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Note

Phospholipid composition of r-DNA hepatitis B surface antigens

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Abstract

The composition of the lipid matrix of the antigenic protein of r-DNA hepatitis B surface antigen (HBsAg) particles was investigated. High-performance liquid chromatography (HPLC) revealed that the lipid fraction contained mainly neutral lipids, as well as phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine. In addition, it was shown that these lipids contained primarily C16 and C18 saturated and mono-unsaturated fatty acids. Comparing these results to literature data concerning blood plasma HBsAg particles, significant differences were observed.

Key words: Phospholipid; Hepatitis B surface antigen; r-DNA vaccine

Human hepatitis B infection constitutes a major worldwide health problem (Sobeslavsky, 1980). As there is no specific and effective therapy against this disease, preventive measures such as vaccination are essential. Until 5 years ago, plasma-derived hepatitis B vaccines had been used. Some major disadvantages of this approach were the limited amount of suitable plasma, the high cost of purification and the residual risk of infection by resistant micro-organisms in the plasma. Therefore, a recombinant DNA vaccine was sought (Tiollais et al., 1985). This search has led to the introduction of a recombinant yeast-derived hepatitis B vaccine.

In this vaccine the hepatitis B surface antigen (HBsAg) particles consist mainly of an antigenic protein, embedded in a lipid matrix. In spite of the fact that the efficacy of the antigen is largely determined by its molecular environment, no data have been reported previously concerning its composition.

In this paper, we report on our experiments to elucidate the composition of the lipid phase of r-DNA HBsAG particles. These were supplied by Smith Kline; they were produced by *Saccharomyces cerevisiae*. The dispersions contained 2.3 mg protein per ml; preliminary experiments revealed that the lipid-to-protein ratio amounted to about 70%. The mean diameter of the HBsAg particles amounted to about 22 nm. The extraction of the lipids from the aqueous dispersions was based on the washing procedure proposed by Folch et al. (1957): methanol and chloroform were added so that a 3:4:8 ratio was obtained. Following two extractions, the combined organic

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fractions were concentrated in a rotavapor and evaporated to dryness under a gentle stream of nitrogen. Finally, the lipids were dissolved in chloroform.

The analytical HPLC method used was described previously (Van der Meeren et al., 1988). Only some minor modifications were applied. A 100 × 4.6 mm stainless-steel column, packed with 3 μm Spherisorb silica gel (Alltech, Eke, Belgium) was used. The mobile phase consisted of hexane, 2-propanol and water. Before injection, the column was equilibrated with a 58:39:2.0 (by vol.) mixture. Upon injecting the solvent ratio was changed to 58:39:3.3. After 5 min a 55:44:4.8 ratio was chosen. Finally, after 22 min the first mobile phase was selected again. The flow rate amounted to 1.8 ml/min throughout.

Analysing the lipid composition of *S. cerevisiae* HBsAg particles, four main peaks were observed (Fig. 1). Comparing their retention time to those of standard phospholipids (Sigma, St. Louis, MO, U.S.A.), it was concluded that the major components were neutral lipids (NL), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylcholine (PC). In addition, a smaller (split) peak could be discerned whose retention time approximated 18 min; from the retention behaviour of phospholipid standards, it was deduced that this peak could correspond to either phosphatidic acid (PA) or phosphatidylserine (PS). In Fig. 1 it is observed that all phospholipid peaks are split. This effect was especially pronounced in the case of PC: in addition to a split peak, a small hump preceding the two major peaks was also observed. Detailed inspection led to the same pattern being recognised for PE and PI. According to our opinion, this behaviour was due to the partial resolution of phospholipid molecular species. In this respect, it was shown previously that species containing shorter or more unsaturated fatty acids eluted later during normal phase chromatography (Van der Meeren et al., 1992). The determination of the fatty acid composition was accomplished by gas chromatography of the methyl esters according to the method proposed by Christie (1982). Considering the fatty acid composition of an r-DNA HBsAg extract, significant differences were observed as compared to the composition of soybean PC (Epikuron-200, Lucas Meyer, Hamburg, Germany), which was used as the PC standard (Table 1); the most striking phenomenon was the great abundance of palmitoleic acid in the HBsAg lipids. In fact, this observation is typical for yeast lipids (Bui and Galzy, 1990). Taking the above-mentioned rule of thumb into account, it appears obvious that molecular species containing this fatty acid have a longer retention time, thus ex-

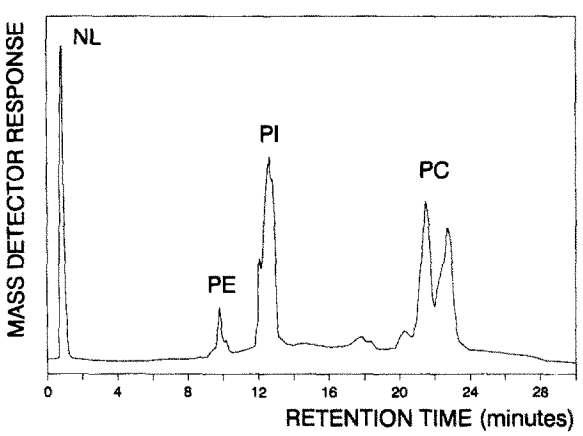

Fig. 1. Evaporative light scattering chromatogram of the lipid fraction of r-DNA HBsAg particles produced by *S. cerevisiae;* the evaporator set of the detector amounted to 70 and the gas pressure was 12 lb/inch^2.

Table 1
Fatty acid composition of lipids of different origin

Fatty acid	r-DNA HBsAg	Soybean PC	Plasma HBsAg [a]
C12:0	3.8	0.0	0.0
C14:0	3.0	0.0	0.0
C16:0	18.8	13.6	27.0
C16:1	38.0	0.0	2.6
C18:0	6.4	3.4	12.8
C18:1	25.9	10.8	20.5
C18:2	0.3	66.2	27.9
C18:3	0.0	6.0	0.0
C20:4	0.0	0.0	5.7
C22:1	0.0	0.0	2.6
C22:6	0.0	0.0	1.1

The fatty acid composition (in wt%) of the lipid fraction of r-DNA HBsAg particles was compared to those of soybean phosphatidylcholine and of blood plasma hepatitis B antigens.
[a] According to Gavilanes et al. (1982).

Table 2
Lipid composition of HBsAg particles of different origin

	r-DNA HBsAg (1)	r-DNA HBsAg (2)	Plasma HBsAg [a]
NL	16.0	22.8	33.1
PE	2.4	2.5	1.1
PI	32.4	27.0	0.0
PA + PS	2.3	2.2	tr.
PC	42.8	44.4	58.7
SPH + LPC	0.0	0.0	7.0

The composition (in wt%) of the lipid fraction of two batches of r-DNA HBsAg particles was compared to those of blood plasma antigens. tr., trace.
[a] According to Gavilanes et al. (1982).

plaining the emergence of split peaks having an additional, slightly later eluting component in the HBsAg phospholipids as compared to the phospholipid standards of vegetable origin.

In order to enable the comparison of the above-mentioned results with literature data on the lipid composition of blood plasma antigens (Gavilanes et al., 1982), the reproducibility of the analytical procedure had to be evaluated. Based on three succesive determinations of the lipid composition of r-DNA HBsAg particles, it was observed that the coefficient of variation amounted to about 10%. Subsequently, the batch-to-batch reproducibility was investigated. Comparing the first two columns of Table 2 and bearing the above-mentioned reproducibility in mind, it was concluded that the phospholipid composition of different batches was not significantly different. On the other hand, significant differences were observed on comparing the lipid composition of r-DNA and blood plasma HBsAg. As far as the fatty acid composition of the lipid fraction is concerned, Table 1 demonstrates that considerable deviation in the results appeared for both types of antigens. Above all, the content of palmitoleic acid (C16:1) was markedly different: whereas this typical yeast lipid mono-unsaturated fatty acid (Bui and Galzy, 1990) was the major component of the lipid fraction of the r-DNA particles, it represented only a minor fraction of the fatty acids of the lipids of blood plasma HBsAg. In addition, the antigens produced by *S. cerevisiae* contained almost no polyunsaturated fatty acids. In fact, this pattern is typical for the *Ascomycetes,* subclass *Hemiascomycetes* (of which *S. cerevisiae* is a representative), which are generally regarded as primitive or degenerate and appear to be unable to synthesize polyunsaturated fatty acids (Rattray et al., 1975).

Comparing the lipid composition data of r-DNA and blood plasma particles (Table 2), several similarities were observed. PC and neutral lipids are major components of both types of HBsAgs, whereas PE represents only a minor fraction of the total lipids. The most striking difference is the abundance of PI. Whereas Gavilanes et al. (1982) did not detect this phospholipid in any of their preparations, it appeared to be the second most important lipidic substance of the r-DNA particles. On the other hand, neither LPC nor sphingomyelin (SPH) could be detected in the r-DNA antigens. The latter observation could be expected, since SPH is known to be a major component of the complex lipids of all animal tissues, but is not present in plants or microorganisms.

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