CHAPTER - III
PURIFICATION AND CHARACTERIZATION OF CHICK EMBRYONIC LIVER CYTOSOLIC GLUTATHIONE S-TRANSFERASES

Introduction

Liver is one of the main target organs of environmental pollutants and xenobiotics. The study on the influence of these pollutants on hepatic GSTs is necessary. GSTs are generally involved in the detoxication of activated, electrophilic xenobiotics. Thus the investigation on the capacity of these xenobiotics and environmental pollutants to alter the activation and detoxication balance has great importance. Chick embryos have functional biotransformation enzymes, which are highly inducible by xenobiotics (Lorr and Bloom, 1987; Hamilton et al., 1992).

Knowledge of the type of GSTs in normal tissues is a prerequisite for a sound understanding of their variations in abnormal conditions, as seen in many diseased states. The presence of mu (μ), theta (θ) and alpha (α) class GSTs was reported in chick livers (Hsiao et al., 1995; Liu and Tam, 1991 and Chang et al., 1992). Theta is considered the most ancient among the GSTs and theta-like GSTs are found in mammals, fish, insects, plants, unicellular algae, and bacteria. It is thought that an ancestral theta-gene underwent an early duplication before the divergence of fungi and animals and further duplications generated the variety of the other classes of GSTs (alpha, mu, pi, etc.). The comparison of the amino
acidic homologies among mammals suggests that a duplication of an ancient GST theta occurred before the speciation of mammals and resulted in the subunits GSTT1 and GSTT2. The ancestral GST theta has a dehalogenase activity towards several halogenated compounds, such as the dichloromethane. Therefore, although GST theta behaves as a scavenger towards electrophiles, such as epoxides, it acts also as metabolic activator for halogenated compounds, producing a variety of intermediates potentially dangerous for DNA and cells. Interestingly, the liver and kidney are two organs that express the highest level of GST theta in the human body. Thus, the GSTT1-1 genotype is suspected to confer decreased or increased risk of cancer in relation to the source of exposure (Stefano, 2000).

Hence in the present study GSTs are purified from 11th day old chick embryonic liver by affinity chromatography. Polyclonal antibodies raised against chick embryonic liver GSTs in rabbit were employed for probing the variations in GST subunits in acrylamide and cadmium chloride treatments.

Objectives:

To study the effect of acrylamide and cadmium chloride on liver GSTs of chick embryo, the following objectives were studied:

1. To purify 11th day old chick embryonic liver GSTs by using affinity chromatography.
2. To characterize both affinity and individual GST subunits of acrylamide and cadmium chloride treated chick embryonic liver by using SDS-PAGE analysis, a wide array of substrates and western and dot-blot analysis.

Materials and methodology of this chapter were mentioned in the chapter "Materials and Methods".

Results
Affinity purification:

Glutathione S-transferases have been purified from 11th day old chick embryonic liver by affinity chromatography.

A 10% homogenate was prepared and centrifuged at 10,000 x g for 30 min and later the 10,000 x g supernatant was subjected to 1,05,000 x g for 1 hr and the following supernatant was termed as cytosol. The cytosol was further subjected to overnight dialysis against 25 mM Tris-HCl buffer, pH 8.0, to remove the endogenous GSH. The dialyzed cytosol was recentrifuged at 10,000 x g for 30 min at 4°C to remove precipitated proteins. The supernatant thus obtained was loaded on to the Glutathione-CL agarose column, previously equilibrated with 50 mM Tris containing 0.2 M KCl, pH 7.4. Subsequently the column was washed thoroughly with 50 mM Tris with 0.2 M KCl, pH 7.4 to remove proteins bound non-specifically. The proteins with GST activity were eluted with 50 mM Tris-HCl containing 0.2 M KCl, 5 mM GSH, pH 8.0. The typical elution profile of GSTs from glutathione affinity column was represented in Table 21. As shown in Fig. 27 GSTs eluted in a single sharp peak and all the active fractions pooled and were concentrated to 5 ml by ultrafiltration using Amicon concentrators with 30 KDa cut off. The purification achieved was with an overall yield of 31% (Table 22).
Table 21: The Typical Elution Profile of GSTs

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Protein at 280nm</th>
<th>GST Activity at 340nm (Units*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.158</td>
<td>0.73</td>
</tr>
<tr>
<td>2.</td>
<td>0.165</td>
<td>0.86</td>
</tr>
<tr>
<td>3.</td>
<td>0.192</td>
<td>4.69</td>
</tr>
<tr>
<td>4.</td>
<td>0.188</td>
<td>3.75</td>
</tr>
<tr>
<td>5.</td>
<td>0.171</td>
<td>2.82</td>
</tr>
<tr>
<td>6.</td>
<td>0.152</td>
<td>1.84</td>
</tr>
<tr>
<td>7.</td>
<td>0.108</td>
<td>0.96</td>
</tr>
<tr>
<td>8.</td>
<td>0.068</td>
<td>0.62</td>
</tr>
<tr>
<td>9.</td>
<td>0.042</td>
<td>0.53</td>
</tr>
<tr>
<td>10.</td>
<td>0.036</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*One Unit is defined as micromoles of GSH conjugate formed per min.
Figure 27: The Typical Elution profile of 11th day chick embryonic liver GSTs

![Graph showing elution profile with fractions on the x-axis and activity in units on the y-axis.]

Table 22: Purification profile of 11th day old chick embryonic liver GSTs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Protein (mg)</th>
<th>Total Activity (Units*)</th>
<th>Specific Activity (Units/mg protein)</th>
<th>Fold Purification</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>576.0</td>
<td>1918</td>
<td>3.32</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Affinity Purified sample</td>
<td>16.8</td>
<td>593.6</td>
<td>35.33</td>
<td>10.64</td>
<td>31</td>
</tr>
</tbody>
</table>

*One Unit is defined as micromoles of GSH conjugate formed per min.
SDS- PAGE Analysis of Affinity Purified Chick Embryonic Liver GSTs

The affinity purified GSTs were subjected to 12% SDS- PAGE to check the purity of the preparation and analyze the molecular weights of the GSTs. Calibration of the relative molecular weight was done by loading the authentic standards along with the affinity purified GSTs side by side in a single gel (Fig. 28).

As shown in the figure 28, the affinity purified GSTs (Lane- 2 & 3) resolved into three bands CL1, CL2 and CL3 with relative molecular weights of 27.0, 26.0 and 25.0 KDa respectively.

Substrate Specificities of Affinity Purified GSTs of Control Liver

Apart from the structural characterization, functional studies of chick embryonic liver GSTs were also conducted by studying the activity levels of affinity purified GSTs with nine different substrates (Table 23). As shown in the table, highest activity of affinity purified GSTs was observed with CDNB and lowest activity was observed with BSP and DCNB. Higher activity of isomerase was found in chick embryo liver due to the presence of alpha GST. The presence of high activity with CDNB indicated the existence of mu GST gene product and the activity towards EPNP represented the existence of theta GST in chick liver.
Figure 28: SDS-PAGE Analysis of Affinity purified 11th day old Chick Embryonic Liver GSTS

Lane 1: Marker Proteins
Lane 2-3: 11th day old chick embryonic liver purified GSTs
Table 23: Substrate specificities of Affinity Purified 11th day old Chick Embryonic Liver GSTs

<table>
<thead>
<tr>
<th>Sample</th>
<th>CDNB*</th>
<th>DCNB*</th>
<th>PNBC*</th>
<th>PNPA*</th>
<th>BSP*</th>
<th>EPNP*</th>
<th>$\Delta^5$ A*</th>
<th>CHP#</th>
<th>$\text{H}_2\text{O}_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity purified protein</td>
<td>35.33</td>
<td>0.26</td>
<td>0.34</td>
<td>0.47</td>
<td>0.16</td>
<td>0.33</td>
<td>1.31</td>
<td>0.57</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* μ moles of GSH conjugate formed /min/ mg protein

# μ moles of NADPH oxidized /min/ mg protein

Effect of Acrylamide and Cadmium chloride on liver GSTs

After studying the significant increase of GST activity in chick embryo liver in AC and CdCl$_2$ treatments in a dose and time dependent manner in chapter II, it was felt worth to extend the study to characterize GSTs under the influence of these two chemical carcinogens. The doses of AC and CdCl$_2$ in 72 hr treatment caused significant increase in the induction of GST activity compared to 24 and 48 hr treatment (Fig. 21 & 22). Hence, for characterization studies 0.1, 0.2 and 0.3 mg acrylamide and 0.01, 0.02 and 0.03 mg cadmium chloride in 72 hr treatment were selected.
Substrate Specificity studies of Acrylamide and Cadmium chloride Treated Liver GSTs

The specific activities of GSTs of acrylamide and cadmium chloride treated chick embryonic liver with the substrates CDNB, DCNB, pNPA, pNBC, EPNP, BSP, Δ⁵ A, CHP and H₂O₂ were determined.

In acrylamide treated samples higher activities were observed with CDNB, pNBC, EPNP, BSP, Δ⁵ A and pNPA. Total GPx levels were decreased with CHP in liver samples.

In 0.1mg AC (72 hr) treatment, GST activities with substrates CDNB, pNBC, EPNP, BSP, pNPA, Δ⁵ A and DCNB found to be increased by 1.13, 2.68, 1.85, 1.62, 5.4, 2.22 and 2.36 fold when compared to control, respectively. The GPx levels with substrates CHP decreased by 1.21 folds and with substrate H₂O₂ 1.15 fold in liver with respect to controls (Table 24).

In acrylamide treatment with 0.2mg, increase in activities with CDNB, pNBC, EPNP, BSP, pNPA and DCNB by 1.36, 2.8, 3.86, 1.9, 7.55 and 3.45 fold respectively, when compared to controls was observed. Activity with Δ⁵ A decreased by 1.27 fold compared to control. The GPx levels with substrates CHP and H₂O₂ decreased by 1.25 and 1.23 fold respect to controls (Table 24).
In 0.3mg AC treatment activities with CDNB, pNBC, EPNP, BSP, pNPA, $\Delta^5$A and DCNB found to be increased by 1.52, 3.79, 4.87, 2.18, 9.96, 1.48, and 7.54 fold when compared to controls, respectively. The GPx levels with substrates CHP and $H_2O_2$ decreased by 1.31 and 1.36 fold respect to controls (Fig. 29 to 36).

In 0.01mg CdCl$_2$ (72hr) treatment, GST activities with substrates CDNB and EPNP found to be increased by 1.1 and 1.01 folds respectively compared to controls. The activities with substrates pNBC, BSP and $\Delta^5$A decreased significantly by 5.01, 2 and 1.42 fold compared to control. Where as with DCNB, the activity remained the same as that of control. The GPx levels with substrates CHP decreased by 1.33 fold and with $H_2O_2$ 1.09 fold in liver with respect to control (Table 25).

In 0.02mg CdCl$_2$ (72hr) treatment GST activity with CDNB, EPNP and DCNB increased significantly by 1.34, 2.01 and 2.27 fold compared to control. The activities with pNBC, BSP and $\Delta^5$A increased from 0.01mg treatment by 3.13, 1.93 and 1.07 folds; but, they showed less activity compared to controls. The GPx levels with substrates CHP and $H_2O_2$ decreased by 1.42 and 1.15 fold with respect to controls (Table 25).
In 0.03mg CdCl₂ (72hr) treatment GST activities with CDNB, DCNB, EPNP and BSP increased significantly by 1.5, 3.9, 2.7 and 1.12 folds compared to control, respectively. Activities with pNBC and Δ⁵A decreased by 3.0 and 1.42 fold with respect to controls. The GPx levels with substrates CHP decreased by 1.51 fold and with H₂O₂ 1.21 fold in liver compared to controls (Fig. 37 to 43).
Table 24: Effect of Acrylamide Treatment on the Levels of GSTs and GPx of 11th day old Chick Embryonic Liver with Different Substrates

<table>
<thead>
<tr>
<th>Liver</th>
<th>CDNB*</th>
<th>DCNB*</th>
<th>pNPA*</th>
<th>pNBC*</th>
<th>BSP*</th>
<th>EPNP*</th>
<th>Δ 3 Androstenedione*</th>
<th>CHP*</th>
<th>Hydrogen peroxide*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47.91±0.48</td>
<td>0.011±0.003</td>
<td>0.029±0.010</td>
<td>0.471±0.09</td>
<td>0.032±0.008</td>
<td>0.358±0.03</td>
<td>0.074±0.04</td>
<td>0.158±0.03</td>
<td>0.136±0.03</td>
</tr>
<tr>
<td>0.1mg AC</td>
<td>54.03±0.17*</td>
<td>0.026±0.003*</td>
<td>0.157±0.019*</td>
<td>1.264±0.16*</td>
<td>0.052±0.005*</td>
<td>0.664±0.05*</td>
<td>0.165±0.03*</td>
<td>0.130±0.04*</td>
<td>0.118±0.01*</td>
</tr>
<tr>
<td>0.2mg AC</td>
<td>65.62±0.75*</td>
<td>0.038±0.007*</td>
<td>0.219±0.038*</td>
<td>1.319±0.17*</td>
<td>0.061±0.007*</td>
<td>1.385±0.37*</td>
<td>0.058±0.003*</td>
<td>0.126±0.01*</td>
<td>0.110±0.04*</td>
</tr>
<tr>
<td>0.3mg AC</td>
<td>73.02±0.49*</td>
<td>0.083±0.008b</td>
<td>0.289±0.054*</td>
<td>1.789±0.33*</td>
<td>0.070±0.012*</td>
<td>1.745±0.84*</td>
<td>0.110±0.02*</td>
<td>0.120±0.01*</td>
<td>0.100±0.01*</td>
</tr>
</tbody>
</table>

*One unit is defined as micromoles of GSH conjugate formed/min/mg protein

+ One unit is defined as micromoles of NADPH oxidized/min/mg protein

Values are average of six separate experiments of six samples. Mean±SE

t-test: Values are significant at a=p<0.001, b=p<0.01, c=p<0.05
Figure 29: Effect of Acrylamide on Levels of GSTs of 11th day old Chick Embryonic Liver with CDNB

Figure 30: Effect of Acrylamide on Levels of GSTs of 11th day old Chick Embryonic Liver with DCNB
Figure 31: Effect of Acrylamide on Levels of GSTs of 11th day old Chick Embryonic Liver with pNPA

Figure 32: Effect of Acrylamide on Levels of GSTs of 11th day old Chick Embryonic Liver with pNBC
Figure 33: Effect of Acrylamide on Levels of GSTs of 11th day old Chick Embryonic Liver with EPNP

Figure 34: Effect of Acrylamide on Levels of GSTs of 11th day old Chick Embryonic Liver with BSP
Figure 35: Effect of Acrylamide on Levels of GSTs of 11th day old Chick Embryonic Liver with Δ5-Androstene 3,17-dione

Figure 36: Effect of Acrylamide on Levels of GPx of 11th day old Chick Embryonic Liver with CHP and H2O2
Table 25: Effect of Cadmium chloride treatment on the Levels of GSTs and GPx of 11th day old Chick Embryonic Liver with Different Substrates

<table>
<thead>
<tr>
<th>Liver</th>
<th>CDNB*</th>
<th>DCNB*</th>
<th>pNPA*</th>
<th>pNBC*</th>
<th>BSP*</th>
<th>EPNP*</th>
<th>Δ5 Androstenedione*</th>
<th>CHP*</th>
<th>Hydrogen peroxide*</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>47.91±0.48</td>
<td>0.011±0.003</td>
<td>0.029±0.010</td>
<td>0.471±0.09</td>
<td>0.032±0.008</td>
<td>0.358±0.03</td>
<td>0.074±0.004</td>
<td>0.158±0.03</td>
<td>0.136±0.03</td>
</tr>
<tr>
<td>0.01mg CdCl₂</td>
<td>52.76±0.36⁶</td>
<td>0.011±0.002⁴</td>
<td>0.094±0.02⁴</td>
<td>ND</td>
<td>0.016±0.002⁴</td>
<td>0.364±0.04⁴</td>
<td>0.052±0.001⁴</td>
<td>0.118±0.03⁴</td>
<td>0.121±0.03⁴</td>
</tr>
<tr>
<td>0.02mg CdCl₂</td>
<td>64.23±0.42⁵</td>
<td>0.025±0.01⁵</td>
<td>ND</td>
<td>0.295±0.08⁵</td>
<td>0.031±0.004⁵</td>
<td>0.721±0.15⁵</td>
<td>0.056±0.001⁵</td>
<td>0.111±0.04⁴</td>
<td>0.118±0.04⁴</td>
</tr>
<tr>
<td>0.03mg CdCl₂</td>
<td>71.45±0.28⁴</td>
<td>0.043±0.004⁴</td>
<td>ND</td>
<td>0.157±0.03⁴</td>
<td>0.036±0.001⁴</td>
<td>0.969±0.30⁵</td>
<td>0.052±0.002⁴</td>
<td>0.104±0.006⁵</td>
<td>0.112±0.01⁴</td>
</tr>
</tbody>
</table>

* One unit is defined as micromoles of GSH conjugate formed/min/mg protein

⁺ One unit is defined as micromoles of NADPH oxidized/min/mg protein

Values are average of six separate experiments of six samples. Mean±SE, ND: Not detectable

t-test: Values are significant at a=p<0.001, b=p<0.01, c=p<0.05

130
Figure 37: Effect of Cadmium chloride on Levels of GSTs of 11th day old Chick Embryonic Liver with CDNB

![Graph showing the effect of Cadmium chloride on GST levels.](image)

Figure 38: Effect of Cadmium chloride on levels of GSTs of 11th day old Chick Embryonic Liver with DCNB

![Graph showing the effect of Cadmium chloride on GST levels.](image)
Figure 39: Effect of Cadmium chloride on Levels of GSTs of 11th day old Chick Embryonic Liver with pNBC

Figure 40: Effect of Cadmium chloride on Levels of GSTs of 11th day old Chick Embryonic Liver with BSP
Figure 41: Effect of Cadmium chloride on Levels of GSTs of 11th day old Chick Embryonic Liver with EPNP

Figure 42: Effect of Cadmium chloride on Levels of GSTs of 11th day old Chick Embryonic Liver with \( \Delta^5 \)Androstene 3, 17-dione
Figure 43: Effect of Cadmium chloride on Levels of GPx of 11th day old Chick Embryonic Liver with CHP and H₂O₂

Immunological studies

Antisera was prepared against affinity purified GSTs of 11th chick embryonic liver. Immunoprecipitin band was observed when cross reactivity between raised antisera and affinity purified chick embryonic liver GST protein was checked by Ouchterlony double immuno diffusion method (Fig. 44). The chick embryo liver GST antibody cross reactivity did not show any precipitin band with the rat liver GST on double immunodiffusion.

Approximately 100 μgms each of cytosolic extract was taken as enzyme source from acrylamide and cadmium chloride treated liver for transblot procedure. Immunoblot analysis of AC and CdCl₂ treated liver samples showed a dose dependent increase in the induction of GSTs (Fig. 45 - 47). CL1 of theta class, CL2 of Mu class and CL3 of alpha class were induced with AC and CdCl₂ treatment. The induction of GSTs in AC and CdCl₂ treated liver in a dose dependent manner was also confirmed by dot-blot analysis (Fig. 48).
Figure 44: Ouchterlony Double Immuno Diffusion of purified Chick Embryonic Liver GST and Polyclonal Chick Embryonic Liver GST Antibodies

Figure 45: Immuno blot Analysis of 11th day old Chick Embryonic Liver GSTs of Control, Acrylamide and Cadmium chloride Induced GSTs probed with Chick Embryonic Liver GST specific Antibodies (BCIP/ NBT staining)

Lane 1: control liver
Lane 2: 0.1mg AC treated liver
Lane 3: 0.2mg AC treated liver
Lane 4: 0.3mg AC treated liver
Lane 5: 0.01mg CdCl₂ treated liver
Lane 6: 0.02mg CdCl₂ treated liver
Lane 7: 0.03mg CdCl₂ treated liver
Figure 46: Band Density patterns of Acrylamide Induced Liver GSTs in Immuno Blot Analysis

Figure 47: Band Density patterns of Cadmium chloride Induced Liver GSTs in Immuno Blot Analysis
Figure 48: Dot-blot Analysis of 11th day old Chick Embryonic Liver GSTs of Control, Acrylamide and Cadmium chloride Induced GSTs probed with Chick Embryonic Liver GST specific antibodies

(Data represented as triplicates)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1" alt="Control liver" /></td>
<td><img src="image2" alt="0.1mg AC treated liver" /></td>
<td><img src="image3" alt="0.2mg AC treated liver" /></td>
<td><img src="image4" alt="0.3mg AC treated liver" /></td>
<td><img src="image5" alt="0.03mg CdCl2 treated liver" /></td>
<td><img src="image6" alt="0.02mg CdCl2 treated liver" /></td>
<td><img src="image7" alt="0.01mg CdCl2 treated liver" /></td>
</tr>
</tbody>
</table>

Lane 1: control liver
Lane 2: 0.1mg AC treated liver
Lane 3: 0.2mg AC treated liver
Lane 4: 0.3mg AC treated liver
Lane 5: 0.03mg CdCl2 treated liver
Lane 6: 0.02mg CdCl2 treated liver
Lane 7: 0.01mg CdCl2 treated liver
**Discussion:**

Glutathione S-transferases are encoded by a multigene family and are differentially regulated in a tissue specific manner (Abramovitz et al., 1988) to meet the special detoxification needs of various organs (Awasthi and Singh, 1985).

Glutathione S-transferases as the detoxifying enzymes play an important protective role in embryonic tissues. In the present study 11th day old chick embryonic liver cytosolic GSTs were purified and individual subunits were characterized. The molecular masses of CL1, CL2 and CL3 subunits are estimated to be 27, 26 and 25 KDa respectively and are with in the range of molecular mass reported for mammalian cytosolic GSTs (Mannervik and Danielson, 1985). The substrate specificity study of affinity purified GSTs showed increased activity with CDNB, Δ^5A, EPNP, CHP, pNPA and pNBC (Table 23) indicating the presence of theta, alpha and mu isoforms. In view of the detoxification role of the GSTs against electrophilic compounds, the presence of three GST isoenzymes (CL1, CL2 and CL3) in the liver tissue might contribute to protection of the embryo during development. The major isoenzymes of the cytosolic liver GSTs was the theta (CL1), mu (CL2) and alpha class (CL3). Chang et al., 1990 in chick liver, isolated GSTs and designated as CL1 – CL5 according to their electrophoretic mobilities on denaturing gels. Chang et al., 1990 also proposed that subunits CL2 and CL3 belong to the mu and alpha family respectively, on the basis of immuno cross-reactivity, substrate specificity and N-terminal sequencing data. Hsiao et al., 1995; Liu and Tam, 1991 and Chang et al., 1992 by cloning.
experiments have shown that CL1, CL2 and CL3 are class theta GST (cGSTT1),
class mu (cGSTM1) and class alpha (cGSTA1), respectively. Meyer et al., 1991
has reported theta, a new class of GST purified from liver of rat and man. Uma in
2006 reported alpha and Mu class GSTs in rat liver. Strange et al., 1984 reported a
continuous expression of GST alpha in human fetal liver during development.
Lisa et al., 1992 reported in rat liver development, the two subunits of GST alpha.

The chick embryo liver GST antibody cross reactivity did not show any
precipitin band with the rat liver GST on double immunodiffusion. This shows
that the chick embryonic liver GSTs lack any homogeneity with rat liver GSTs.
Liu et al., 1992 reported that the theta GST cannot cross react with antibodies of
GST mu and alpha.

Glutathione S- transferases are a super gene family of ubiquitous
distribution and versatile enzymes which differ in the isozyme and the constituent
subunits. Therefore, they differ in the enzyme activity with different substrates
due to the difference in the C-terminal domain (Wilce and Parker; 1994). The
substrate specificity studies were aimed towards the analysis of levels of
induction of different isozymes of GST after the administration of acrylamide and
cadmium chloride in a dose dependent manner in liver of d11 chick embryo.

The specific activity profile of liver to different doses of acrylamide and
cadmium chloride are analyzed by using different substrates like CDNB, DCNB,
pNPA, pNBC, EPNP, BSP, Δ5A, CHP and H2O2
When the activity profiles of acrylamide and cadmium chloride treated liver with different substrates were analyzed. The acrylamide showed increase in activities with CDNB, DCNB, pNBC, BSP, pNPA, and $\Delta^3$A and Cadmium chloride treated liver GSTs showed increase in activities with CDNB, and DCNB in a dose dependent manner indicating the induction of mu, and alpha form of GSTs. Increased activities with EPNP indicates that theta GST exists and is induced in the liver of chick embryo. The theta GST (cGSTT1) that is expressed in chick liver may participate in detoxification by binding to the acrylamide end product glycidamide and also acts as dehalogenase enzyme in the case of CdCl$_2$ to remove chlorine from the system. Decrease in activities was observed with CHP and H$_2$O$_2$. Mouse, rat and human alpha class GSTs have high peroxidase activity (Mannervik and Danielson, 1988) but the chick alpha class GST (cGSTA1) has no peroxidase activity, this might be the reason for decrease in peroxidase activities in acrylamide and cadmium chloride treated chick embryonic liver. Chang et al., 1992; Liu et al., 1997 has reported that CL3 (cGSTA1) has no peroxidase activity and a relatively high activity with ethacrynic acid and CDNB. Similar results were obtained in the present study (Table 24 & 25). A similar observation was reported for rat alpha GST (GST 8-8) by Jensson et al., 1986. Mannervik and Danielson, 1988 reported that rat liver alpha class GSTs (GST 1-1 & GST 2-2) has shown high peroxidase activity and very high activity with CDNB. Whittington et al., 1999 has reported that theta class GST (GSTT1) is catalytically active towards EPNP, pNBC and dichloromethane and low activity towards CDNB. The western blot and dot-blot analysis revealed the presence
and induction of theta (CL1), mu (CL2) and alpha (CL3) class GSTs more predominantly. This confers protection in the liver from cytotoxic agents. The differential expression of GST isoforms in actively proliferating normal and pathological tissues, may be due to the varied environments to which the tissues are exposed.

Casalino et al., 2002 reported that cadmium administration causes a parallel increase in expression of alpha-GST protein in rats. An investigation by the Agency for Toxic Substances and Disease Registry, USA, to evaluate the toxicity of some metals, showed that in HepG2 cells, Cd^{2+} respectively, exhibited a significant dose dependent transcriptional activation capacity of the GST Ya subunit and of several other enzymes (Tully et al., 2000). Shukla et al., 2000 reported that cadmium induced increased mRNAs for two isoforms of glutathione S-transferases in rats. Reduced glutathione and other thiol containing proteins play a key role in cellular defense against Cd toxicity and carcinogenesis. It has been demonstrated that exposure to cadmium results in the induction of the genes for glutathione S-transferases (GST α and GST π) so as to result in rapid and efficient detoxification of Cd as well as the ROS generated from this metal (Chin and Templeton, 1993; Eneman et al., 2000).

Numerous reports have been published about the induction of GST isoforms during cancer (Batist et al., 1986; Shea et al., 1988; Tsuchida et al., 1989). The elevated expressions of GSTs is also observed in spontaneous cancers (Mitaka et al., 1987; Oyamado et al., 1988; Masuda et al., 1989). The induction is
attributed to more than one factor like increase in the half-life of the protein mRNA stability (Hongxie et al., 1995) and gene amplification (Chao et al., 1987). A promoter element has been identified, known as the "antioxidant responsive element" (ARE) that regulates the expression of genes encoding glutathione S-transferase and other various enzymes in response to oxidants or xenobiotics (He et al., 2001; Chanas et al., 2002). It has been shown that the transcriptional factor Nrf2 binds to ARE and regulates ARE-mediated gene expression and induction of these enzymes (Cho et al., 2000; Thimmulappa et al., 2002).

Acrylamide is converted to glycidamide in the organism. The epoxide formed is a good substrate for mu (Cl.2) and theta (Cl.3) GSTs, which is present in liver of chick embryo. It is largely this substance that is made responsible for the carcinogenic effect of acrylamide. High levels of expressions of GST are associated with increased tolerance of cells to noxious chemicals and failure to express GSTs is associated with increased risk of disease.

Generally carcinogenesis is classified into initiation, promotion and progression. Initiation is the covalent modification of DNA with electrophilic metabolites derived from carcinogens. The role of GSTs is particularly important in the prevention of initiation of carcinogenesis, as they remove the harmful electrophilic molecules from the body.

Acrylamide and cadmium chloride doses caused increase in the induction of theta, mu and alpha class GSTs in a dose dependent manner in the liver. The effect of inducers on the expression of phase II enzymes revealed that animal
systems on exposure to chemical carcinogens have evolved various defense mechanisms to protect themselves from the oxidative damage. The theta, mu and alpha classes induced by acrylamide and cadmium chloride might inhibit the initiation of carcinogenesis.