THE FATTY ACID COMPOSITION OF INCOMPLETE INFLUENZA VIRUS

H. A. Blough, J. P. Merlie and J. M. Tiffany†

Department of Microbiology, University of Pennsylvania
School of Medicine, Philadelphia, Pennsylvania 19104

Received February 19, 1969

Summary

The fatty acid compositions of standard and incomplete influenza virus (von Magnus type) were analyzed by gas-liquid chromatography. Incomplete virus had appreciable increases in short chain fatty acids and in the relative amounts of long chain unsaturated fatty acids. It is suggested that these changes are responsible, in part, for the pleomorphism of incomplete virus and reflect alterations in the amount and/or configuration of envelope proteins.

The fatty acid compositions of various strains of influenza virus are different, and it has been suggested that the fatty acyl chains are a sensitive index of differences in primary amino acid sequence and hence the tertiary structure of virus envelope proteins (Tiffany and Blough, 1969 a, b). Such a concept fits with the lipoprotein-complex theory of membrane structure as proposed by Benson (1966). Incomplete, multiplicity-dependent influenza virus of the von Magnus type is characterized by its pleomorphism, low infectivity-hemagglutinin ratio (von Magnus, 1951), and decreased amounts of envelope proteins (Paucker, et al., 1959; Seto, 1964; Pons and Hirst, 1969). The purpose of this present study is to test the hypothesis that the pleomorphism of enveloped viruses is related to the nature of lipids which constitute the envelope.

MATERIALS AND METHODS

The A_0/PR8/34 strain of influenza virus was used throughout. Standard virus was prepared as previously described (Blough et al., 1967); incomplete virus of von Magnus type was prepared by passing undiluted virus three times intra-allantoically. The infectivity: hemagglutinin ratio ($log_{10}EID_{50}/log_{10}HA$) was 5.89 for standard virus and 3.94 for incomplete virus. The purification of virus, the extraction of lipids, and the separation of lipids into neutral (NL)

and polar lipid (PL) classes were done as previously described (Blough et al., 1967). The methyl esters of fatty acids were prepared by catalytic transesterification using BCl_3-CH_3OH (Hyun et al., 1965) and analyzed by gas-liquid chromatography on a 6 foot (1.8 m) column of 17% ethylene glycol succinate polyester on Anakrom ABS in a Barber-Colman 5000 instrument.

RESULTS AND DISCUSSION

A comparison of the total saturated, monoenoic and polyenoic acids showed little difference for both standards and incomplete virus (Table I) attesting to the relatedness of these two virus preparations (Tiffany and Blough, 1969 a). Increases in the short chain fatty acids ($C_{12:0}$) and in the ratio of long chain ($\geq C_{20}$) saturated to unsaturated fatty acid were noted for incomplete virus when compared to standard virus (Tables 1 and 2). The long

TABLE I

A COMPARISON OF FATTY ACIDS OF STANDARD AND INCOMPLETE INFLUENZA VIRUS (A_o/PR8/34)*

Acyl Group	Standard Virus		Incomplete Virus	
	PL	NL	PL	NL
12:0	trace	—	4.8	8.6
14:0	1.6	4.1	5.5	8.1
16:0	15.1	28.5	21.9	24.8
16:1	5.2	6.6	1.6	10.8
18:0	14.8	18.8	13.6	6.5
18:1	15.8	22.0	18.9	23.0
18:2	4.3	4.6	3.8	5.2
18:3	0.9	trace	trace	1.4
20:0	8.4	6.1	2.5	2.0
20:1	trace	—	0.8	1.0
20:4	4.2	4.9	5.4	2.2
22:0	13.7	4.4	4.8	trace
22:1	trace	—	—	—
22:polyene	8.4	—	8.8	—
24:0	6.8	trace	5.8	6.2
24:2	—	—	1.0	—
26:0	—	—	trace	—
Uncharacterized	0.6	—	—	—

* Values represent percentage composition and are the mean of duplicate determinations. Detector response was determined using quantitative methyl ester standards (Applied Science Laboratories). PL = polar lipids; NL = neutral lipids.

TABLE 2

SUMMARY OF DIFFERENCES BETWEEN ACYL CHAIN COMPOSITION OF STANDARD AND INCOMPLETE (von MAGNUS) VIRUS (A_0/PR8/34)

	Standard		Incomplete	
	PL	NL	PL	NL
Total Saturates	60.4	61.9	58.9	56.2
Total Unsaturates	38.8	38.1	40.5	43.6
Monoenoic	21.0	28.6	21.3	34.8
Polyenoic	17.8	9.5	19.6	8.8
Short Chains	Trace	—	4.8	8.6
Long Chain Unsaturated/Saturated	0.45	0.47	1.24	0.39

chain unsaturated/saturated ratio of 1.24 for the polar lipids of incomplete virus was appreciably higher than for three strains (Tiffany and Blough, 1969a) of standard virus (range, 0.45-0.8) and this increase was primarily due to a decrease in long chain saturates (Table 1).

It has been suggested that concentrated virus inoculum is "toxic" and interferes with the maturation and the differentiation of influenza virus (Horsfall, 1955; Morgan et al., 1962). Incomplete influenza virus contains less RNA than does standard virus (Ada and Perry, 1956), and it has been suggested that this may be due to an accumulation of soluble antigen (nucleocapsid) in the nuclei of cells infected with high multiplicities of an avian influenza virus, fowl plague virus (Rott and Scholtissek, 1963). Recent studies by Pons and Hirst (1969) have shown that incomplete virus of the von Magnus type is consistently deficient in a large genomic fragment. While these studies account for the lower infectivity of incomplete virus, they fail to explain why incomplete particles are "pleomorphic, flattened and appear as empty sacs" (Werner and Schlesinger, 1954).

Film-balance studies of synthetic phospholipids have shown that the molecular packing of phospholipids is dependent upon the fatty acid chain length and the number of unsaturated bonds (van Deenen, 1966). A high content of short chain acids and a relative

increase in the amount of long chain unsaturated fatty acids, as found in incomplete virus, would tend to reduce van der Waals' interactions between the hydrophobic regions of envelope proteins and adjacent paraffinic chains. Such changes in the interaction of lipid and protein would tend to produce a more fluid and expanded viral envelope and would explain the pleomorphism observed with incomplete virus. The heterogeneity of morphology may be related to the degree of change in envelope proteins and hence fatty acids; it is anticipated that localized changes may yield viral particles which are relatively "normal", whereas widespread changes would produce markedly pleomorphic particles. The results of this study add further support to the hypothesis that the packing of lipids plays an important role in the determination of the molecular architecture of enveloped viruses.

ACKNOWLEDGEMENT

This study was done under the sponsorship of the Commission on Influenza of the Armed Forces Epidemiological Board and was supported, in part, by the U.S. Army Medical Research & Development Command, Department of the Army (Contract No. DADA 17-C-67-7128) and grants from the American Thoracic Society and the Damon Runyon Memorial Fund for Cancer Research (DRG-948A). † J. M. T. is a Research Fellow of the Damon Runyon Memorial Fund for Cancer Research.

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