Mechanism and Stereochemistry of Vinyl Group Formation in Haem Biosynthesis

By Z. ZAMAN, M. M. ABBOUD (in part), and M. AKHTAR*

(Department of Physiology and Biochemistry, The University, Southampton SO9 5NH)

Summary It is shown that both vinyl groups of haem are formed through the loss of S hydrogen atoms located at β -positions of the propionic acid side chains; the hydrogen atoms at the α -positions of the side chains are not involved in the biosynthesis of haem. β -hydrogen is retained. A mechanism of the latter type is suggested by the work of Sano.³

The presence of vinyl groups as well as intact propionic acid side chains within the same molecule (5) may serve as reliable indicators for giving information on the reaction, $-CH_2-CH_2-CO_2H \rightarrow -CH=CH_2$, in isotopic studies.

CONTINUING studies on the mechanisms of enzymic act reactions involved in porphyrin biosynthesis,^{1,2} we now 8 h

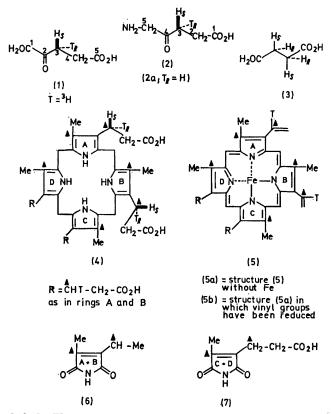
When 5-amino- $[3,5^{-3}H_4]$ laevulinic acid [(2a), 5 mg, 13 × 10⁶ c.p.m./mg], containing 43% of the total radioactivity at C-5 and the remainder at C-3, was incubated for 8 h at 37° with a haemolysed preparation⁴ of anaemic[†] chick

SU . 14C in (6)

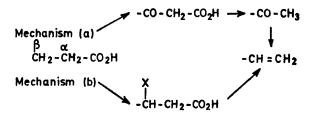
				Predicted for
		⁸ H : ¹⁴ C		conclusions
Substrate		in (7)	Found	drawn
[2RS- ³ H ₂]Succinic acid	 ••	1.00	0.85	0.83
$[2R-^{3}H_{1}]$ Succinic acid	 ••	1.00	0.940.96	1.00
[2S- ⁸ H ₁]Succinic acid	 	1.00	0.70	0.67

report results on the decarboxylation reactions involved in the formation of vinyl groups in haem (5) biosynthesis. Analogies suggest that conversion of the propionic acid portion of a precursor porphyrinogen, such as coproporphyrinogen (4), into a vinyl group may occur by one of at least two broad mechanisms, (a) and (b). Mechanism (a) requires that both β -hydrogens of the propionic acid side chain are removed, while mechanism (b) predicts that one blood (25 ml), 1.35×10^6 c.p.m. of tritium were incorporated into haem.⁵ The C-3 of (2a) with its associated hydrogen atoms occupies the position indicated by (\blacktriangle) in haem (5). The tritiated haem was mixed with another sample of haem biosynthesised from [4-1⁴C](2a). The doubly labelled material was subjected to removal of iron to give protoporphyrin-IX^{6a} (5a). The latter, after reduction ^{6b} to mesoporphyrin-IX (5b), was oxidised with ylhydrazine (5 mg/kg body weight) every alternate day for a week.

† Chickens were made anaemic by subcutaneous injections of phenylhydrazine (5 mg/kg body weight) every alternate day for a week.



CrO₃.7 The reaction mixture was separated into neutral and acidic fractions, and sublimed to obtain (6), derived from the vinyl group-containing rings A and B, and haematinic acid (7), originating from the propionic acid side



X = H, OH, or a good leaving group

chain-containing rings c and D, of haem." It should be noted that the tritium located at C-5 of (2a) will not be present in the degradation products. The ³H: ¹⁴C ratios of (7) and (6) were 1.0 and 0.58, respectively. The 42% loss of tritium in (6) compared to that in (7) agrees adequately with the theoretical loss of 50%, if the vinyl group formation occurs through the mechanism of the type (b). Mechan-

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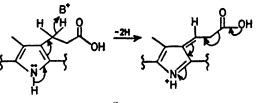
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ism (a) requires (6) to be completely devoid of tritium radioactivity.

The presence of an enzyme system in the anaemic chick reticulocytes for the conversion of 2-oxoglutarate into 5-aminolaevulinic acid suggested an additional approach for studying the mechanism of the vinyl group formation. This involved the biosynthesis of haem from stereospecifically labelled 2-oxoglutarate. 2-Oxo-[3R-3H]glutarate8,9 (1) was converted into haem as described, mixed with ¹⁴C-haem, and degraded to (7) and (6), which had ³H:¹⁴C ratios of 1.0 and 0.92-0.95, respectively. Since in this experiment either a complete retention or a complete loss of tritium was expected in the formation of vinyl groups, the presence of more than 92% of tritium in (6) supports mechanism (b) or a variant of it as shown in the Scheme. The path of the tritium atom during the conversion of 2-oxo- $[3R-^{3}H]$ glutarate into haem is shown by the sequence $(1) \rightarrow (2) \rightarrow (4) \rightarrow (5).$

One complication of the work with 2-oxoglutarate is that randomisation of the label occurs, and this was shown by an indirect method to be about 10% in our experiments. Use of labelled succinic acid throws further light on the mechanism and stereochemistry of reactions involved in the biosynthesis of vinyl groups of haem.



SCHEME

 $[2RS^{3}H_{9}]$ -, $[2R^{3}H]$ -, and $[2S^{3}H]$ -Succinic acid^{8,10} (3) were biologically converted by anaemic chick blood into haem (5), which was mixed with ¹⁴C-haem to act as internal reference. The subsequent degradation of haem biosynthesised from $[2RS^{-3}H_4]$ succinic acid gave (7) and (6) which had ³H:¹⁴C ratios of 1.0 and 0.85, respectively. These values may be rationalized by assuming that for six tritium atoms present in (7), (6) contained only five. The results for $[2R-^{3}H]$ - and $[2S-^{3}H]$ -succinic acid are summarised in the Table, and allow two main conclusions to be drawn. Firstly that both the vinyl groups in haem (5) are formed through the loss of S hydrogen atoms. Secondly, in all cases the relative tritium content of (7) and (6) show that hydrogen atoms at the α -carbon atom of the propionic acid side chains are not disturbed during the biosynthesis of haem, thus eliminating the involvement of an acrylic acid intermediate. The non-involvement of α -hydrogen atoms in protoporphyrin-IX (5a) biosynthesis in Euglena has recently been reported.11

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