Binding of benzo(a)pyrene to rat lung glutathione *S*-transferases in vivo

Shivendra V. Singh, Satish K. Srivastava and Yogesh C. Awasthi*

C3-16 Child Health Center, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550, USA

Received 10 September 1984; revised version received 27 October 1984

A highly selective in vivo binding of benzo(a)pyrene to rat lung glutathione *S*-transferases is demonstrated. Benzo(a)pyrene or its metabolites are specifically bound to Ya′ and Yc subunits of rat lung glutathione *S*-transferases.

Lung *Glutathione S-transferase* *Subunit* *Benzo(a)pyrene* *Polycyclic aromatic hydrocarbon* *Detoxification*

1. INTRODUCTION

Benzo(a)pyrene and other polycyclic aromatic hydrocarbons are known carcinogens. In rodents, benzo(a)pyrene has been demonstrated to produce cancer in lung and in other organs [1]. The carcinogenic effect of benzo(a)pyrene and other polycyclic aromatic hydrocarbons is primarily due to highly toxic metabolites of these compounds formed during their metabolism by the enzymes of the mixed function oxygenase system [2]. Glutathione (GSH) *S*-transferases (EC 2.5.1.18) probably represent one of the major defense systems for the detoxification of these compounds [3–5]. GSH *S*-transferases can catalyse the conjugation of highly toxic epoxides of polycyclic aromatic hydrocarbons to GSH thereby preventing their conversion to highly carcinogenic polyols. It has been suggested that GSH *S*-transferases may also non-catalytically bind carcinogens and precarcinogens and remove them from circulation [4]. Here, we report for the first time that rat lung GSH *S*-transferases bind benzo(a)pyrene or its metabolites in vivo and that the binding is highly selective to the subunits Ya′ and Yc of lung enzymes.

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

Male Sprague-Dawley rats (225 ± 10 g) used in this study were obtained from Timco Harlan Sprague Dawley Breeding Laboratories, Houston, TX. ^{14}C-labelled benzo(a)pyrene was purchased from New England Nuclear and non-radiolabelled benzo(a)pyrene was purchased from Sigma, St. Louis, MO. Sources of other chemicals and antibodies were the same as in [6]. The protocol for purification of rat lung GSH *S*-transferases was similar to that of [6]. GSH *S*-transferase activity was determined by the method of [7]. Radioactivity counting was performed in a liquid scintillation counter (model LS-230) [8].

3. RESULTS AND DISCUSSION

When the rats were intraperitoneally injected with ^{14}C-labelled benzo(a)pyrene (total counts per min (cpm), 4.7×10^6) dissolved in corn oil (specific activity, 4.7×10^5 counts per mg benzo(a)pyrene), a steady increase in the levels of benzo(a)pyrene in blood was observed up to 28 h (fig.1). Thirty h after injection, the rats were killed and the lungs were excised, perfused and washed with 10 mM potassium phosphate buffer containing 140 mM sodium chloride. Lungs (total wet wt of both

Published by Elsevier Science Publishers B.V.
00145793/85/$3.30 © 1985 Federation of European Biochemical Societies

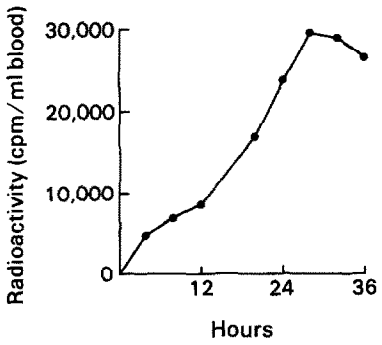

Fig.1. Benzo(a)pyrene levels in blood after injecting ^{14}C-labelled benzo(a)pyrene to rats. Blood was drawn from tail vein at 4 h intervals. 20 μl blood was spotted on a piece of filter paper, dried and radioactivity determined.

lungs, 1.74 g) were homogenized in a Sorvall omnimixer at 4000 rpm to make a 10% homogenate. Aliquots of the homogenate (100 μl) were used for radioactivity determinations. The homogenate was centrifuged at 10000 × *g* for 40 min and the radioactivity was determined in the supernatant. The supernatant was dialysed against 22 mM potassium phosphate (pH 7.0), and passed over an affinity column (0.9 × 10 cm) of epoxy activated Sepharose 6-B to which GSH was linked. GSH *S*-transferases were eluted with 5 mM GSH in 50 mM Tris–HCl buffer, pH 9.6. Upon urea/SDS/2-mercaptoethanol–polyacrylamide-slab gel electrophoresis [9] of this preparation only 3 polypeptide bands corresponding to Ya and Ya′ (M_r 22000), Yb (M_r 23500), Yc (M_r 25000) subunits were observed.

A significant amount of radioactivity was found to be associated with the lung tissue after 30 h of benoz(a)pyrene injection. Out of 69620 cpm present in 1 g of lung tissue (wet wt), 44039 cpm were recovered in the soluble fraction (table 1). In a separate experiment when rats were injected intraperitoneally with ^{14}C-labelled benzo(a)pyrene (total cpm, 1.0 × 10^6) about 18660 cpm were detected in 1 g lung tissue. This indicates a dose response interrelationship between benzo(a)pyrene injected and the amount bound to lung proteins. About 75% of total cpm present in the soluble fraction were found in GSH *S*-transferase fraction purified by affinity chromatography (table 1). Since GSH-affinity columns specifically bind GSH *S*-transferases [6,10], a highly selective binding of

Table 1

Binding of benzo(a)pyrene with GSH *S*-transferases in rat lung

Fraction obtained from 1 g of lung tissue	Subunit structure	cpm
Whole homogenate	–	69620
10000 × *g* supernatant	–	44039
Total GSH *S*-transferases obtained by affinity chromatography[a]	–	32778
GSH *S*-transferase I[b] (p*I* 8.8)	Yc/Yc	9398
GSH *S*-transferase II (p*I* 7.2)	Ya/Ya′	7948
GSH *S*-transferase III (p*I* 6.8)	Ya/Yb or	ND
GSH *S*-transferase IV (p*I* 6.0)	Ya/Yb′	ND
GSH *S*-tranferase V (p*I* 5.3)		ND
GSH *S*-transferase VI	Ya′/Ya′	11431

[a] Details of the affinity chromatography are described in the text. The yield of the enzyme activity during the affinity step was 63%. During the affinity chromatography the recovery of radioactivity (cpm) in absorbed and unabsorbed fractions ranged from 92–95%

[b] Total cpm subjected to isoelectric focusing were 28407. Details of isoelectric focusing were the same as in [6]

^{14}C-labelled benzo(a)pyrene (total cpm 4.7 × 10^6, specific activity 4.7 × 10^5 cpm/mg benzo(a)pyrene) was injected. The efficiency of the machine ranged from 85–89%

benzo(a)pyrene to these enzymes is indicated. The binding of benzo(a)pyrene with GSH *S*-transferases was confirmed by gel filtration studies. When the affinity pool was subjected to Sephadex G-100 gel filtration, coincident peaks of GSH *S*-transferase activity and radioactivity were obtained corresponding to an M_r value of about 47000 (fig.2). The binding of benzo(a)pyrene and other xenobiotics to cytosolic receptors present in rodent lung, liver, and kidney [11,12] has been demonstrated. These receptors have M_r values of 87000–245000 [12]. A coincident peak of radioactivity and GSH *S*-transferase activity corresponding to an M_r value of 47000 indicates that the

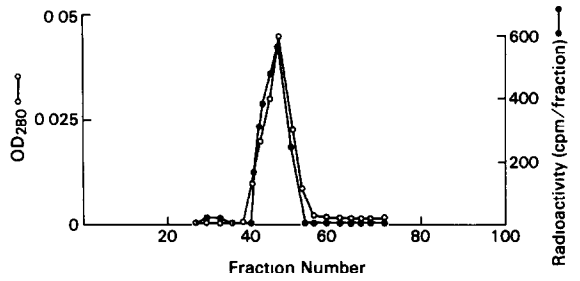

Fig.2. Sephadex G-100 gel filtration profile of rat lung GSH *S*-transferases. The enzyme sample having 5000 cpm was passed through a 1.5 × 90 cm column at an upward flow rate of 15 ml/h. 4 ml fractions were collected.

observed binding of benzo(a)pyrene to GSH *S*-transferases in this study is not due to the presence of these receptors. These results, therefore, strongly indicate that upon initial exposure, a major portion of benzo(a)pyrene or its metabolites present in the lung is bound to GSH *S*-transferases.

Since rat lung has several forms of GSH *S*-transferases, the binding of benzo(a)pyrene with specific enzymes/subunits was studied. We have previously [6] demonstrated 6 forms of GSH *S*-transferases in rat lung: I (p*I* 8.8), II (p*I* 7.2), III (p*I* 6.8), IV (p*I* 6.0), V (p*I* 5.3), and VI (p*I* 4.8). Form I is a dimer of Yc subunits (M_r 25000) whereas form II is a heterodimer of two different subunits, Ya and Ya′, having similar M_r values of 22000. The subunit structures of forms III, IV, and V are not completely understood. However, all of these forms have Ya subunits along with either Yb or Yb′ (M_r 23500) subunits. Form VI is a homodimer of Ya′ subunits. We have demonstrated that the Ya′ subunit is immunologically distinct from Ya subunits [6]. This subunit is expressed in the lung but not in liver. To investigate the binding of benzo(a)pyrene with individual forms of rat lung GSH *S*-transferases, the purified GSH *S*-transferases were subjected to isoelectric focusing. Six peaks of enzyme activity corresponding to the forms I to VI were observed (fig.3). The peaks of radioactivity were associated only with the forms I, II, and VI (fig.3, table 1). These results indicate that only Ya′ and Yc subunits of GSH *S*-transferase bind benzo(a)pyrene because no radioactivity was associated with forms III, IV, and V which contain Ya, Yb and Yb′ subunits. Of

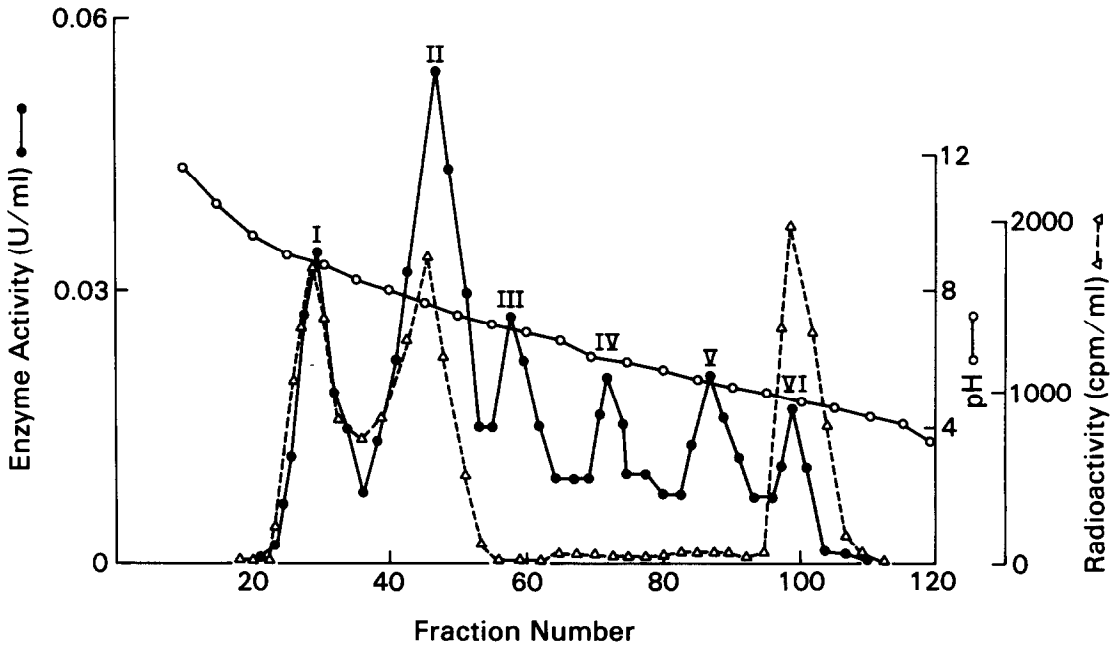

Fig.3. Isoelectric focusing (IEF) profile of purified rat lung GSH *S*-transferases. A total of 28407 cpm were present in the enzyme sample used for IEF. Experimental details were as in [6].

the two benzo(a)pyrene binding subunits the Ya' appear to have a higher affinity for benzo(a)pyrene.

The selective binding of benzo(a)pyrene to Ya' and Yc subunits of lung GSH *S*-transferases was confirmed by immunoaffinity studies. For these studies antibodies specific to different constituent subunits of rat lung GSH *S*-transferases were used. These antibodies were the same as those used in [6]. An aliquot of the affinity pool having total GSH *S*-transferases of lung (prior to electrofocusing) was passed through a column of CNBr-activated Sepharose 4B to which antibodies raised against GSH *S*-transferase VI of rat lung (Ya'Ya') were linked. More than 70% of total radioactivity associated with the affinity pool was retained by this column. These results indicate that the Ya' subunits present in GSH *S*-transferase VI (Ya'Ya') and GSH *S*-transferase II (YaYa') account for the binding of about three fourths of the total benzo(a)pyrene bound to lung GSH *S*-transferases. When an immunoaffinity column of antibodies against Yc subunits was used, only about 20% of total radioactivity was retained by the column. Immunoaffinity columns of antibodies raised against a mixture of GSH *S*-transferases III, IV, and V (Ya, Yb and Yb' subunits) did not retain any radioactivity. This further indicated that the subunits Ya, Yb, and Yb' of lung GSH *S*-transferases do not bind benzo(a)pyrene. The results of immunoaffinity chromatography indicate a highly specific binding to Ya' and Yc subunits. High affinity of the Ya' subunit for benzo(a)pyrene binding indicates that this subunit may play an important noncatalytic role in the protection of lung tissue against chemically induced carcinogenesis. However, the metabolic fate of benzo(a)pyrene–enzyme complex and whether or not this complex is translocated to nucleus should be studied before assigning a definitive protective role to these enzymes. We have shown previously that the Ya' subunit is present in lung but not in liver. Selective expression of Ya' subunit in lung may have evolved to meet the special detoxication needs of this tissue. In case human lung GSH *S*-transferases also bind benzo(a)pyrene the noncatalytic role of these enzymes may be very important in the detoxication of polycyclic aromatic hydrocarbons present in air as pollutants.

ACKNOWLEDGEMENTS

This investigation was supported by PHS grant CA 27967, awarded by the National Cancer Institute, grants EY 04396 and EY 01677, awarded by the National Eye Institute, and grant GM 32304, awarded by Institute of General Medical Sciences.

REFERENCES

[1] Shimkin, M.B. and Stoner, G.D. (1975) Adv. Cancer Res. 21, 1–49.
[2] Miller, E.C. and Miller, J.A. (1969) Prog. Exp. Tumor Res. 11, 273–297.
[3] Booth, J., Boyland, E. and Sims, P. (1961) Biochem. J. 79, 516–524.
[4] Jakoby, W.B. (1978) Adv. Enzymol. Related Areas Mol. Biol. 46, 383–414.
[5] Chasseaud, L.F. (1979) Adv. Cancer Res. 29, 175–274.
[6] Singh, S.V., Partridge, C.A. and Awasthi, Y.C. (1984) Biochem. J. 221, 609–615.
[7] Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974) J. Biol. Chem. 249, 7130–7139.
[8] Bray, G.A. (1960) Anal. Biochem. 279, 1–60.
[9] Laemmli, U.K. (1970) Nature 227, 680–685.
[10] Simons, P.C. and Vander Jagt, D.L. (1977) Anal. Biochem. 82, 334–341.
[11] Okey, A.B., Bondy, G.P., Mason, M.E., Kahl, F.G., Eisen, H.J., Guenthner, T.M. and Nebert, D.W. (1970) J. Biol. Chem. 254, 11636–11648.
[12] Hannah, R.R., Nebert, D.W. and Eisen, H.J. (1981) J. Biol. Chem. 256, 4584–4590.