High Resolution Field Desorption Mass Spectrometry of Disodium Deoxyfluoro-D-glucose 6-Phosphates

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Summary High resolution field desorption mass spectrometry (FD-MS) of the disodium salts of D-glucose 6-phosphate and the corresponding 2-, 3-, and 4-deoxyfluoro-derivatives gives quasimolecular ions inclusive of cations.

UNLIKE EI, FI, and CI mass spectrometry the field desorption technique does not require vaporisation of samples prior to ionisation.[1] Briefly, FD-MS involves the deposition of a substance (by concentration of a solution) on an emitter comprising a tungsten wire (10 μm diameter) on which semi-conducting organic micro-needles are grown.[2] In the ion source at *ca.* 10^{-6} Torr, the wire is given a high positive potential (10 kV). Loss of electrons from the adsorbed substance occurs by tunnelling and the residual positive ions are repelled from the wire. FD-MS can be effected on sub-microgram amounts of material and useful spectra can often be obtained at relatively low temperatures (*ca.* 20°).

Thus, FD-MS may be usefully applied[3,11] to molecules

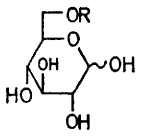
Field desorption mass spectra[a] *of* D-*glucose* 6-(*disodium phosphate*) *and deoxyfluoroglucose* 6-(*disodium phosphate*) (II)—(IV)[b]

Mass		Relative intensity						
Found[c,d]	Theory	(I)	(II)	(III)	(IV)	Composition	Origin	Ion type[f]
308·007	308·010		7	10	9	$^{13}C_5H_{11}FNa_2O_8P$	(M + 2)	Isotope
307·004	307·006		**100**	**100**	**100**	$C_6H_{11}FNa_2O_8P$	(M + 1)	Quasimolecular
306·017	306·015	8				$^{13}C_5H_{12}Na_2O_9P$	(M + 2)	Isotope
305·014	305·012	**100**				$C_6H_{12}Na_2O_9P$	(M + 1)	Quasimolecular
286·028	286·029		14	11	15	$^{13}C_5H_{12}FNaO_8P$		Isotope
285·025	285·025		82	66	82	$C_6H_{12}FNaO_8P$	[(M − Na + H) + H]	
284·036	284·034	10				$^{13}C_5H_{13}NaO_{10}P$		Isotope
283·031	283·030	78				$C_6H_{13}NaO_{10}P$	[(M − Na + H) + H]	
267·008	267·013		18	13	18	$C_6H_{10}FNaO_7P$	285 − H_2O	
263·042	263·044		20	19	20	$C_6H_{13}FO_8P$	[(M − 2Na + 2H) + H]	
261·045	261·049	10				$C_6H_{14}O_9P$	[(M − 2Na + 2H) + H]	
245·031	245·032		40	36	40	$C_6H_{11}FO_9P$	263 − H_2O	
244·898	244·899	15	52	48	52	$H_2Na_3O_7P_2$	$(Na_3PO_4)(HPO_3)H^+$	Inorganic cluster[12]
243·035	243·037	40	85	80	85	$C_6H_{11}O_8P$	263 − HF (I, 261 − H_2O)	
222·925	222·917	10	28	20	22	$H_3Na_2O_7P_2$	$(Na_2HPO_4)(HPO_3)H^+$	Inorganic cluster[12]
204·906	204·905	30	50	16	43	$HNa_2O_6P_2$	$(NaPO_3)(NaPO_3)H^+$	
200·936	200·936	18	31	20	27	$H_4NaO_7P_2$	$(NaH_2PO_4)(HPO_3)H^+$	
164·933	164·931	30	26	20	35	HNa_3O_4P	$(Na_3PO_4)H^+$	
142·942	142·950	52	70	56	22	$H_2Na_2O_4P$	$(Na_2HPO_4)H^+$	
124·939	124·938	16	60	10	7	Na_2O_3P	Na_2PO_3	Fragment
120·967	120·969	40	65	16	13	H_3NaO_4P	$(NaH_2PO_4)H^+$	
83·051	83·056	12	17	16	21	C_5H_7O	Methylfuran + H^+	Pyrolysis product
80·974	80·975	20	40	35	56	H_2PO_3	$(HPO_3)H^+$	
63·025	63·027		12	15		C_2H_3FO	Fragments (see text)	
61·030	61·033	32			35	C_2H_5O		
60·025	60·024	31			8	C_2H_4O		
47·016[e]	47·016	50		36		CH_3O_2		
46·006[e]	46·007			7		CH_2O_2		

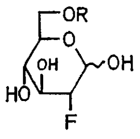
[a] Measured at high resolution (15—20,000, 10% valley definition) on a modified CEC21-110B spectrometer (Mattauch-Herzog geometry, photoplate detection) with a field anode heating current of 0—50 mA. Other operating conditions were as in ref. 5.

[b] Compounds adsorbed on the emitter from aqueous solution. [c] From the spectrum of (I) and the 2-fluoro-derivative (II). [d] High relative intensities are observed at m/e 22·990 for Na^+ and 38·964, 40·962 for $^{39}K^+$ and $^{41}K^+$, the latter ions were probably introduced when the sugar phosphates were eluted from an anion-exchange resin in the borate form which had been generated using potassium borate.[13] [e] From the spectrum of (I) and the 3-fluoro-derivative (III). [f] Apart from m/e 125 (Na_2PO_3), all ions occurred preponderantly as the protonated forms.

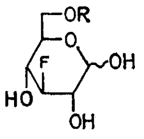
(*e.g.* amino-acids, peptides, monosaccharides, and nucleotides) which have low volatility and/or are thermally unstable, and may be of particular value in studies of drug and carcinogen metabolism[4] where frequently only microgram amounts of material are available[5] and derivatisation is often inconvenient. We now report an extension of the method to D-glucose 6-(disodium phosphate) and the corresponding 2-,3-, and 4-deoxyfluoro-derivatives.[6] Previously, aldohexose phosphates have been examined by EI-MS as their hexakis-*O*-trimethylsilyl derivatives.[7]



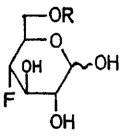
(I)



(II)



(III)



(IV)

R = PO(ONa)$_2$

A successful application of low and high resolution FD-MS to D-glucose 6-(disodium phosphate) (I) (Table) and the concurrent development[8] of high resolution FD-MS, aided by the use of emitters which are not inactivated by the commonly used fluorinated reference compounds, made possible an investigation of the deoxyfluoro-D-glucose phosphates. The data are recorded in the Table; only peaks of relative intensity >5% of that of (M + 1) are included. Although the data in the Table cannot be fully interpreted at present, certain facts emerge. Thus, for the deoxyfluoro-D-glucose phosphates (II)—(IV), there are strong peaks at m/e 307 for (M + 1) (inclusive of sodium ions) and 285 for $[(M - \text{Na} + \text{H}) + \text{H}]^+$, and a moderate peak at m/e 263 for $[(M - 2\text{Na} + 2\text{H}) + \text{H}]^+$. The FD-MS of (I) displays analogous ions in the upper mass range due to an exchange of Na^+ and H^+. Thus, FD-MS may have considerable value for the identification of intermediates in carbohydrate metabolism (see also ref. 1)

An EI-MS study of a series of deoxyfluorosugar acetates[9] and related hexitol derivatives[10] has shown that cleavage of the C–C bond in a C(F)–C grouping is relatively disfavoured. On the basis of the available data, it was not possible to establish the operation of a similar effect in the fragmentation of compounds (II)—(IV) under FD conditions. At low emitter temperatures the molecular ion and fragments are observed but as the temperature is raised pyrolysis fragments become more abundant and this hinders the detection of the smaller organic ions arising from FD-induced fragmentation. It is expected that the protonation of the molecule and the generation of ions from the adsorbed surface layer in the extremely high field used in the FD method may result in novel fragmentation pathways.

It seems likely, however, that C-2 and C-3 are present in the C_2 fragments in the FD spectra of the fluorosugar phosphates. These fragments have an m/e value of 63 for the 2 (II) and the 3-fluoro-derivatives (III), and 61 for the 4-fluoro-analogue (IV). This inference is supported also by the occurrence of fragments at m/e 46 and 47 (containing C-1 and the ring oxygen) and m/e 60 and 61 (containing C-2 and C-3) in the spectra (low and high resolution) of D-glucose 6-(disodium phosphate) (I). Moreover, the FD-MS of free nucleotides[11] display signals at m/e 60·021 [$(CHOH)_2$] and 61·029 [$(CHOH)_2 + H$], probably indicating a ring opening between C-1 and C-2 following the field-induced proton transfer to the ring oxygen.

Thus, low and high resolution FD-MS will be of particular value for the determination of molecular weights and molecular formulae of sugar phosphates on amounts of material as small as *ca.* 5×10^{-8} g without derivatisation. Owing to the limited data on fragmentation pathways of carbohydrates under FD conditions and on the relative contribution of pyrolytic processes,[12] the scope of the method for defining the position of functional groups needs further investigation.

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