Hydroxylation of Phenylalanine by Aqueous H_2O_2 in the Presence of an Artificial Water-soluble Iron Porphyrin Complex

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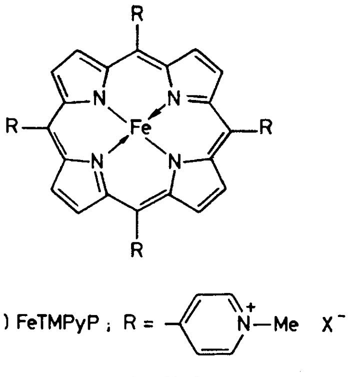
Summary Phenylalanine is hydroxylated by aqueous H_2O_2 in the presence of a catalytic amount of an artificial water-soluble iron porphyrin complex (**1**) to give monohydroxylated and dihydroxylated products, *i.e.*, tyrosine and dihydroxyphenylalanine, which are obtained in good yields.

HYDROXYLATION of aromatic amino-acids is involved in the biosynthesis of tyrosine, catechol amines and serotonins, and also in metabolic sequences such as the oxidative cleavage of aromatic rings. Since Udenfriend and his coworkers reported that aromatic compounds were hydroxylated by O_2 or H_2O_2 in the presence of Fe^{II}–ascorbic acid–ethylenediaminetetra-acetic acid (EDTA),[1] several other investigators have demonstrated this kind of hydroxylation, which is one of the enzymic reactions catalysed by peroxidase.[2] However, there have been few hydroxylation systems reported using artificial iron porphyrin complexes because these complexes are labile to H_2O_2.[3] In general peroxidase uses iron protoporphyrin IX as its prosthetic group. We report that an artificial water-soluble iron porphyrin complex[4] catalyses the hydroxylation of phenylalanine (Phe) by aqueous H_2O_2 to generate tyrosine (Tyr) and dihydroxyphenylalanine (DOPA) in good yields.



(1) FeTMPyP ; R = X⁻

X = Cl, OTs

To a mixture of compound (**1**) ($2{\cdot}0 \times 10^{-3}$ M), and Phe ($2{\cdot}0 \times 10^{-2}$ M), a sufficient amount of H_2O_2 was added to initiate the reaction at room temperature in neutral non-buffered solution with stirring. The analysis of the products was carried out by paper chromatography and u.v. absorption spectroscopy.

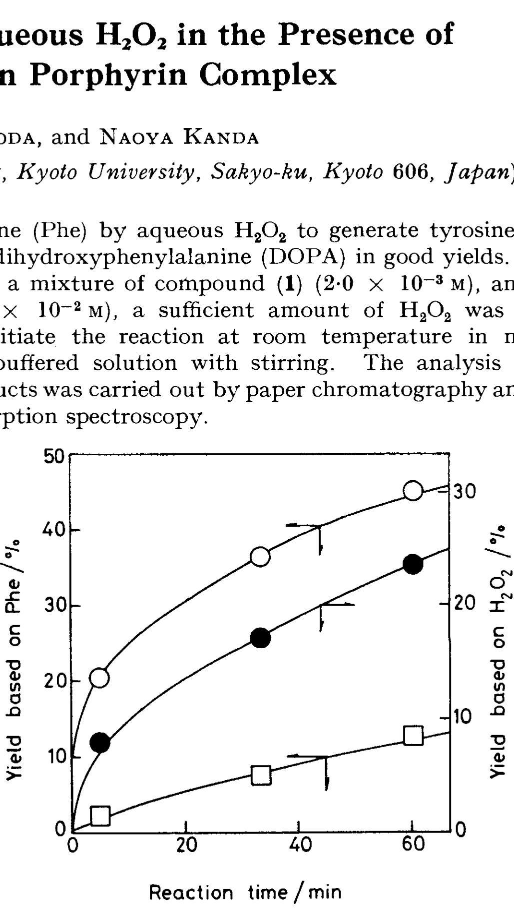


FIGURE 1. Time course of the hydroxylation yield. ○ and □, yields of Tyr and DOPA respectively based on Phe; ●, total yield of Tyr and DOPA based on H_2O_2.

Efficient hydroxylation of Phe with H_2O_2 occurred when compound (**1**) was present in a molar ratio Phe : H_2O_2 : (**1**) of 10 : 30 : 1. The dark-brown homogeneous solution of (**1**) and Phe foamed slightly on addition of H_2O_2, and Tyr and DOPA, which have one and two hydroxy-groups, respectively, were obtained as the main products, in addition to a brown precipitate which appeared after *ca.* 1 h. Figure 1 shows an example of the development of the yields of Tyr and DOPA with time based on Phe and H_2O_2 under the conditions Phe : H_2O_2 : (**1**) = 10 : 30 : 1. As DOPA has two hydroxy-groups, we define the yield of the introduction of a hydroxy-group into the phenyl ring of Phe as the sum of the yield of Tyr and twice that of DOPA. The yield reached *ca.* 70% within 1 h under these conditions, and turnover number was estimated to be 7 h^{-1}. This shows that compound (**1**) acts as a catalyst for the present hydroxylation. The yield based on H_2O_2 was 23% for 1 h. From the point of view of model hydroxylation systems for peroxidase, this yield can be regarded as good since part of the H_2O_2 is decomposed without the occurrence of hydroxylation.

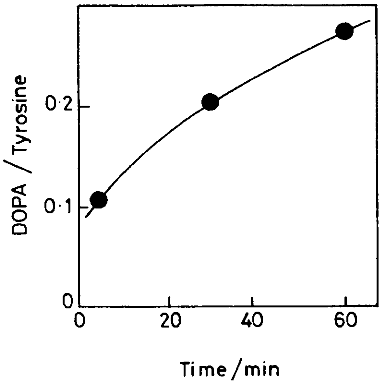


FIGURE 2. Time dependence of the ratio of DOPA to Tyr, both of which resulted from the hydroxylation of Phe.

Further, the ratio of the yield of Tyr to that of DOPA increased with reaction time (Figure 2). This result suggests that consecutive hydroxylation occurs in the present reaction. When Tyr was used as a starting material, hydroxylation proceeded and DOPA was obtained. Hydroxylation catalysed by $FeCl_3$ instead of (**1**) also occurred but the total yield based on H_2O_2 was one-third of that catalysed by (**1**), and with the $FeCl_3$–H_2O_2 system Tyr was the product and no DOPA was formed. Compound (**1**) is a superior catalyst for the hydroxylation of Phe with aqueous H_2O_2 in comparison with $FeCl_3$. This hydroxylation system is characterized by the large dependence of the yield on the molar ratio of (**1**), H_2O_2, and Phe. For example, the hydroxylation hardly occurred under the condition Phe : H_2O_2 : (**1**) = 10 : 10 : 1, while the decomposition of (**1**) was observed with a ratio of 10 : 50 : 1, and efficient hydroxylation occurred with a ratio of 10 : 30 : 1. This leads to the speculation that an active intermediate of compound (**1**) in a higher oxidation state, as found in peroxidase *in vivo*, is produced in the present hydroxylation process and that the stability of the intermediate governs an efficient hydroxylation.

Finally, the present study offers a possible technique for the hydroxylation of other aromatic compounds. Studies are in progress to investigate this possibility and to explore the efficiency and scope of this model system for peroxidase.

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