THE INTERACTION OF MITOCHONDRIAL FERROCHELATASE WITH A RANGE OF PORPHYRIN SUBSTRATES

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1. Introduction

The enzyme ferrochelatase catalyses the insertion of metal ions into porphyrins to form metalloporphyrins; the presumed natural substrates are ferrous iron (Fe^{2+}) and protoporphyrin which yield protohaem. It is likely that other natural iron porphyrins (e.g., *c*-type cytochromes and haem *a*) are formed by modification of protohaem (discussed in [1]). Ferrochelatase in mammals is located on the inner mitochondrial membrane with the active site facing the mitochondrial matrix [2]. In photosynthetic organisms ferrochelatase acts at a branch point of biosynthesis; insertion of magnesium into protoporphyrin is the first committed step to chlorophyll synthesis whilst iron insertion by ferrochelatase leads to haem and cytochrome synthesis. In mammals there is considerable evidence that protohaem exercises important regulatory functions on protein synthesis [3].

Despite its important position in metabolism little is known of the mechanism of mitochondrial ferrochelatase. In part this is due to the difficulty of stabilising the enzyme during purification and in part to the problem of synthesising a range of modified substrates. In this communication we describe the use of a number of differently substituted porphyrins in a study of the specificity of ferrochelatase of sheep liver mitochondria. Striking differences between porphyrins were found. A propionate substituent at positions 6 and 7 of the porphyrin nucleus (see fig.1) is necessary for maximum activity. Hydrophobic substitution in positions 1,2,3 and 4 lowered the V_{max} of the enzyme and also lowered its K_m. A porphyrin with hydrophobic substituents in these positions and lacking a propionate substituent at positions 6 and 7 is likely to be a competitive inhibitor of ferrochelatase.

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2. Methods

Protoporphyrin IX was prepared from protohaem, as in [4]. Protoporphyrin I was a gift from Dr K. Smith, γ-phylloporphyrin XV, rhodoporphyrin XV and 1,4,5-triethyl, 2,3,6,8-tetramethyl, 7 (2′ carboxyethyl) porphyrin were gifts from the late Professor G. W. Kenner, FRS, Department of Organic Chemistry, Liverpool University. Mesoporphyrin I was prepared from protoporphyrin I by controlled reduction of the vinyl groups [5] and its identity confirmed by ^1H NMR and mass spectroscopy. Mesoporphyrin IX dimethyl ester and deuteroporphyrin IX dimethyl ester were purchased from Koch-Light Labs. Ltd. Colnbrook, Bucks. Protoporphyrin XIII was kindly given by Professor A. H. Jackson, Department of Organic Chemistry, University College, Cardiff. All other porphyrins were synthesised as in [6].

Esters of porphyrins were hydrolysed, aqueous solutions prepared, spectra recorded and extinction coefficients for metalloporphyrin formation determined in each case as in [2]. Ferrochelatase activity was measured using a dual wavelength spectrophotometer with Co^{2+} as metal ion substrate as in [2]. Incubation was at 37°C and the incubation buffer 100 mM Tris–HCl (pH 7.2) containing 0.1% (v/v) Tween 80.

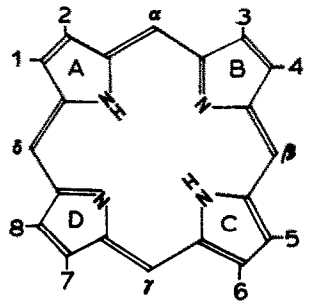
Mitochondria, prepared by conventional methods

[2] from liver cut from recently killed sheep, were used immediately or stored at −15°C before use.

3. Results and discussion

The rates of metalloporphyrin synthesis given by the range of porphyrin substrates are shown in table 1. In a series of porphyrins with vicinal propionate substuents at positions 6 and 7, the highest rates were given by porphyrin 1 which has no substituents in positions 1,2,3 or 4. Indeed, this is the best substrate yet found for ferrochelatase. The next highest rates were given by deuteroporphyrin IX, which has no methyl groups at positions 1 and 3. Mesoporphyrin IX, which has hydrophobic substituents at positions 1,2,3 and 4 gives lower rates and the addition of vinyl groups at 2 and 4, as in protoporphyrin IX lowers the rate even further. In this series of porphyrin substrates, as the V_{max} declines the K_m too diminishes, and protoporphyrin has the lowest K_m of the group (table 1). A possible explanation for this effect is that the active site of the enzyme has a hydrophobic region to which hydrophobic substituents in positions 1,2,3 and 4 bind; the product metalloporphyrin would also bind and the rate of its release would control the overall reaction rate. As predicted by this explanation, a change in the location of a vinyl group of protoporphyrin from position 2 in Proto IX to position 1 in Proto XIII produced relatively little effect.

Table 1



Porphyrin	Substituents								K_m (μM)	V_{max}[a] (nmol.min^{-1}. mg protein^{-1})
	1	2	3	4	5	6	7	8		
1	H	H	H	H	Me	Pr	Pr	Me	5.0	2.8
2	H	H	H	H	Pr	Me	Pr	Me	0.3	0.4
3	H	H	H	H	Pr	Me	Me	Pr	–	0
Deutero-IX	Me	H	Me	H	Me	Pr	Pr	Me	4.0	1.4
Meso-IX	Me	Et	Me	Et	Me	Pr	Pr	Me	1.9	1.0
Proto-IX	Me	V	Me	V	Me	Pr	Pr	Me	0.8	0.11
Proto-XIII	V	Me	Me	V	Me	Pr	Pr	Me	1.4	0.25
Meso-I	Me	Et	Me	Et	Me	Pr	Me	Pr	–	0
Proto-I	Me	V	Me	V	Me	Pr	Me	Pr	–	0
4	Et	Me	Me	Et	Et	Me	Pr	Me	–	0
Rhodo-XV	Me	Et	Me	Et	Me	COOH	Pr	Me	–	0
γ-Phyllo-XV	Me	Et	Me	Et	Me	H(Me)	Pr	Me	–	0

[a] V_{max} given as 0 where rates were too low for accurate measurement

Abbreviations: Me, methyl; Et, ethyl; V, vinyl; Pr, 2′-carboxyethyl

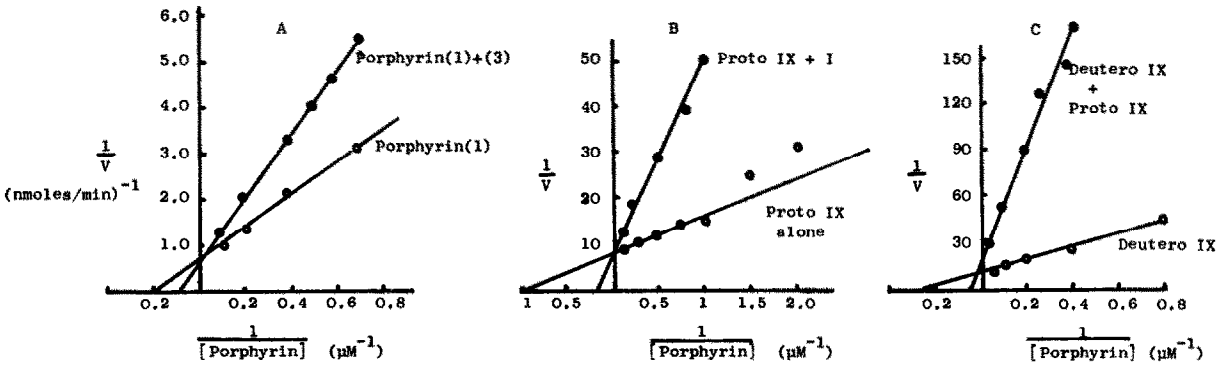


Fig.1. Kinetic properties of sheep liver ferrochelatase with different porphyrin substrates, alone and in mixtures. Assay conditions were as in section 2 with porphyrin substrates provided at the concentrations shown. (A) Porphyrin 3 added at 2 μM. Measure at 496 minus 511 nm. ΔE_{mM} = 8.0. [Co^{2+}] = 20 μM. 30°C. (B) Protoporphyrin I added at 0.2 μM. Measure at 505 minus 511 nm. ΔE_{mM} = 7.32. [Co^{2+}] = 20 μM. 30°C. (C) Protoporphyrin IX added at 6 μM. Measure at 498 minus 510.5 nm. ΔE_{mM} = 10.0. [Co^{2+}] = 20 μM. 30°C.

The effect of propionate substituents at position 6 and 7 can be clearly seen in table 1. Moving the propionate from positon 6 to position 5 lowered the rate of metal incorporation to 1/7th (see results from porphyrins 1 and 2). Moving both propionates to position 5 and 8 caused a complete loss of activity. Porphyrin 4, which has only one propionate substituent, at position 7, was not a substrate, nor was γ-phylloporphyrin XV a porphyrin derived from chlorophyll, with only one propionate substituent, or rhodoporphyrin XV which has a carboxylate substituent at position 7. It seems likely that two propionate substituents at positions 6 and 7 are necessary for forming an effective enzyme substrate complex. In agreement with this mesoporphyrin I was not a substrate nor was protoporphyrin I, confirming the harmful effect of moving a propionate from position 7 to 8.

These experiments extend and clarify the work in [7] where there was no access to such a range of model substrates. One apparent inconsistency is that the earlier work reported that mesoporphyrin I was an excellent substrate. The results described here were obtained using porphyrins characterised by NMR spectroscopy and synthesised by unequivocal routes. It is likely that the sample of mesoporphyrin I available in earlier work [7] would not have met such rigorous tests.

Insufficient material was available for detailed studies of effects of porphyrins as inhibitors but some interesting effects have been observed. Porphyrin 3 is a competitive inhibitor of protoporphyrin I (fig.1). Similarly protoporphyrin I was a competitive inhibitor of protoporphyrin IX (fig.1) and protoporphyrin IX is a competitive inhibitor for deuteroporphyrin IX (fig.1) and for porphyrin 3 (results not shown).

It can be seen that porphyrins which are not substrates may bind to the enzyme and act as competitive inhibitors and that a substrate porphyrin with hydrophobic substituents at positions 1,2,3 and 4 is an effective inhibitor of a substrate porphyrin without.

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