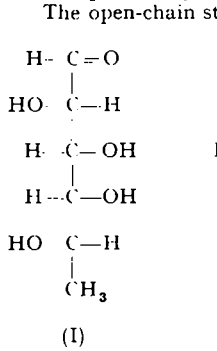
On the biosynthesis of L-fucose by *Aerobacter aerogenes*

L-Fucose is known to occur in a variety of microorganisms, plants and animal tissues. The sugar is present in the capsular material of bacteria[1], and in certain marine algae[2]. In mammalian systems it is found as a component of blood group substances[3]. In spite of its widespread occurrence, practically no information has been adduced regarding its biosynthesis.

The open-chain structures of L-fucose and D-glucose are given by (I) and (II), respectively. It is apparent that L-fucose (I) differs from D-glucose (II) in the configuration about C-2, C-3 and C-5, and also in the state of oxidation of C-6. This report attempts to delineate the biosynthetic relationships between the two sugars.



(I) (II)

Aerobacter aerogenes, strain A3S1 (ATCC 12657), which is known to produce a polysaccharide containing L-fucose, D-glucose, galactose, and an unknown uronic acid[1], was grown on a synthetic medium with either glucose-1-^{14}C or glucose-6-^{14}C as the sole carbon source. Both L-fucose and D-glucose were isolated from the combined slime and capsular polysaccharides by the method of WILKINSON, DUDMAN AND ASPINALL[1], except that 90% *iso*propyl alcohol, instead of butanol–water, was used for elution from the cellulose column. The fucose peak coincided with that of authentic L-fucose§, and this fraction was shown, by descending paper chromatography (methyl ethyl ketone–acetic acid–water (6:1:1); R_F of L-fucose = 0.17), to be free from other sugars. Further identification of fucose was provided by positive 6-deoxyhexose tests[4,5]. By chromatographic techniques similar to those mentioned above, the glucose fraction was found to be free from other sugars. In addition, the copper-reducing capacity of this fraction was completely destroyed by glucose oxidase. The D-glucose was oxidized to potassium gluconate (C-1,2,3,4,5,6), which was then degraded with periodate[6] to give C-1 as carbon dioxide, C-2,3,4,5 as formic acid, and C-6 as formaldehyde, isolated as its dimedon derivative. Carrier L-fucose was added to the fraction containing the 6-deoxyhexose, effecting a 7–8-fold dilution in the first experiment and approximately a 12-fold dilution in the second experiment. The intact deoxysugar was isolated as the diphenylhydrazone (C-1,2,3,4,5,6)[3]. C-1 was obtained as carbon dioxide and C-2,3,4 were obtained as formic acid after the periodate treatment of barium fuconate. Acetaldehyde (C-5,6) was isolated as its dimedon derivative after subjecting L-fucose to periodate oxidation. Iodoform (C-6) was obtained upon treatment of the 6-deoxyhexose with iodine-sodium hydroxide. The results are presented in Table I.

TABLE I

^{14}C DISTRIBUTION* IN D-GLUCOSE AND L-FUCOSE DERIVED FROM BACTERIAL POLYSACCHARIDE

Carbon source in medium	*D-Glucose*			*L-Fucose*		
	Position	*c.p.m./mmole*	*% total radioactivity*	*Position*	*c.p.m./mmole*	*% total radioactivity*
Expt. 1. Glucose-1-^{14}C	C-1,2,3,4,5,6	28,200		C-1,2,3,4,5,6	3,965	
$5.4 \cdot 10^{-2}$ μc/mmole	C-1	19,440	68	C-1	2,490	63
(72 h incubation)	C-2,3,4,5	1,380	5	C-2,3,4	230	6
	C-6	6,460	23	C-5,6	890	22
Expt. 2. Glucose-6-^{14}C	C-1,2,3,4,5,6	72,430		C-1,2,3,4,5,6	6,715	
$1.26 \cdot 10^{-1}$ μc/mmole	C-1	9,400	13	C-1	705	11
(48 h incubation)	C-2,3,4,5	1,370	2	C-2,3,4	645	10
				C-5**	515	8
				C-5,6	5,385	80
	C-6	68,135	94	C-6	4,870	72

* All samples were converted to CO_2 and counted as hyamine carbonate[7].

** Calculated: (C-5,6) – (C-6) = C-5.

§ We gratefully acknowledge receipt of a gift of authentic L-fucose from Dr. H. B. WOOD, Jr., Section on Carbohydrates, Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.

D-Glucose obtained from the polysaccharide in each case still had much of the asymmetric labeling of the corresponding substrate. The smaller degree of isotope redistribution in the second experiment is presumably due to the shorter incubation time. The labeling pattern in the L-fucose closely parallels that of the D-glucose. With glucose-1-^{14}C as the sole carbon source the fucose is primarily labeled in C-1, whereas C-6 is predominantly labeled when glucose-6-^{14}C is the carbon source. In the second experiment, however, there was a somewhat greater redistribution of isotope in fucose than was observed in glucose.

The latter observation suggests the presence of a *minor* pathway for fucose biosynthesis from small fragments which can give rise to both the top and bottom halves of the molecule. However, the asymmetric labeling observed in L-fucose indicates that in the conversion of D-glucose to the deoxypentose either non-interconvertible small molecules derived from glucose act as intermediates or utilization of the intact hexose carbon-skeleton is involved.

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The inertness of the ammonium salt of N-acetyl-L-cysteic acid carboxamide in systems containing alpha-chymotrypsin

According to SANGER AND THOMPSON[1] α-chymotrypsin, or possibly another closely associated enzyme which is also inhibited by di*iso*propylfluorophosphate, causes the hydrolysis of three of the twenty peptide bonds present in the oxidized A-chain of bovine insulin, *i.e.*, Gly-Ileu-Val-Glu-Glu(NH_2)-$CySO_3H$-$CySO_3H$-Ala-Ser-Val-$CySO_3H$—Ser-Leu-Tyr—Glu(NH_2)-Leu-Glu-Asp(NH_2)-Tyr—$CySO_3H$-Asp(NH_2), when an aqueous solution of this substance and crystalline α-chymotrypsin, adjusted to pH 7.5 with ammonium hydroxide, is allowed to stand at 37° for 24 h. While a precedent for the hydrolytic cleavage of the two peptide bonds involving the carboxyl groups of the two tyrosine residues can be found among the many synthetic specific substrates of α-chymotrypsin which are simple peptides or amides derived from a variety of α-N-acylated L-tyrosines[2,3], no similar derivatives involving the carboxyl group of an N-acylated-L-cysteic acid have been examined for specific substrate activity. Therefore, it appeared desirable to prepare such a derivative and to examine its behavior with α-chymotrypsin.

The ammonium salt of N-acetyl-L-cysteic acid carboxamide was prepared. When aqueous solutions of this compound and crystalline α-chymotrypsin, adjusted to pH 6.2, 6.8, 7.3, 7.9 or 8.3 with aqueous NaOH, were allowed to stand at 25° for as long as 27.5 h, the extent of apparent hydrolysis was no more than would have been observed had the intended specific substrate been absent (Table I).

The absence of any significant hydrolysis raised the question whether the lack of reactivity of N-acetyl-L-cysteate carboxamide was due to an inability of this anion to combine with the catalytically active site of the enzyme or alternatively was due to combination with the active site in a mode or modes which did not lead to the subsequent formation of reaction products. To answer this question the ammonium salt of N-acetyl-L-cysteic acid carboxamide was examined with respect to its ability to function as a competitive inhibitor in the α-chymotrypsin-catalyzed hydrolysis of α-N-acetyl-L-tyrosinehydrazide[5] in aqueous solutions at 25° and pH 7.9 and 0.5 M in the THAM (tris(hydroxymethyl)aminomethane) component of a THAM–HCl buffer. It will be seen from the data given in Table II that no evidence was obtained for competitive inhibition by the N-acetyl-L-cysteate carboxamide anion and it may be concluded that, if this compound can so function, the value of K_I (the enzyme-inhibitor dissociation constant), is substantially greater than 0.1 M.

The inertness of the ammonium salt of N-acetyl-L-cysteic acid carboxamide in the above