the disproportionate loss of polyribosomes during erythroid cell maturation remain yet to be resolved.

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Chemical Evidence for the Phillips Model of the Action of Hen Egg White Lysozyme

A THREE-dimensional model of the ES-complex formed between a chito-oligosaccharide and hen egg white lysozyme was presented by Blake et al. as a result of X-ray crystallographic investigations[1]. We reported that partially O-carboxymethylated chitin, which had satisfactory solubility, was highly susceptible to the action of lysozyme[2] where the contents of the carboxymethyl groups were very roughly 0·3 mole/mole of N-acetoglucosamine residue. It is to be expected that the fairly bulky O-substituents will not permit such a free binding as that between unsubstituted chito-oligosaccharides and the enzyme, but will force the enzyme to select some suitable portions of the substrate molecules for making the ES-complex. Although it is impossible to know the exact location of the O-substituents on the high molecular substrate, the location of those on the enzymatic reaction products—which are low molecular compounds—can be determined. The orientation of the O-substituents in the product molecules must be reflexions of the conformation of the binding sub-sites in the ES-complex, because those suitable portions of the substrate molecule must have been fitted in the sub-sites before being released as low molecular saccharides.

Among the reaction products, we identified N-acetoglucosamine (I), di-N-acetochitobiose (II), tri-N-acetochitotriose (III), and 6'-O-carboxymethyl-di-N-acetochitobiose (IV).
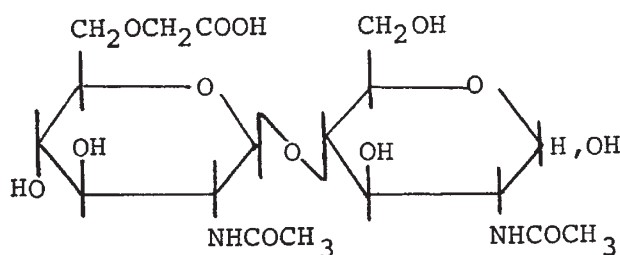
These products were isolated by 'Sephadex G-15' gel filtration and 'Dowex-1' anion exchanger chromatography. Determination of the structures of these saccharides was performed by the usual method of borohydride reduction and acid hydrolysis followed by quantitative analyses of the sugar and the sugar alcohol using a cation exchanger automatic amino-acid analyser. The results of the analyses are summarized in Table 1. The product saccharides I, II and III were further identified on a paper chromatogram by comparison with authentic specimens. The fractions in the cation exchanger chromatography of the O-substituted glucosamines and the corresponding sugar alcohols were identified by comparison with synthetic specimens[3]. The monomer fraction in the gel filtration was quite pure and it is most probable that no O-substituted monomer was produced by the enzyme. The reducing end residues of all the saccharides produced by the enzyme were likewise analysed by borohydride reduction and acid hydrolysis followed by the determination of the sugar alcohols on a cation exchanger chromatogram. The unsubstituted residue and 3-O-substituted residue were identified as reducing ends, whereas the 6-O-substituted residue was not.

Table 1

Saccharide	Molar ratio Sugar alcohol/sugar
I	1·00/0·00
II	1·00/1·00
III	1·00/2·64
IV	1·00/1·12

Table 2. ORIENTATION OF THE O-SUBSTITUENTS

"IN" means that a substituent is directed toward inside of the "cleft", thus exerting steric hindrance, and "OUT" means the contrary

Substituent	Binding sub-site A	B	C	D	E	F
3-O-	IN	OUT	IN	OUT	IN	OUT
6-O-	OUT	IN	OUT	IN	OUT	IN

All the facts mentioned previously are well interpreted when we take into account the orientation of the O-substituents in the Phillips model of the ES-complex which is presented in Table 2. The reducing ends of the saccharides produced by the enzyme had to be on the sub-site D which could accommodate only an unsubstituted residue or a 3-O-substituted one. The saccharide I had to be on D as well as on E before it was released as a monomer. No O-substituted residue can do this because it will suffer steric hindrance either on D or on E. Likewise the saccharide IV had to be on C–D as well as on E–F, both of which could accommodate this O-substituted disaccharide before it was released as a dimer.

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Glucuronation of the Liver in Premature Babies

ONE of the principal factors causing neonatal jaundice is the incomplete ability of liver to conjugate the bilirubin which is produced in greater amounts than that normally found in the older infant or adult. This condition has been extensively studied in vitro in numerous species of animals