchange chromatography, the purity of AFP was increased up to 339 fold. However, the volume was lost considerably to only 13% left. But the albumin was completely discarded from the sample. The separation was still preserved the antigenicity.

ภาควิชา.....

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