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Biochemical Characteristics of Alpha Fetoprotein from Ascitic Fluid of Hepatoma

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ABSTRACT

In this study, we successfully purified the carbohydrate moiety of the alpha fetoprotein from ascites fluid of hepatoma patients by immunoaffinity chromatography and gel filtration chromatography. The results of the amino acid composition analysis showed that Glu had the highest proportion. Mannose, Galactose, Glucose, N-Acetylglucosamine and Sialic acid were found in the alpha fetoprotein from hepatoma patients. No N-Acetylneuraminic acid was found in this study, even though it is an amino sugar content presenting in bovine alpha fetoprotein. The absence of N-Acetylneuraminic acid of alpha fetoprotein from hepatoma patients is probably due to the fact that the quantity was too low to detect . A molecular weight of 67,000 was found by SDS polyacrylamide. The amino acid sequence of this purified alpha fetoprotein, analyzed by the Department of Microbiology, School of Allied Health Science, the University of Texas Health Science Center at Dallas is RTLHRNEYGIASILDSYQCTA; the results of the first 10 amino acid sequence are in good agreement with the results made by Y. Koyama, K. Taketa, M. Azuma et al. in 1996.

Eventually we will provide the alpha fetoprotein's data as a standard reference for clinical laboratories and research laboratories for other studies such as epidemiological studies of hepatocellular carcinoma in Taiwan.

Key words: ascites fluid, alpha fetoprotein, glycoprotein, amino acid sequence.

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Introduction

Alpha fetoprotein has a well established clinical significance in the diagnosis of certain tumors (1). The serum level of alpha fetoprotein in normal adults is usually less than 20 ng/ml. However, it increases in certain tumors, such as hepatocellular carcinoma and tetratocarcinoma (2). In recent years assays of serum alpha fetoprotein levels have been proved useful for diagnosis of such tumors especially with HBs antigen positive patients (3). Alpha fetoprotein is structurally related to albumin(4).Both alpha fetoprotein and albumin are known to have amino acid sequence homology; in the fetus, alpha fetoprotein has transport function similar to albumin (5). Unlike albumin, alpha fetoprotein is a glycoprotein containing 4-7% carbohydrate (6).

Because of the importance of the alpha fetoprotein for clinical diagnosis, we decided to study the molecular basis of this specific glycoprotein(7). We report here on the carbohydrate moieties and the amino acid compositions of alpha fetoprotein from ascites fluid of hepatoma patients(8). Alpha fetoprotein is a fetal serum protein produced by the yolk sac and the liver; it is known as a marker for the detection of tumors(9). The concentration of alpha fetoprotein is extremely low in sera and ascitic fluids of nonpregnant adult mammals, but it is elevated in sera and/or ascites fluid of hepatoma and yolk sac tumor patients (10).

Materials and Methods

Purification of Alpha Fetoprotein

Ascites fluid of hepatoma patients was supplied by the Department of Internal Medicine, National Taiwan University Hospital. The alpha fetoprotein concentration in the ascites fluid ranged from 250,000 to 400,000 ng/ml as assayed by RIA using anti-human alpha fetoprotein serum. The ascitic fluids were kept frozen at -25°C until the start of purification. Ascites fluid was applied to the anti-alpha fetoprotein sepharose column. Further purification was performed on a DEAE-Sephadex A-50 column. Next, sephadex G-150 gel filtration was done. Finally, purified alpha fetoprotein was dialyzed against deionized water and lyophilized.

Electrophoresis

Electrophoresis was performed with sodium dodecyl sulfate polyacrylamide gel according to the method of Y. Aoyagi et al. Bovine serum albumin, β-subunit of RNA polymerase and α -subunit of RNA polymerase were used as standard proteins.(11)

Amino Acid Analysis

Lyophilized alpha fetoprotein (1.10mg) was hydrolyzed with 6N HCl at 110°C for 20 and 60 hrs, and then amino acids were analyzed on a Shimadz amino acid analyzer. For the determination of tryptophan, the alpha fetoprotein was first treated with 3N *p*-toluenesulfonic acid containing 0.2% 3-(2-aminoethyl) indole in an evacuated tube at 110°C for 24 hrs. After hydrolysis, the tryptophan was analyzed(12). Values for Valine and Isoleucine were obtained from analysis of the 60 hrs hydrolysate. Those values for other amino acids were determined by averaging the values from the analysis of 20 and 60 hrs hydrolysates.

Carbohydrate Analysis

The analysis was performed by gas chromatography with the use of a 3-m glass column packed with 3% SE-30 on Chromosorb W. Alpha fetoprotein (1.20mg) was hydrolyzed with 2N H_2SO_4 at 100°C for 4hrs. Sugars were converted into methylglycosides and trimethylsililated for determination of the neutral sugars. For analysis of the amino sugars, alpha fetoprotein (1.60 mg) was hydrolyzed with 4N HCl at 110°C for 6 hrs. To determine the sialic acid content, we used the α -acid glycoprotein as a control substance (13).

Results

We successfully purified the alpha fetoprotein from ascites fluid of hepatoma patients, and the results of amino acid compositions along with data of other investigators and human serum albumin are shown in Table 1. Like human serum albumin, Glutamic acid(Glu) is the major amino acid content in ascites fluid of hepatoma, and Tryptophan is the minor one.

Table 1 Comparison of amino acid compositions of alpha fetoprotein from hepatoma patients and human serum albumin (moles/mole)

Amino acid	Present study	Nishi et al.	Rouslahti et al.	Human serum albumin
Asp	45	49	44	54
Thr	36	35	34	30
Ser	30	36	39	22
Glu	97	104	101	83
Pro	23	22	23	25
Gly	28	27	35	12
Ala	50	49	50	63
Cys	17	13	32	35
Val	25	29	35	39
Met	6	6	7	6
Ile	30	26	30	8
Leu	55	54	54	61
Tyr	17	16	17	18
Phe	27	29	29	30
Trp	1	2	-	1
Lys	37	35	46	58
His	12	12	16	16
Arg	19	17	21	23

The carbohydrate compositions of alpha fetoprotein in the present study are shown in Table 2. Mannose, Galactose, Glucose, N-Acetylglucosamine and sialic acid are found in ascites fluid of hepatoma.

Table 2. Carbohydrate compositions of alpha fetoprotein from hepatoma patients (moles/ mole)

Carbohydrate	Present study	Aoyagi et al.
Mannose	3.7	3.5
Galactose	3.0	2.9
Glucose	2.8	3.3
N-Acetylglucosamine	4.9	5.1
Sialic acid	1.9	2.2

A molecular weight of 67,000 was estimated by electrophoresis with sodium dodecyl sulfate polyacrylamide gel(14). We sent this sample to the Department of Microbiology, University of Texas Health Science Center at Dallas for determining the amino acid sequence, and obtained the following sequence as the result: Arg-Thr-Leu-His-Arg-Asn-Glu-Tyr-Gly-Ile-Ala-Ser-Ile-Leu-Asp-Ser-Tyr-Gln-Cys-Thr-Ala. This sequence was established by automated amino acid analyzer.

Discussion

In the present study, the compositions of amino acid and carbohydrate of alpha fetoprotein from hepatoma patients were analyzed. Amino acid compositions of alpha fetoprotein from hepatoma patients in Taiwan were found to be similar and in agreement with the results of other investigators(15).The results of the amino acid composition analysis showed that Glutamic acid was the highest in content. The reason for Glutamic acid being the highest in content in alpha fetoprotein is still unclear. Like albumin, Glutamic acid is the major amino acid component in alpha fetoprotein from ascites fluid of hepatoma patients. Some investigators reported that Glu had a high affinity to anti-alpha fetoprotein, Poly Glutamic acid was used to bind anti-alpha fetoprotein with anti-cancer drug as an intermediate drug carrier (16), and conjugates (formed by Glu and anti-alpha fetoprotein) may cause inhibition of both in vitro and in vivo tumor growth of AFP-causing target tumor cells. To increase the selective localization of anti-cancer drugs to the target tumor cells, Poly Glutamic acid conjugates will show highly selective cytocidal effects against the hepatoma cells.

A molecular weight of 67,000 was estimated; this value is in good agreement with the value reported by Nishi and Aoyagi(17), and this is slightly different from the molecular size of alpha fetoprotein in rat (72,000~74,000), mouse (72,000~73,000) and normal human (70,000)(18). The amino acid sequence of alpha fetoprotein in this study was made by the University of Texas Health Science Center at Dallas, and the results of the first 10 amino acids are in good agreement with the results made by Y Koyama, K Taketa, M Azuma, et al.(19). No difference was observed in the amino acid compositions between fetal alpha fetoprotein and hepatoma alpha fetoprotein from previous reports (20). Although a small difference in the carbohydrate composition of these glycoproteins was recognized(21), the alpha fetoproteins from different sources were distinct from those of proteins derived from cord serum, hepatoma, yolk sac tumor and gastric cancer.

N-Acetylneuraminic acid is an amino sugar content presenting in bovine alpha fetoprotein; however, we did not find this in alpha fetoprotein from hepatoma patients. The mechanism for the absence of N-Acetylneuraminic acid of alpha fetoprotein from hepatoma patients is not yet known. N-Acetylneuraminic acid was not found in serum alpha fetoprotein neither, probably due to the fact that the quantity was too low in the hepatoma patients to detect. These results will be used as a standard reference for clinical laboratories and research laboratories, and also for epidemiological study of hepatocellular carcinoma in Taiwan(22).

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肝癌病患腹水中甲胎蛋白之生化特性研究

吳正文*

摘 要

甲胎蛋白是某些內科領域腫瘤診斷上之重要指標,在一般成人正常值約為 20ng/ml 以下，而在肝細胞癌症中它的含量會明顯增加，本實驗利用肝癌病患腹水中提取並精製甲胎蛋白,精製的方法包含利用免疫層析精製甲胎蛋白，再以氣相層析分析儀作甲胎蛋白碳水化合物的側鏈成份分析,並以氨基酸分析儀決定甲胎蛋白的氨基酸含量,與以前發表過的文獻數據作了比較,本實驗的結果顯示 Glutamic acid 的含量最高,糖鏈上含有五種碳水化合物，但是沒有發現 N-Acetylneuraminic acid 的存在,另外以 SDS 方法測得分子量約在 67,000。

同時我們將精製後的甲胎蛋白送請美國德州達拉斯醫學中心作了氨基酸的排序,得到的結果為 RTLHRNEYGIASILDSYQCTA,前面 10 個氨基酸序列與 1996 年 Y Koyama 等人的結果一致,將有助於我們對甲胎蛋白的生化特性之瞭解,並作為今後肝癌患者流行病學研究的重要參考。

關鍵詞：腹水，甲胎蛋白,醣蛋白，氨基酸序列。

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