Coplanar PCB induces a selenium binding protein as a major cytosolic protein in rat liver

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1. Introduction

Selenium binding protein (SeBP) is a cytosolic protein that binds to selenium in a manner distinct from proteins involving selenocysteine.[1] This protein has been postulated to exhibit an important role in biological activity of selenium, such as tumor prevention.[1] Coplanar PCBs are extremely toxic chemicals which are widespread environmental pollutants[2] and causal agents of Yusho.[3] We recently discovered that the most toxic coplanar PCB, 3,3',4,4',5'-pentachlorobiphenyl (PenCB) strongly induces SeBP in liver cytosol of rats. Induction of the protein deserves attention for preventive mechanism for toxicity of coplanar PCB. Here we report that a coplanar PCB induces selenium binding protein as a major protein in liver cytosol. This induction response may modulate the biological effects by PenCB.

2. Materials and Methods

Male Wistar rats (4 weeks old) were treated with PenCB (25 mg/kg/4 ml corn oil, i.p.). Free-fed and pair-fed animals were treated as described previously.[4] Four animals were used in each group. Liver cytosol was prepared at five days after treatment. SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli[5] and proteins were stained with 0.1% Coomasie brilliant blue R-250. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was performed according to the method of O'Farrell *et al.*[6] Peptide mapping was done according to the method of Cleveland *et al.*[7] Amino acid sequence was analyzed by the method of Matsudaira.[8]

3. Results

Figure 1 shows that PenCB treatment induces 54k protein. PenCB treatment also induced a 58k protein. Table I summarizes molecular mass of proteins that were induced by PenCB treatment. Other proteins were decreased by treatment with PenCB. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) indicated that the protein was a major protein in liver cytosol of rats treated with PenCB. The protein exhibited a pI of 6.8 and was barely detectable in liver cytosol of free-fed and pair-fed control. We tried to determine N-terminal amino acid sequence of the 54k protein after separation on 2D-PAGE and blotted onto PVDF membrane, but the N-terminal was blocked. Then, the 54k protein separated on 2D-PAGE was digested *in situ* by *S. aureus* V_8-protease. Several fragments derived from the 54k protein were obtained, which were blotted onto PVDF membrane and sequenced. Amino acid sequence of

three analyzed fragments was highly homologous to mouse liver 56k SeBP[1)] and its isoform, acetaminophen binding protein.[9)] This result demonstrates that coplanar PCB inducible 54k protein is the counterpart of the mouse liver 56k SeBP, although amino acid sequence of rat liver 56k SeBP has not yet been elucidated.

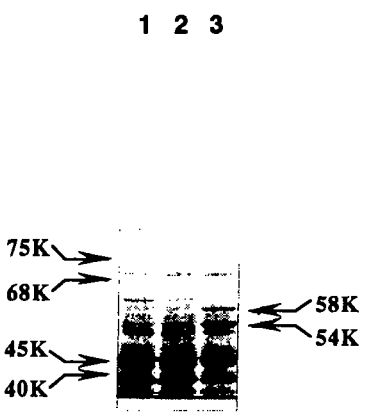

Fig. 1 SDS-Polyacrylamide gel electrophoresis (7.5%) of liver cytosol of rats. Lanes: 1, free-fed control (30 μg); 2, pair-fed control (30 μg); 3, PenCB-treated rat (30 μg). Left hand arrows represent migration of molecular weight marker proteins. Right hand arrows represent PenCB-inducible proteins, 54k and 58k, respectively. Migration is from top to bottom. The column represents the sample of an animal from each group.

Table I
Summary of the effects of 3,3',4,4',5-pentachloro-biphenyl treatment on cytosolic proteins in rat liver

response	molecular weight of protein
increased	54K, 58K
decreased	40K, 45K, 62K, 66K

4. Discussion

Selenium is a trace essential element in nutrition.[10)] Selenium is translationally incorporated into selenium labeled protein as selenocysteine encoded by a unique termination codon, such as glutathione peroxidase,[11,12)] type I iodothyronine deiodinase,[13)] and selenoprotein P.[14)] Contrary to this, SeBP binds to selenium in a manner distinct from selenocysteine[1)], but the binding mechanism is still unclear.

Selenium compounds inhibit chemical carcinogen-induced tumorigenesis in a broad spectrum of epithelial tissues, as well as the growth of mammalian cells in cell culture.[15] *In vitro* experiments have demonstrated that selenite is a potent, non toxic and reversible inhibitor of DNA synthesis.[16, 17] SeBP could mediate important role in tumor prevention by selenite, since the degree of inhibition of DNA synthesis by selenite was closely correlated with [^{75}Se]-labeling of SeBP in five different murine mammary epithelial cell lines.[18] SeBP distribution parallels selenite's anticarcinogenic effects *in vivo.* Selenite is effective against hepatic, gastric, colon and mammary carcinogenesis, and the SeBP is found in these tissues (reviewed in reference 18). Selenium compounds may be regulating DNA synthesis in cell cycle.[18, 19] The major [^{75}Se]-labeling was shown in G1 period of cell cycle.[19]

In early studies, non-toxic dose of dioxin and PCB (Aroclor 1254) was found to be strong inhibitors for skin tumors that were induced by 7,12-dimethylbenz[*a*]anthracene or benzo[*a*]pyrene in mice.[20-22] The inhibition of skin tumorigenesis was correlated with the qualitative changes of carcinogen-DNA interactions by the altered metabolism.[20-22] Though SeBP was shown not to be inducible by selenium compound,[23] Garberg and Högberg suggested that hydrogen peroxide generating system raises Se-labeling of SeBP in primary cultured hepatocyte of rats.[24] It is still uncertain whether oxidative stress induced by coplanar PCB is enough to elevate Se-labeling of SeBP. Kouri *et al.* reported that dioxin was a cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in *Ah*-nonresponsive mice.[25] On the contrary, dioxin did not affect the 3-methylcholanthrene carcinogenic index in *Ah*-responsive mice when animals were given dioxin two days before or simultaneously with 3-methylcholanthrene.[25] In addition, dioxin has been, recently, demonstrated as an anticarcinogen of mammary cancer in female Sprague-Dawley rats.[26] These inhibitory effects of dioxin on tumorigenesis are interesting in relation to our result of the 54k protein induction with PenCB.

However, reports are contradicting whether dioxin promotes chemical carcinogens induced carcinogenesis.[20-22, 25, 27] Dioxin was a promoter of hepatocarcinogenesis, rats that had received diethylnitrosamine following partial hepatectomy.[27] Dioxin and PCB have been characterized as tumor promoters rather than initiators.[25, 27] The levels of selenium and SeBP may be determinants of the biological function for this binding protein.

Further studies are required for the understanding of inducing SeBP. The studies on this protein could provide new aspects for the toxicity of polycyclic aromatic hydrocarbons and toxic polychlorinated aromatic hydrocarbons.

5. Conclusions

Coplanar PCB induced SeBP as a major protein in liver cytosol of rats. This significant induction may correlate with important biological events. This is the first report on the induction of SeBP by coplanar PCB.

6. References

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