IN VIVO SYNTHESIS OF IRON-FREE CYTOCHROME *c* DURING LEAD INTOXICATION

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Received 20 December 1976

1. Introduction

Over fifty years ago, Warburg established that iron is the central metal in heme proteins [1]. In spite of this, there is evidence that during lead intoxication and iron deficiency, other metals are substituted for iron in one hemeprotein, hemoglobin [2,3]. This result suggests that 'mistakes' may sometimes be made so that the hemeprotein is synthesized without iron.

In this report we show that cytochrome *c* isolated from lead intoxicated rats exhibits red luminescence. The emission spectrum of the cytochrome *c* corresponds with the emission spectrum of porphyrin cytochrome *c*, indicating that some cytochrome *c* molecules have been synthesized without iron. Evidence is presented that porphyrin is indeed associated with the protein.

2. Materials and methods

Male Sprague-Dawley rats initially weighing about 100 g were fed Wayne Lab-Blox rat chow. Lead acetate (5000 ppm) was added to the drinking water of the experimental animals and no additions were made to the drinking water of the control animals. The rats were weighed daily; no difference was detected in the growth rate of the experimental and control animals. After four to five weeks, the animals were sacrificed and liver mitochondria were isolated [4]. To isolate cytochrome *c*, the mitochondrial pellet was suspended in 0.015 M KCl and incubated at 0°C for 30 min, followed by centrifugation at 9000 × *g* for 15 min. The sediment was resuspended in 0.6 M KCl and incubated for 5 min with stirring. The suspension was centrifuged at 9000 × *g* for 15 min. The supernatant was dialyzed against water and then centrifuged at 70 000 × *g* for 1 h. The supernatant was applied to a CM-cellulose column (0.5 × 3 cm) equilibrated with 10 mM phosphate, pH 7.0. Elution with 50 mM phosphate removed a greenish-colored material Final elution with 0.6 M KCl gave a material which is identified as cytochrome *c* from its absorption spectra.

Iron-free porphyrin cytochrome *c* and zinc cytochrome *c* were prepared from Type III horse heart cytochrome *c* (Sigma Chemical Co., St. Louis MO) as previously described [5,6]. Other reagents were the highest quality commercially available.

Fluorescence spectra were obtained using a Hitachi MPF-2A spectrofluorimeter equipped with an Hammamatsu HTV R636 photomultiplier operated at 1000 V.

3. Results

The absorption spectrum of rat liver cytochrome *c* isolated from the lead intoxicated animals is indistinguishable from the absorption of normal cytochrome *c*. However, a small fraction of the cytochrome *c* is not normal since the sample exhibits red luminescence. The emission maxima at 620 nm and 680 nm correspond with the emission maxima of iron free porphyrin cytochrome *c* which has been synthesized in vitro from native cytochrome *c* (fig.1). The excitation maximum for the 620 nm fluorescence is at 404 nm, which is the absorption maximum for porphyrin cytochrome *c* [5]. The identical fluorescence excitation and emission spectra of porphyrin cytochrome *c* and the cytochrome *c* from the lead intoxicated animals, permits identification of porphyrin cytochrome *c* which has been synthesized during lead intoxication.

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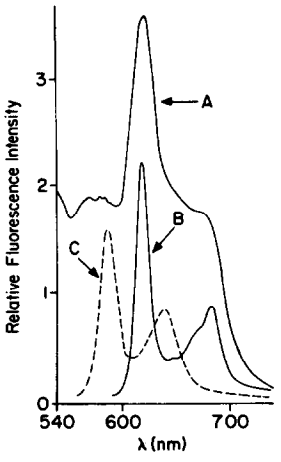

Fig.1. Fluorescence of cytochrome *c* derivatives. (A) Emission spectrum of rat cytochrome *c* (about 50 μM) isolated from lead intoxicated rats as described in Materials and methods. Excitation was at 405 nm, 15 nm half band pass for excitation and emission monochrometers. (B,C) Emission spectra of 1 μM horse heart porphyrin cytochrome *c* (B) and horse heart zinc cytochrome *c* (C). Excitation was at 405 nm, 5 nm half band pass for excitation and emission monochrometers. Buffer was 10 mM phosphate, pH 7.0. Temperature 22°C.

In fig.1, the emission spectrum of zinc cytochrome *c* is also presented. The sample of rat liver cytochrome *c* shows weak luminescence at around 580 nm. Since the presence of a closed shell metal shifts the emission spectrum to lower wavelength [6], the emission at 580 nm may be due to cytochrome *c* in which the iron has been replaced by zinc or another closed-shell metal.

The tryptophan fluorescence of native cytochrome *c* is quenched because of its proximity to the heme [7]. However, in porphyrin cytochrome *c*, excitation of tryptophan at 282 nm results in emission of the porphyrin fluorescence due to energy transfer [5]. It is important to mention that the excitation spectrum of the luminescent cytochrome *c* from the lead intoxicated rats has a peak at 282 nm, using 622 nm as emission wavelength. This result can be interpreted to mean that the porphyrin is protein-bound and that the protein conformation is such that a tryptophan is near the porphyrin ring, as it is in native cytochrome *c*.

Under conditions indicated in fig.1. no fluorescence could be observed from cytochrome *c* isolated from the control rats.

4. Discussion

Iron quenches luminescence from the porphyrin by paramagnetic and spin–spin interactions [8]. Consequently, the detection of red luminescence in a preparation of a hemeprotein is an indication that some of the porphyrins lack iron.

The finding that cytochrome *c* is deficient in iron during lead intoxication is of interest on several levels. Metal substitution for iron should be considered as a possible cause of metal toxicosis. Furthermore luminescence techniques could provide a means to diagnose metal toxicosis. Because the turnover of cytochromes is 5–10 days [9], as compared with 120 days for hemoglobin [10], iron displacement in the cytochromes is likely to be observed at the onset of lead or other metal poisoning and in iron deficiency. Of additional interest, fluorescence parameters are environmentally sensitive, therefore, fluorescence of the cytochromes can be used to obtain information concerning the physical organization of the mitochondrial and microsomal electron transfer carriers.

Antagonism between lead and iron is known to occur in intestinal absorption and at the level of transport in the blood by transferrin [11]. Also, ferrochelatase, the enzyme responsible for inserting iron into porphyrins, is inhibited by heavy metal ions, including lead [12]. The result that hemeproteins are synthesized without iron suggests that some symptoms of lead intoxication can be reversed by supplementing the diet with iron.

Acknowledgements

This work was supported in part by grant US PHS GM 12202. J.M.V. is supported by NIH Career Development Award GM 0053.

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