logical pH, which obviously would be required for the performance of a cytoskeletal function.

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Partial Reaction of Thymine 7-Hydroxylase*

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Thymine 7-hydroxylase (EC 1.14.11.6) from *Neurospora crassa* catalyzes the sequential oxygenation of thymine to 5-carboxyuracil *via* 5-hydroxymethyluracil and 5-formyluracil. In each step 2-oxoglutarate is oxidatively decarboxylated to carbon dioxide and succinate.[1] A reaction mechanism has been proposed in which 2-oxoglutarate reacts with oxygen to produce monopersuccinic acid as the reactive intermediate which acts as hydroxylating agent on the other substrate.[2] This mechanism is supported by reports on uncoupling of the 2-oxoglutarate decarboxylating activity from hydroxylation in reactions catalyzed by thymine 7-hydroxylase[3] and proline hydroxylase.[4,5] In preparations of thymine 7-hydroxylase, we have observed a 2-oxoglutarate decarboxylating activity, which depends on pyrimidines that are noncompetitive inhibitors of the thymine hydroxylase activity.[6]

In the presence of these inhibitors the formation of $^{14}CO_2$ from 2-oxo [1-^{14}C] glutarate is linear with enzyme concentration and linear with time for 10 min. The pyrimidines which are active in promoting the reaction are listed in Table 1. The same cofactors are required as for the thymine hydroxylase activity (Table 2) but there is an absolute requirement for ascorbate. Possibly, ascorbate protects the enzyme from

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Table 1. 2-Oxoglutarate decarboxylating activity in the presence of various pyrimidines. For incubating conditions and specific activity of enzyme in the complete systems see Experimental.

Pyrimidine added	Specific activity μmol min^{-1} g^{-1}	Relative activity %
Thymine	74	100
5-Aminouracil	4.9	6
5-Hydroxyuracil	2.3	3
5-Mercaptouracil	2.1	3
Uracil	0.7	1
None	0.1	0.1

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Table 2. Formation of $^{14}CO_2$ from 2-oxo [1-^{14}C] glutarate relative to that in the complete system with thymine and 5-aminouracil. For incubating conditions and specific activity of enzyme in the complete systems see Experimental.

Compound omitted	% Activity	
	Thymine	5-Aminouracil
None	100	100
Pyrimidine	0	0
Ascorbate	22	0
Fe^{2+}	1	2
Catalase	20	52

inactivation by a reactive intermediate formed in the oxidative decarboxylation of 2-oxoglutarate. When the enzyme was stored at −20 °C the thymine hydroxylating activity decreased more rapidly than the activity catalyzing decarboxylation of 2-oxoglutarate in the presence of pyrimidine inhibitors. When the protein was heated at 42 °C in 10 mM potassium phosphate buffer (pH 6.5) containing KCl (50 mM), EDTA (0.1 mM) and glycine (100 mM) the half lives for decrease of the two activities was about the same (~13 min). The two activities could not be separated in a purification procedure where thymine hydroxylase had been enriched more than 2 000 times. Succinate has been indentified as a product in the partial reaction by capillary gas chromatography.

Although we have not identified a reaction product of the pyrimidines added there remains a possibility that they are oxidized in some way. The most likely interpretation of the results is that the 2-oxoglutarate decarboxylating activity obtained with inhibitors of thymine hydroxylase is an example of a partial reaction catalyzed by the native or a damaged form of the enzyme.

Experimental. The incubation system (0.2 ml) contained purified thymine hydroxylase (3.7 μg with added thymine and 37 μg with other pyrimidines). The concentrations of substrates and cofactors were: Pyrimidines 0.5 mM, 2-oxo[1-$^{14}C_1$]glutarate 0.25 mM, ascorbate 5 mM, $FeSO_4$ 1 mM, EDTA 0.05 mM, glycine 50 mM in 50 mM potassium phosphate buffer pH 7.5. The incubations were carried out for 10 min at 37 °C and stopped with an equal volume of 0.3 M trichloroacetic acid. The $^{14}CO_2$ was trapped on a piece of filter paper soaked in 20 ul of 1 M Hyamine solution.

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