Cytochrome P-450 gene expression in the common mussel *Mytilus edulis*

J. ANDY SPRY,* DAVID R. LIVINGSTONE,† ALAN WISEMAN,* G. GORDON GIBSON* and PETER S. GOLDFARB*

*Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, U.K. and †Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, U.K.

The cytochrome P-450-dependent mono-oxygenase system catalyses the oxidation of many structurally diverse compounds. In vertebrate systems, different species are known to express a number of different isoenzymes, each showing different substrate specificity. It has been observed that different isoenzymes may be induced in response to exposure to xenobiotics and drugs. It is hoped that this phenomenon, if exhibited in the mollusc P-450 system, will allow the mussel to be used as a biomonitor of environmental pollution in the marine environment.

P-450 enzyme activity in the mussel is primarily located in the digestive gland (hepatopancreas), with trace amounts found in mantle (storage and reproductive) and gill tissues. Levels of enzyme activity and specific content are, however, approximately 10-fold lower than in mammalian tissue [1].

Purification of P-450 from mussel digestive gland tissue has been attempted, and partial purification has been achieved [2]. However, problems attributed to low P-450-specific content and to amending techniques used for purification of the mammalian enzyme have made the traditional route to gene isolation (i.e. via protein purification) difficult [3].

In this study, we demonstrate the feasibility of an alternative route to gene isolation, using mammalian gene probes to detect homologous DNA and RNA sequences in the mussel. Mussels were collected from Whitsand Bay (Cornwall, U.K.) and the digestive gland removed and immediately frozen in liquid nitrogen. After storage at −70°C, RNA was purified according to the method of Cathala et al. [4] and DNA was purified according to Maniatis et al. [5]. Northern blotting was undertaken using the methods given in Maniatis

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Fig. 1. Hybridization of $^{125}$I-labelled rat P-450 cDNA probes to mussel and rat RNAs

(a) P-450 RLM6; (b) P-450 IVA1; (c) P-450 IVA1.
et al. [5] on to nylon (Hybond-N, Amersham). Dot blots using a BRL apparatus were on to nitrocellulose (BRL) for RNA and nylon (Amersham) for DNA.

Gene probes isolated from rat cDNA libraries corresponding to two members of cytochrome P-450 superfamily, namely a 1.2 kb fragment of P-450 IVAI (P-450 LAw, P-452) and a 1.1 kb fragment of P-450 RLM6 (probably the same as the alcohol inducible P-450 IIIE1/P-450ij; T. H. Richardson, personal communication) were used to detect homologous sequences.

The results shown in Fig. 1(a) demonstrate that sequences homologous to rat P-450 RLM6 cannot be detected in mussel, even under less stringent washing conditions (42°C, 2 × SSC [5]). In contrast, the P-450 IVAI probe does bind to mussel RNA (Fig. 1b), even under more stringent washing conditions (55°C, 0.5 × SSC). These observations are in agreement with the proposal that the P-450 II family is of comparatively recent origin and might not be present in lower organisms, whereas the P-450 IV family is of more ancient origin [6, 7]. It might be expected therefore that sequences homologous to P-450 IVAI would be expressed in lower organisms and indeed preliminary work in this laboratory has detected homology to RNA from a range of marine invertebrates.

We have also detected sequences homologous to the P-450 IVAI probe in mussel genomic DNA; however, much less stringent washing conditions were required (37°C, 10× SSC) owing to the presence of intron sequences.

In a Northern blot comparing rat and mussel RNA (Fig. 1c), hybridization of the P-450 IVAI probe is again observed. The size of the mRNA would appear to be smaller than the major 2.1 kb rat P-450 IVAI band. Conclusions about the identity of this mRNA and corresponding protein function must therefore await the isolation of the cDNA.

J. Andy Spry is indebted to the Natural Environment Research Council for a Ph.D C.A.S.E. award.

Received 19 June 1989

Metabolic activation of 7,12-dimethylbenz(a)anthracene: role of cytochrome P-450 isoenzymes in the formation of DNA and protein adducts in vitro

M. CUSACK,* A. K. BURNETT,† V. M. MORRISON* and J. A. CRAFT*

*Department of Biological Sciences, Glasgow College, Glasgow G4 0BA, U.K. and †Leukaemia Research Fund Laboratories, Glasgow Royal Infirmary, Glasgow G4 0SF, U.K.

The carcinogenic properties of 7,12-dimethylbenz(a)anthracene (DMBA) are dependent on metabolic conversion to the bay region diol-epoxide, DMBA-3,4-diol-1,2-oxide (DMBA-DO) and this ultimate carcinogen is formed by sequential oxidation during ultimate carcinogen formation [2]. The current study was initiated to identify other P-450s which may be important in the formation of DMBA metabolites and adducts and has utilized microsomal membranes from animals treated with selective inducers of P-450-isoenzymes. A protocol has been developed for the measurement of DMBA-protein and DMBA-DNA adducts in the same reaction mixtures. In parallel with these studies, rates of formation of DMBA diols have also been determined. Adduct formation was determined in reaction mixtures containing membranes (1 mg of protein/ml), NADPH, [14C]DMBA and exogenous calf-thymus DNA. After a 1 h incubation, reaction was stopped by cooling and the membranes were recovered by centrifugation at 100 000 g for 1 h. DNA was recovered from the supernatant by precipitation with ethanol and the resulting pellet was washed with 70% (v/v) ethanol before determination of radioactivity and DNA content. The membrane pellet was extracted with chloroform/methanol to remove unreacted DMBA, DMBA metabolites and DMBA-lipid adducts. The solvent-extracted proteins were hydrolysed with sodium hydroxide before determination of radioactivity and protein content. Rates of adduct formation are expressed as pmol of DMBA min⁻¹ mg⁻¹ protein or DNA.

Microsomal membranes were prepared from the livers of untreated Long-Evans rats and animals pretreated with phenobarbital, Sudan III, dexamethasone, pyrazole or clofibrate. The effect of these inducing agents on the rates of formation of DMBA-diols is shown in Fig. 1(a). Pretreatment of animals with phenobarbital and Sudan III produces distinctive changes in the regio-selective metabolism of DMBA which we have previously reported [2, 3]. The formation of 1'-450(P-IIB1) may play some role in the second P-450-mediated oxidation during ultimate carcinogen formation [2].