Volume 234, number 2, 379–382 FEB 06080 July 1988

Selective inhibition of oat glutathione-*S*-transferase activity by tetrapyrroles

Bal Ram Singh* and Robert W. Shaw

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409, USA

Received 18 March 1988

Inhibition of glutathione-*S*-transferase activity by various tetrapyrroles (bilirubin, biliverdin, chlorophyllin and hemin) has been studied. Addition of 77 μM bilirubin or biliverdin inhibited glutathione-*S*-transferase activity only by 15–19% whereas 77 μM chlorophyllin or hemin inhibited the enzyme activity by 83–86%. The data suggest that oat glutathione-*S*-transferases can distinguish between open and closed tetrapyrroles. Possible physiological significance of the observed results is discussed.

Bilirubin; Biliverdin; Chlorophyllin; Glutathione-*S*-transferase; Hemin; Enzyme inhibition; Tetrapyrrole; (Oat)

1. INTRODUCTION

Glutathione-*S*-transferases are reported to be present in plants [1,2], insects [3] and animals [4–6]. This group of enzymes is known to catalyze the conjugation of glutathione to a wide variety of xenobiotics [7]. Glutathione-*S*-transferases are also reported to possess glutathione peroxidase activity in animals [8]. Thus, this family of enzymes exhibits a detoxifying role in the cells by conjugating toxic agents with glutathione. Extensive studies have been performed on glutathione-*S*-transferases in animal systems [9] but very little research has been carried out in plants. For example, in the animal system, glutathione-*S*-transferases (sometimes also called ligandin) are thought to act as bilirubin-binding proteins [10] or heme-transporting proteins [11] but no such roles have been postulated in plants, so far.

In this communication, the effect of four different tetrapyrroles (bilirubin, biliverdin, chlorophyllin and hemin) on the activity of oat glutathione-*S*-transferases has been presented. It is hoped that this work will trigger further studies on the roles of glutathione-*S*-transferases in plants.

Correspondence (present) address: B.R. Singh, Food Research Institute, University of Wisconsin, 1925 Willow Drive, Madison, WI 53706, USA

2. MATERIALS AND METHODS

A crude extract of etiolated oat seedlings (*Avena sativa*) was prepared by homogenizing 10 g of tissue in 50 ml of grinding buffer (100 mM potassium phosphate, pH 6.5, containing 300 mM sucrose and 1 mM EDTA) in a Waring blendor at full speed for 30 s. The homogenate was filtered through eight layers of cheesecloth. The filtrate was centrifuged at 24000 × *g* for 20 min to remove cell debris. The supernatant was used to assay the glutathione-*S*-transferase activity.

The enzyme activity was measured according to Habig and Jakoby [12] by taking 50 μl of 20 mM 1-chloro-3,5-dinitrobenzene (CDNB) and 50 μl of 20 mM reduced glutathione in a 1 ml reaction mixture and monitoring the absorbance change at 340 nm. In order to determine the inhibition of the enzyme activity by tetrapyrroles (bilirubin, biliverdin, chlorophyllin and hemin), appropriate concentrations of the tetrapyrroles were added to the reaction mixture before the addition of reduced glutathione.

Aqueous solutions of all the tetrapyrroles except chlorophyllin (which is readily soluble in aqueous buffer) were made by first making a suspension of the tetrapyrroles in 1 ml (5 mg/ml) of 20 mM potassium phosphate buffer, pH 7.8, containing 1 mM EDTA. One drop of 2 N KOH was added to the suspension and a homogeneous and clear solution was obtained. The solution was diluted 50 times and the pH was adjusted to 6.5 before use.

The protein content of the crude supernatant was determined using a Bio-Rad protein assay kit [13].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50 © 1988 Federation of European Biochemical Societies

CDNB and glutathione (a gift from Dr D. Roy, UTMB, Galveston, TX) were from Sigma (St. Louis, MO). All other chemicals were of the highest purity grade available commercially. All buffers and solutions were made in deionized doubly distilled water. All the experiments were repeated at least three times.

3. RESULTS AND DISCUSSION

Glutathione-*S*-transferase activity has been associated with metabolism of detoxification in both animal and plant cells [1,7,14]. This group of enzymes has been known for a long time to bind to tetrapyrroles in animal systems [10] and recently has been shown to contain different binding sites for hematin and bilirubin [15]. Tetrapyrrolic compounds such as chlorophyll and heme are widely present in plants and their binding to glutathione-*S*-transferases may be physiologically significant. Whereas several studies have been carried out to investigate the binding of tetrapyrrolic compounds to glutathione-*S*-transferases in animal systems, this is the first report for such a study in a plant system.

Four tetrapyrrolic compounds (bilirubin, biliverdin, chlorophyllin and hemin) have been used to investigate the effect of tetrapyrrole addition on the activity of oat glutathione-*S*-transferases.

Fig.1 shows the time course of glutathione-*S*-transferase catalyzed conjugation of glutathione to CDNB in the absence and presence of tetrapyrrolic compounds. The activity is shown in terms of change in absorbance at 340 nm (due to conjugation of glutathione to CDNB) as a function of incubation time at 25°C. Chlorophyllin and hemin are the most effective tetrapyrroles in inhibiting the activity of glutathione-*S*-transferases whereas bilirubin and biliverdin are the least effective. Thus, the degree of inhibition is related to whether

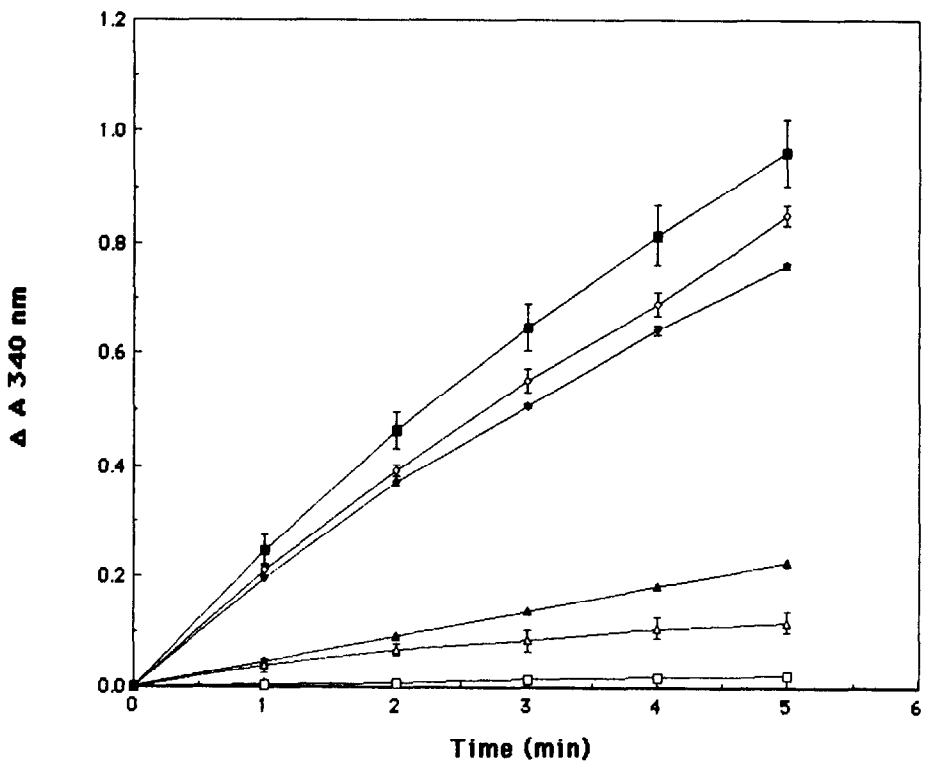

Fig.1. Time course for the glutathione-*S*-transferase catalyzed conjugation of glutathione to CDNB in the presence and absence of various tetrapyrrolic compounds. The bars represent the standard deviation of the measurements. (■) Control activity without inhibition; (◇) activity in the presence of bilirubin; (◆) activity in the presence of biliverdin; (▲) activity in the presence of chlorophyllin; (△) activity in the presence of hemin; (□) control in which no enzyme is added.

Table 1

Inhibition of glutathione-*S*-transferase activity by tetrapyrrolic compounds: bilirubin, biliverdin, chlorophyllin and hemin

Condition	Enzyme activity/mg protein (μM/min)	Relative ratio
Control	1085 ± 119	1.00
+ bilirubin	923 ± 8.5	0.85
+ biliverdin	877 ± 8.5	0.81
+ chlorophyllin	186 ± 13	0.17
+ hemin	155 ± 55	0.14

The enzyme activity (initial velocity) is shown in terms of concentration of CDNB conjugated to glutathione per minute as determined from the absorbance change at 340 nm. The concentrations of tetrapyrroles used in enzyme inhibition were 77 μM in all experiments

the tetrapyrroles are closed or open chain compounds. Chlorophyllin and hemin are closed chain tetrapyrroles whereas bilirubin and biliverdin are open chain tetrapyrroles.

The results of inhibition of glutathione-*S*-transferases by tetrapyrroles are summarized in table 1. The open chain tetrapyrroles inhibited the enzyme activity by only 15–19% while closed chain tetrapyrroles inhibited 83–86% of the control glutathione-*S*-transferase activity. This clearly shows the specificity of this group of enzymes for closed chain tetrapyrroles in plants. Though the exact mechanism of the inhibition of the enzyme activity is not known, it has been suggested that the conformational changes introduced upon binding of tetrapyrrolic compounds to the enzyme may be responsible [15]. It is interesting also that whereas in animal systems, the enzymes were inhibited equally by bilirubin and hemin [15], in plant systems, there is a discrimination between bilirubin and hemin or for that matter between open and closed tetrapyrrolic compounds. This observation may be of physiological importance because only chlorophyll and heme are present in abundance in plants. In contrast, in animal systems, bilirubin, biliverdin and heme are all present. Although with the present data it is not possible to conclude whether chlorophyllin and hemin (i.e. closed tetrapyrroles) share the same binding site on the enzyme, it is clear that open tetrapyrroles (i.e. bilirubin and biliverdin) do not strongly inhibit plant glutathione-*S*-transferases. Further study on this aspect will advance the understanding of tetrapyrrolic inhibition of glutathione-*S*-transferase activity at the molecular level.

As far as the physiological implication of this study is concerned, it can be argued that glutathione-*S*-transferases might be involved in the transport of heme or other tetrapyrroles between the subcellular compartments of plant cells similar to the process reported in animal systems [11]. This would be a hitherto unexplored interesting area of research. However, the most significant implication of this study concerns the possible role of glutathione-*S*-transferases in the process of plant senescence. It should be noted here that, to our knowledge, glutathione-*S*-transferases have not been implicated in the molecular processes involved in plant senescence. Since both mitochondrial and chloroplast membranes are known to be altered drastically during senescence [16], it is possible that heme groups and chlorophyll derivatives become available to bind with glutathione-*S*-transferases. Chlorophylls have been shown to be detached from the chloroplast membranes upon heating of chloroplast membranes [17]. Once the detoxifying activity of glutathione-*S*-transferases is inhibited, plant senescence is likely to be enhanced. Glutathione-*S*-transferases are shown to protect plants from toxic chemical pesticides [1] and are likely to guard plant cells from the toxic byproducts of natural metabolism. It is, therefore, believed that this work will be of interest to those working on plant senescence.

REFERENCES

[1] Mozer, T.J., Tiemeir, D.C. and Jaworski, E.G. (1983) Biochemistry 22, 1068–1072.
[2] Diesperger, H. and Sanderman, H. jr (1979) Planta 146, 643–648.
[3] Clark, A.G., Smith, J.N. and Spier, T.W. (1973) Biochem. J. 135, 385–392.
[4] Jakoby, W.B. (1978) Adv. Enzymol. Relat. Areas Mol. Biol. 46, 383–414.
[5] Singh, S.V., Dao, D.O., Patridge, C.A., Theodore, C., Srivastava, S.K. and Awasthi, Y.C. (1985) Biochem. J. 232, 781–790.
[6] Singh, S.V., Patridge, C.A. and Awasthi, Y.C. (1984) Biochem. J. 221, 609–615.
[7] Boyland, E. and Chasseaud, H.E. (1969) Adv. Enzymol. Relat. Areas Mol. Biol. 32, 173–219.
[8] Prohaska, J.R. and Ganther, H.E. (1977) Biochem. Biophys. Res. Commun. 76, 437–445.
[9] Habig, W.H. and Jakoby, W.B. (1981) Methods Enzymol. 77, 218–231.

[10] Ketterer, B. (1985) in: Optical Properties and Structure of Tetrapyrroles (Blauer, G. and Sund, H. eds) pp.261–278, Walter de Gruyter, Berlin.
[11] Husby, P., Srai, K.S., Ketterer, B. and Romslo, I. (1981) Biochem. Biophys. Res. Commun. 100, 651–659.
[12] Habig, W.H. and Jakoby, W.B. (1981) Methods Enzymol. 77, 398–405.
[13] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[14] Booth, J., Boyland, E. and Sims, P. (1961) Biochem. J. 79, 516–524.
[15] Vander Jagt, D.L., Hunasker, L.A., Garcia, K.B. and Royer, R.E. (1985) J. Biol. Chem. 260, 11603–11610.
[16] Thimann, K.V. (1980) in: Senescence in Plants (Thimann, K.V. ed.) pp.85–115, CRC Press, Boca Raton, FL.
[17] Murata, N. and Fork, D.C. (1975) Plant Physiol. 56, 791–796.