Degradation of Horse Heart Cytochrome c to a Single Diastereoisomeric Porphyrin c

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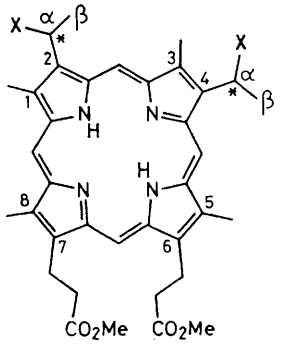
Summary Porphyrin c derived from horse heart cytochrome c was found to be only one isomer of four possible diastereoisomers by comparison with the model compound (**1**) utilizing high-pressure liquid chromatography (h.p.l.c.).

CYTOCHROME C has two sulphide bonds between the iron-porphyrin prosthetic group and the cysteinyl residues of the apoprotein.[1] The stereochemistry of the sulphide linkage was shown to be α by chemical methods.[1] The configuration of the bonds remained to be determined. To this end it must first be determined whether or not cytochrome c contains only one of the four possible diastereoisomers. For this purpose the preparation of an appropriate model compound, which can also be derived from cytochrome c by stereospecific reactions, is a key step. As a candidate for such a compound, a mixture of diastereomers of bis-(*N*-benzyloxycarbonyl)porphyrin c tetramethyl ester (**1**) was prepared[2] from haemin c which was itself synthesized from protohaemin and L-cysteine.[2,3] Compound (**1**) thus obtained



(1) X = $SCH_2C^*HCO_2Me$ (NHCO$_2$CH$_2$Ph)

(2) X = OAc

Chiral atoms are labelled *.

was subjected to h.p.l.c. utilizing a reversed-phase column (μ-Bondapak, Waters) with acetonitrile–water (4 : 1) as eluant and a flow rate of 2 ml/min to give the chromatogram shown in the Figure. The four diastereoisomers were separated into three fractions (A, B, and C), whose retention times (at the top of the peaks) were 14·1, 16·0, and 18·0 min, respectively, in an area ratio (determined by absorbance at 404 nm) of *ca.* 1 : 2 : 1. Compound (**1**) was also prepared by a substitution reaction (S_N2) of haematoporphyrin IX diacetate dimethyl ester (**2**) (a mixture of four diastereomers) and *N*-benzyloxycarbonylcysteine methyl ester and again showed an identical h.p.l.c. chromatogram (conditions as above) to that of (**1**) derived from the reaction between

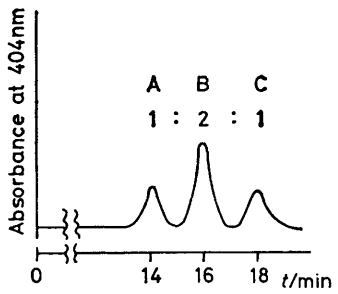


FIGURE. Chromatogram of a mixture of the four diastereoisomers of (**1**) (conditions given in the text).

protohaemin and L-cysteine. These results show that four diastereoisomers of (**1**) are separated by h.p.l.c. into three fractions (A, B, and C), where fractions A and C consist of only one diastereomer and fraction B contains the other two isomers. For comparison, horse heart cytochrome c was subjected to acid hydrolysis to give haemin c,[1] which was further converted into bis-(*N*-benzyloxycarbonyl)porphyrin c tetramethyl ester[2] (30% yield based on cytochrome c used), which was found to be one isomer (corresponding to peak C) of a mixture of diastereoisomers on the basis of h.p.l.c. To eliminate a possible epimerization in the acid hydrolysis of cytochrome c, haemin c was also prepared by hydrolysis utilizing protease (Sigma, Type V) at pH 7·4 and was then converted into (**1**)[2] which also gave a single peak on h.p.l.c. (conditions as above) corresponding to peak C.

Four chiral carbons are present in (**1**). Two of them are α-carbons of the 2- and 4-cysteinylethyl groups as described above. The others are carbons of cysteinyl groups and are assumed to have the *R*-(L)-configuration. Finally, we had to ascertain that the separation by h.p.l.c. was due to the difference in the configuration at the two carbons α to the porphyrin and not due to epimerization of the cysteinyl side-chains. A mixture of the 8 diastereomers of (**1**) and their enantiomers was prepared from DL-cysteine and showed a similar h.p.l.c. chromatogram (conditions as above) to that of (**1**) prepared from L-cysteine. However, recycling of the fraction giving peak C resulted in a gradual separation into a 1 : 1 mixture of two components. On the other hand, the peak C of (**1**) prepared from L-cysteine or the peak of (**1**) derived from cytochrome c could not be resolved and coincided with the first peak of the resolved two peaks by recycling the third fraction of a mixture of all the diastereomers of (**1**). This shows that the epimerization of the cysteinyl moiety in the preparation of (**1**) from cytochrome c, as well as from protohaemin and L-cysteine,[2] was negligible.

These results show that the configuration of the S-substituted chiral centres of horse heart cytochrome c is

only one of the possible combinations *RR*, *RS*, *SR*, or *SS*. Even though the presence of an enzyme which catalyses the formation of the sulphide bond *in vivo* is still unknown, it is concluded that the sulphide bond formation in cytochrome c biosynthesis takes place stereospecifically.

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[2] S. Kojo and S. Sano, *J. Chem. Soc., Perkin Trans.* 1, 1981, in the press.
[3] S. Kojo and S. Sano, *J. Chem. Soc., Chem. Commun.*, 1977, 249.