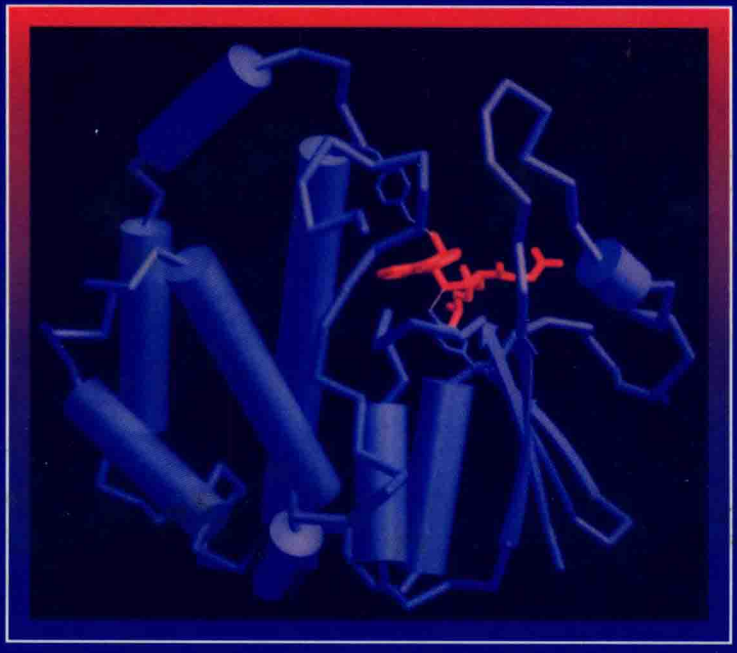
STRUCTURE *and* FUNCTION *of* GLUTATHIONE TRANSFERASES



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On the Cover: *RASTER3D representation of the three-dimensional structure of the type 3 subunit of a class mu glutathione S-transferase, courtesy of Xinhua Ji, Gary Gilliland, and Richard Armstrong, University of Maryland, College Park, MD, and the Center for Advanced Research in Biotechnology. The diagram was generated from the 1.8 Å structure of isoenzyme 3-3 in complex with 9-(S-glutathionyl)-10-hydroxy-9,10-dihydrophenanthrene, a product of the reaction of glutathione with phenanthrene 9,10-oxide. The α-helices are illustrated as cylinders and the β-strands as arrows. The product is shown in red with hydrogen bonding interactions (dashed lines) to the side-chains of two active site tyrosine residues (tyrosines 6 and 115) involved in catalysis.*

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Dedication

The Asilomar meeting and these proceedings are dedicated to the memory of Dr. Kiyomi Sato, Professor and Chairman of the Second Department of Biochemistry, Hirosaki University School of Medicine, Hirosaki, Japan, who passed away on November 4, 1992, after a year and a half struggle with rectal cancer.

Professor Sato was born on August 6, 1931, in Odate, Japan. In 1959, he received his M.D. from Tohoku University in Sendai, and after a year in internship, he entered the Biochemistry Division, Institute for Tuberculosis, Leprosy, and Cancer, Tohoku University. He started to work on isoenzyme changes of glycolysis, gluconeogenesis, and glycogen metabolism in rat primary and transplantable hepatomas. He worked hard, very often until midnight, obtained a Ph.D. in 1965, and was appointed as a research associate in the Division. Three years later, he found the muscle-type isoenzyme of fructose diphosphatase in rat hepatoma. This discovery provided him an opportunity to spend time in Dr. Sidney Weinhouse's laboratory at the Fels Institute in Philadelphia.

During his stay in Dr. Weinhouse's laboratory (1971–1972), he found the fetal type isoenzyme of glycogen phosphorylase in rat hepatoma. Later, Dr. Sato told his staff of an episode from its discovery. He obtained "leftover" samples that somebody else had used to assay for other enzymes and found the isoenzyme in them. He often said "there is luck in the last helping." This discovery provided him with a new and life-long position in Hirosaki. During his stay at Fels, he received a paper on glutathione S-transferase (GST) published in *Nature* from Dr. Litwack, and was much impressed by the paper, later leading to his own study on GSTs in Hirosaki.

During the next five years or so, he continued to work with his staff in Hirosaki on isoenzyme changes of sugar-metabolizing enzymes in rat hepatoma. From the late 1970s, his interest gradually shifted to drug-metabolizing enzymes such as γ-glutamyltranspeptidase and GSTs. In 1984, he discovered that the placental form of GST (GST-P) was an extremely useful marker for rat hepatic preneoplastic and neoplastic lesions. He also found the expression of GST-π in human colon, uterine cervix, and other cancers. In a series of studies he described the importance of pi class GSTs in carcinogenesis and malignancy. He organized with Dr. Henry C. Pitot a conference on GST and carcinogenesis as a U.S.–Japan Cooperative Cancer Research Program in 1988 and stimulated many investigators to study GSTs. His reputable reviews on GSTs (*Jpn. J. Cancer Res.*, 79, 556, 1988; *Adv. Cancer Res.*, 52, 205, 1989;

Crit. Rev. Biochem. Mol. Biol., 27, 337, 1992) have emphasized that he was a true scholar with a broad view of science. His outstanding work has been introduced by Dr. Weinhouse on the cover of the September issue of *Jpn. J. Cancer Res.* 82(9) in 1991 with a photograph of GST-P-positive single hepatocytes, putative initiated cells. Besides the pi class GSTs in carcinogenesis, he also contributed to the field by the discovery of several forms with interesting functions, such as GST-Yn_1Yn_1 of rat brain and GST-M_1M_2 of human aorta.

During his career he has obtained many grants for cancer research, including one from the Princess Takamatsu Cancer Research Fund in 1986. As one of the established investigators and a leader in the field of cancer research, he has organized groups to study gene expression of marker enzymes in preneoplastic hepatocytes since 1987 under the support of the Ministry of Education, Science, and Culture of Japan.

Besides being a devoted scientist, Dr. Sato was a warm and friendly person by nature. Everyone who knew him deeply respected and admired him, not just because of his accomplishments, but because of his friendly and outgoing personality. Dr. Sato's dedication to GST has been, and will be, an inspiration for all of us who have had the privilege and the pleasure of knowing him.

Preface

These Proceedings present a series of up-to-date articles by investigators who have focused on structural and functional studies of the glutathione transferase isozyme family. The chapters in this volume resulted from a meeting held in January, 1993, which brought together some of the top investigators in the area of glutathione transferase enzymology. The glutathione transferases are involved in many endogenous and exogenous functions in cells. For the first time, a series of x-ray crystallographic studies have been brought together in one volume and show detailed information on the structural aspects of these proteins. New information on the forces that play a role in glutathione and substrate binding sites are included. The recent success of the crystallography has provided an important platform for continued investigation of structure/function relationships. A number of molecular studies are also represented; these focus on the regulation of the glutathione transferases by identifying important response elements that control gene expression. Metabolism of drugs and carcinogens is also covered in some detail. The role these enzymes play in determining drug resistance at both the preclinical and clinical levels is discussed. Strategies for modulating drug resistance in tissue culture, animals, and in clinical studies is also well represented. In addition, the design and synthesis of novel inhibitors of glutathione transferases may provide an experimentally and (eventually) clinically useful route for the modulation of intracellular functions governed by the glutathione transferases. Thus, this book should provide a broad range of subject matter for biochemists, pharmacologists, medical oncologists, experimental therapeutic specialists, and structural biologists interested in isozyme families exemplified by the glutathione transferases.

Kenneth D. Tew

Acknowledgments

The ultimate success of this meeting was due in large part to the speakers and participants, Donna Bunch for her organizational skills, and to the record-breaking rainstorms of January 1993, which miraculously subsided during the course of the meeting.

The organizers would particularly like to thank Terrapin Technologies, Inc. for their enthusiastic support of the meeting, including generous financing of the speakers' expenses, without which it would not have been possible to bring together this international roster of leading GST researchers.

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SESSION ONE

FUNCTIONS OF GST

ROLE OF GLUTATHIONE S-TRANSFERASE AND ALDEHYDE REDUCTASE IN RESISTANCE TO AFLATOXIN B_1

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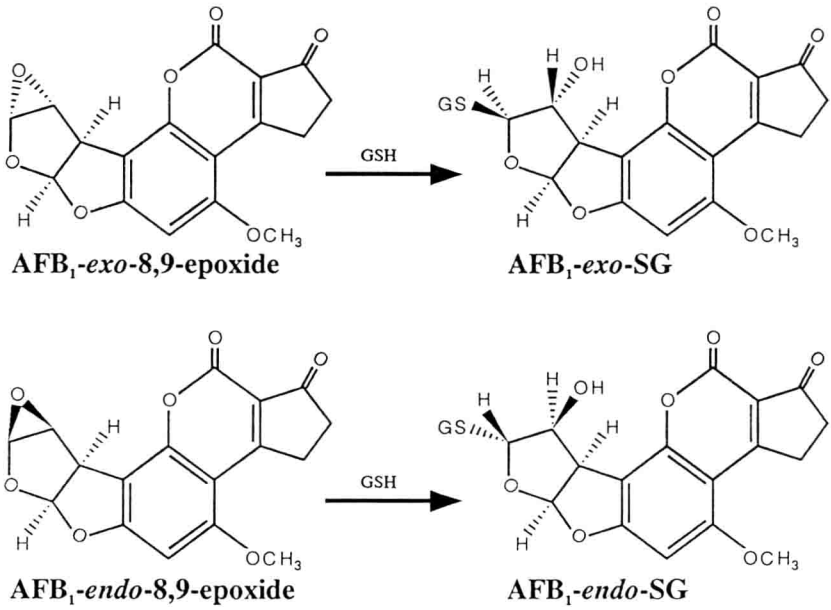
INTRODUCTION

Aflatoxin B_1 (AFB_1), produced by the mould *Aspergillus flavus*, is a potent hepatocarcinogen. It is often found to contaminate grain and nut crops in regions of the world that experience high humidity. Epidemiological studies suggest that AFB_1 is partly responsible for the high incidence of liver cancer in Asia and Africa.

The carcinogenic effects of AFB_1 result from its metabolism by cytochrome P-450 to AFB_1-8,9-epoxide. Recent studies have shown that cytochrome P-450s produce two epoxide isomers of AFB_1, namely an *endo* and an *exo* AFB_1-8,9-epoxide (Raney *et al.*, 1992a). The *exo* 8,9-epoxide is thought to represent the ultimate carcinogen and binds to DNA primarily through the N^7 atom of guanine (Essigmann *et al.*, 1977). Formation of such DNA adducts can result in activation of members of the *ras* gene family (Sinha *et al.*, 1988) and may lead to mutation of the tumour suppressor gene *p53* (Bressac *et al.*, 1991; Hsu *et al.*, 1991).

Selective toxicity of AFB_1

Aflatoxin B_1 displays selective toxicity. Species such as mouse and hamster are resistant to AFB_1 whilst the rat, and probably man, are sensitive to this mycotoxin (Newberne & Butler,1969). Aflatoxin is metabolised extensively in the liver and its toxicity is determined by the relative levels of enzymes responsible for the activation of AFB_1 and those involved in its detoxification (for a review, see Hayes *et al.*, 1991a). Several research groups (Neal & Green 1993; Coles *et al.*, 1985) have shown that the glutathione S-transferases, through their ability to catalyse the conjugation of AFB_1-8,9-epoxide with glutathione (GSH), can provide an important mechanism of resistance to this mycotoxin. Raney *et al.* (1992b) have shown that in the rat the alpha-class GST have the capacity to detoxify the *exo* 8,9-epoxide whilst the mu-class GST are active towards the *endo* 8,9-epoxide (see Figure 1). Since the formation of 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy-AFB_1, would appear to arise primarily from a reaction between DNA and AFB_1-*exo*-epoxide, rather than between DNA and AFB_1-*endo*-epoxide, protection against AFB_1 carcinogenesis is likely to be conferred by alpha-class GST rather than mu-class GST.



AFB_1-*exo*-8,9-epoxide **AFB_1-*exo*-SG**

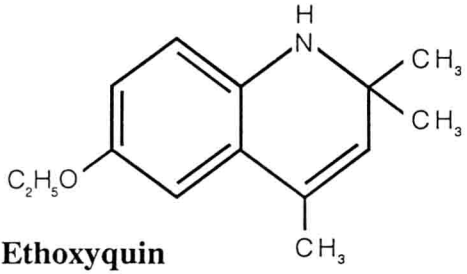
AFB_1-*endo*-8,9-epoxide **AFB_1-*endo*-SG**

Figure 1. Conjugation of glutathione with aflatoxin B_1 exo- and endo-epoxides

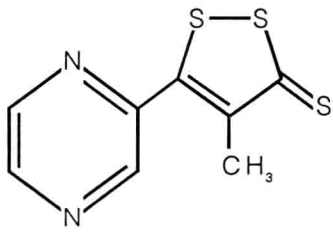
The intrinsic resistance of the mouse to AFB_1 cannot be attributed to failure to activate the mycotoxin as Monroe & Eaton (1987) have shown that murine liver microsomes possess 4-fold greater capacity to form the 8,9-epoxide of AFB_1 than rat liver microsomes. However, mouse liver cytosol exhibits at least 20-fold greater AFB_1-GSH-conjugating activity than does rat liver cytosol suggesting that GST is responsible for the resistant phenotype; this conclusion is supported by the fact that treatment with buthionine-S-sulphoximine and diethyl maleate, which deplete hepatic GSH levels, can produce a marked increase in the sensitivity of the mouse to AFB_1-DNA adduct formation (Monroe & Eaton, 1988) and presumably AFB_1 hepatocarcinogenesis. The high level of AFB_1-GSH-conjugating activity has been attributed to the constitutive expression of a GST Yc subunit in mouse liver (Quinn *et al.*, 1990; Ramsdell & Eaton, 1990) and recently the cDNA encoding this murine GST has been cloned (Buetler & Eaton, 1992; Hayes *et al.*, 1992).

Chemoprotection

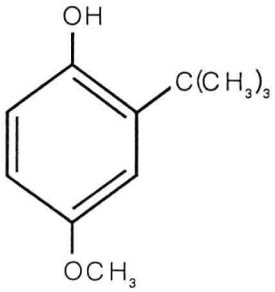
Although the male Fischer 344 rat is sensitive to AFB_1, and will develop liver cancer following exposure to AFB_1, resistance to this carcinogen can be induced by the administration of chemoprotectors. Drugs which serve as chemoprotectors (see Figure 2) include the antioxidants, ethoxyquin (Cabral & Neal, 1983), butylated hydroxyanisole (Kensler *et al.*, 1986; Jhee *et al.*, 1988) and oltipraz (Kensler *et al.*, 1987) as well as the enzyme inducer phenobarbital (Lotlikar *et al.*, 1989). The ability of these chemicals to reduce the sensitivity of the rat to AFB_1 is associated with increased hepatic detoxification capacity for AFB_1 and its metabolites.



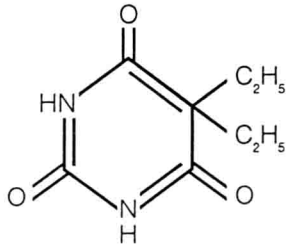
Ethoxyquin



Oltipraz



Butylated hydroxyanisole



Phenobarbital

Figure 2. Structure of chemoprotectors against AFB_1 carcinogenesis

Our laboratories have focussed on chemoprotection conferred by ethoxyquin (EQ) in the rat and we have shown that dietary administration of this antioxidant increases several detoxification enzymes. For example, EQ induces the cytochrome P-450s involved in the hydroxylation of AFB_1, to form AFQ_1 and AFM_1 (Mandel *et al.*, 1987), it induces GST responsible for the conjugation of the 8,9-epoxide with GSH (Hayes *et al.*, 1991b) and it induces an aldehyde reductase which reduces the dialdehydic form of AFB_1-8,9-dihydrodiol (Hayes *et al.*, 1993).

Glutathione S-transferase Yc_2

To allow the identification of the rat GST isoenzymes that are responsible for the EQ-inducible conjugation of AFB_1-8,9-epoxide with GSH, the hepatic transferases were resolved by cation-exchange chromatography on CM-cellulose. Figure 3 shows the CM-cellulose elution profile of hepatic GST from Fischer 344 rats that have been fed diets containing EQ. It is important to note that the AFB_1-GSH-conjugating activity from CM-cellulose does not co-elute with either GST activity towards 1-chloro-2,4-dinitrobenzene or peroxidase activity towards cumene hydroperoxide, indicating that the major isoenzymes responsible for detoxifying AFB_1 are distinct from the most thoroughly characterized rat GST, namely transferases F,L,C,B,A and AA.

Following the CM-cellulose step, we purified the transferases which metabolise AFB_1-8,9-epoxide by elution from hydroxyapatite followed by chromatography on a Waters Protein PAK SP-5PW cation exchanger. The enzymes isolated by this procedure, which are active with the 8,9-epoxide, were found to comprise either Ya_1Yc_2 or Yc_1Yc_2 subunits