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SPECTRAL EVIDENCE FOR THE CYTOCHROME P450 NATURE OF PROSTACYCLIN SYNTHETASE

Volker Ullrich, Laurence Castle and Peter Weber
Department of Physiological Chemistry, University of Saarland
665 Homburg-Saar, Federal Republic of Germany

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Cytochrome P450 is present in many organisms and tissues and so far has always been associated with monooxygenase reactions (1). When Juchau et al. (2) found this cytochrome in aorta microsomes together with an oxidation of benzo(a)pyrene, they suggested an aryl-4-monooxygenase function for cytochrome P450. However, the results reported here indicate that the physiological role of this hemoprotein in arterial walls is prostacyclin synthesis from the corresponding endoperoxide.

MATERIALS AND METHODS

Strips of pig aorta were homogenized in icecold 0.1 M Tris-buffer pH 8.0 containing 1 mM EDTA and 0.1 mM dithiothreitol and the microsomal fraction was obtained by differential centrifugation (3).

9.11-Endoperoxy-15-hydroxy arachidonic acid (PGH_2) was prepared according to a literature procedure (4) as well as 15-hydroperoxy arachidonic acid (5). 7-Ethoxycoumarin O-dealkylation was assayed as described previously (6) and for difference spectra an Aminco DW-2 spectrophotometer was employed. Cytochrome P450 concentrations were calculated using an extinction coefficient of ϵ = 91 mM^{-1} cm^{-1} for the CO-complex (7). Prostacyclin synthetase activity was measured as described (8).

RESULTS

Due to the vigorous homogenization required for the tough tissue, the microsomal fraction contained about 15 % mitochondrial fragments as judged by their succinate oxidase activity. This is also seen from the dithionite-reduced versus oxidized difference spectrum (Fig. 1, dotted line). Since these bands could mask a cytochrome P450-CO complex, the mitochondrial contribution was largely eliminated by the addition of succinate to the CO-gassed anaerobic reference cuvette. After bubbling the sample cuvette with CO and subsequent addition of a small amount of dithionite, an absorption at 450 nm developed slowly over 30 min (solid line). With an average of 12 preparations, a P450 concentration of 13 $\pm$ 5 pmol per mg protein was found. The mitochondrial fraction did not show this CO complex at 450 nm. NADPH could not replace dithionite in the microsomal difference spectrum, although an NADPH-cytochrome c reduction activity of about 11 nmol/mg protein was measured. Also no O-dealkylation of 7-ethoxycoumarin occurred.

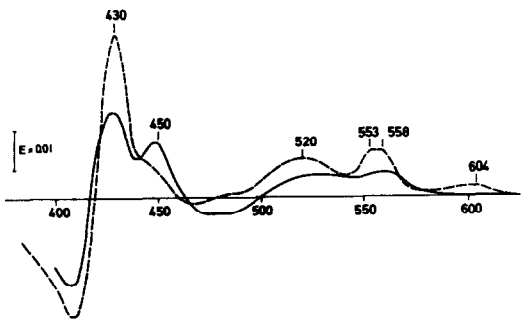


Fig. 1

Difference spectra of aorta microsomes. Dashed line: reduced by dithionite minus oxidized sample. Solid line: reduced by dithionite plus CO minus succinate-reduced plus CO. Succinate, 2 x 10^{-3}M; CO, 10^{-3}M, microsomes, 9.2 mg/ml.

Aorta microsomes are known to contain prostacyclin (PGI_2) synthetase and we also could establish a conversion of PGH_2 to PGI_2 in our preparations. We therefore looked for the influence of the substrate and known inhibitors of PGI_2 synthesis on the spectral properties of the aortic cytochrome P450.

Among the inhibitors it was reported that 15-hydroperoxy arachidonic acid strongly blocked PGI_2 synthesis already at micromolar concentrations (9). Increasing amounts of this compound caused the formation of an absorption band at 442 nm with a trough at 419 nm (Fig. 2). The corresponding spectral dissociation constant calculated from a Lineweaver-Burk plot was 6×10^{-6} mol/l. Again the mitochondrial fraction did not give this effect.

Among a series of amines, only tranylcypromine (phenylcyclopropylamine) was reported to inhibit PGI_2 synthesis, but only at millimolar concentrations (9). A corresponding difference spectrum showed a peak at 435 nm and a trough at 415 nm with a K_s-value of 1.2×10^{-4} mol/l (Fig. 3).

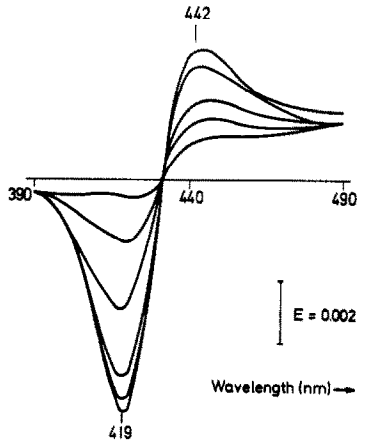


Fig. 2 Difference spectra of aorta microsomes with 15-hydroperoxyarachidonic acid. The curves correspond to concentrations of 1,2,4,8,12 µM, respectively. Microsomes: 9.6 mg/ml.

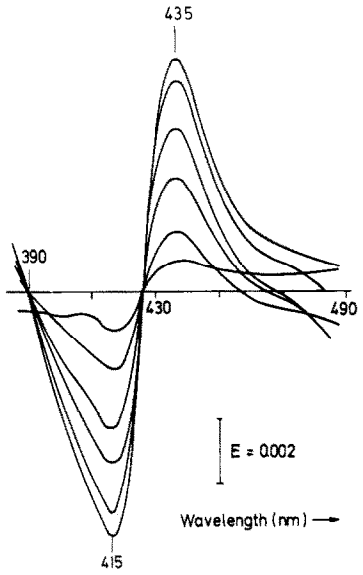


Fig. 3 Difference spectra of aorta microsomes with tranylcypromine. The curves correspond to concentrations of 20,40,80,160,320 and 640 µM, respectively. Microsomes: 9.6 mg/ml.

Finally it was interesting to look for a spectral interaction of PGH_2 as a substrate for PGI_2 synthetase with aorta microsomes (Fig. 4). At a concentration of 10^{-5} mol/l a difference spectrum with a small peak at 432 nm and a trough at 416 nm was formed. This spectrum was completely abolished in the presence of 10^{-3} mol/l tranylcypromine in both cuvettes indicating a competition at the same site. Typical substrates of liver microsomal cytochrome P450 did not give any spectral change in aorta microsomes.

DISCUSSION

Our results confirm the presence of cytochrome P450 in aorta microsomes, but neither an O-dealkylating activity for 7-ethoxycoumarin was detected nor a reduction by NADPH which is a prerequisite for monooxygenase activity. Even the reduction by dithionite was unusually slow and made a function in dioxygen activation unlikely. On the other hand, there is a rather

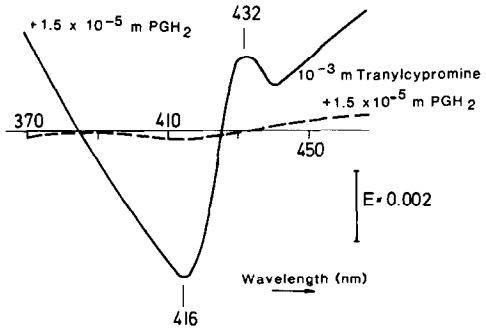
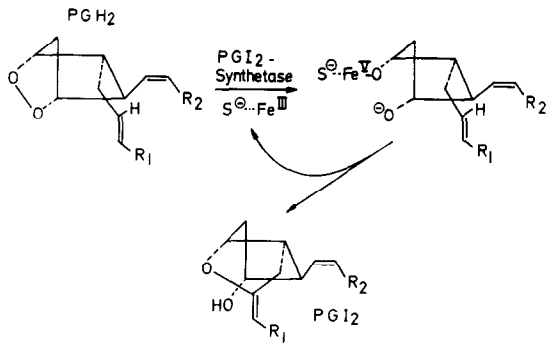


Fig. 4 Difference spectra of aorta microsomes with PGH_2. Microsomes: 9.2 mg/ml

specific and sensitive spectral interaction with PGH_2 and inhibitors of PGI_2 synthesis.

These spectral changes were not seen with the mitochondrial fraction and therefore must be associated with the microsomal fraction. They are typical of a conversion of ferric low spin cytochrome P450 to either a ligand complex with tranylcypromine or in case of the two peroxides of a higher valence state of the iron. It is important to note that the magnitudes of spectral changes at around 417 nm are comparable to that of the CO complex, suggesting the involvement of all of the CO-reacting material in the binding of the amine and the hydroperoxide.

The reaction with peroxides resembles that of ferric liver microsomal cytochrome P450 with cumene hydroperoxide or other oxene donors (10,11) which results in intermolecular oxene transfer as observed with nitromethane (12), and oxene transfer as one of the characteristic features of cytochrome P450 was postulated (13). For the rearrangement of PGH_2, we therefore propose the reaction scheme below.



Proposed mechanism for the participation of a heme-thiolate protein in prostacyclin synthesis

A similar mechanism but with a different cytochrome P450 could also apply to thromboxane A_2 formation in platelets. It is known that platelets contain cytochrome P450 (14) and their thromboxane formation is sensitive to typical P450 inhibitors (15).

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