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GLYCOSYLATION OF RAT LIVER NUCLEOPROTEINS INVOLVED IN THE REGULATION OF HAPTOGLOBIN GENE EXPRESSION IS CHANGED DURING ACUTE PHASE RESPONSE. **Ivana Cvetković, Ilijana Grigorov, Tanja Milosavljević and M. Petrović.** *Department for Molecular Biology, Institute for Biological Research Siniša Stanković,* 11060 Belgrade, Yugoslavia.

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Haptoglobin (Hp) is an acute phase protein (APP) synthesis of which significantly increases during acute phase response (APR) as a consequence of increased transcription of its gene in liver (B a u m a n n and G a u l d i e 1994). It has been established that transcriptional regulation of the rat Hp gene is controlled by a mechanism relying on structural modification of liver trans-acting nucleoproteins (NPs) (M i l o s a v l j e v i ć *et al.* 1997) which act *via* hormone regulatory sequence termed ABC element. Numerous studies implied that glycosylation is a prevalent form of structural modification of many nuclear proteins including transcription factors such as Sp1, HNF-1 and p53 (J a c k s o n and T j i a n 1988; S n a w *et al.* 1996; C o m e r and H a r t 1999). Qualitative carbohydrate content analyses of these transcription factors have shown that N-acetylglucosamine (GlcNAc), mannose (Man) and glucose (Glc) are the most common types of sugar moieties and their presence or absence determines their DNA binding affinity and/or transactivation potential (Eufemi et. al. 1991). Based of these data, the aim of this study was to investigate whether GlcNAc, Man and Glc sugar moieties exist in rat liver trans-acting NPs involved in the Hp gene transcriptional regulation during APR. In order to examine that, we isolated rat liver trans-acting NPs by DNA affinity chromatography.

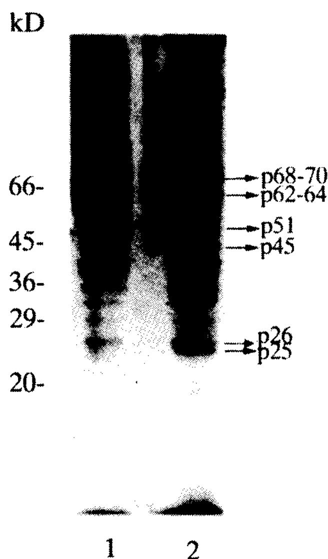

Fig. 1. Daffinity chromatography of nucleoproteins from the livers of control (lane 1) and turpentine treated (lane 2) rats.

DNA affinity column was prepared with regulatory ABC element (-170/-56 bp) linked to a CNBr-activated Sepharose 4B (Pharmacia) according to the method of K a d o n a g a and T j i a n (1986). Nuclear extracts (NEs) were isolated from the livers of control and 12 *h*-turpentine-treated rats (G o r s k i *et al.* 1986) and then applied on to the ABC affinity column. ABC-binding NPs were eluted with the buffer (25 mM Hepes, pH 7.9, 12.5 M MgCl2, 1mM DTT, 20% glycerol) containing 1M KCl. Equal amounts of ABC-binding NPs from the livers of both groups of rats were subjected to 11% SDS-polyacrylamide electrophoresis (SDS-PAGE) as described by L a e m m l i (1970). Silver staining of ABC-binding NPs electrophoretical profiles (Fig. 1) showed that DNA binding affinity of several NPs with molecular weights (Mw) ranging from 25 to 70 kD is changed during the course of APR. Namely, NPs with Mw from 26 to 45 kD displayed increased binding affinity to ABC element (Fig. 1, lane 2) in relation to the control sample (Fig. 1, lane 1), while NPs molecular mass of 25 (p25) and 51 kD (p51) expressed the binding affinity only during APR . At the same time, binding affinity of p62, p64, p68 and p70 was decreased.

In order to detect if GlcNAc, Glc and Man sugar moieties are attached to the rat liver trans-acting proteins amino acids side chains we performed lectin Western blot analyses. This method enabled us to detect GlcNAc moieties with wheat-germ agllutinin (WGA) and Glc and Man moieties with concanavalin A (Con A). Equal amounts of *trans*-acting NPs from the livers of control and turpentine-treated rats were separated by 11% SDS-PAGE and transferred on nitrocellulose membrane (Hybond-C, Amersham) after the elution from ABC affinity column. A membrane was firstly incubated in the TBST buffer (100 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 0.1% Tween-20 with 3 *mg*/mL bovine serum albumin (BSA) for 30 min, and then with biotinylated WGA or Con A (10 *mg*/mL of TBST buffer) for 45 min, with or without D(+)GlcNAc and D(+)Glc as specific competitors. To remove unbound lectin, the membranes were rinsed (3x10 min) with TBST and then incubated with avidine-biotinylated alkaline phosphatase complex (30 min) and rinsed (3x10 min). Binding reaction was visualised with chromofore 5-bromo-4chloro-3-indolyl phosphate and nitro blue tetrazolium (BCIP/NBT) (INC kit SP-2001, Vector Laboratories).

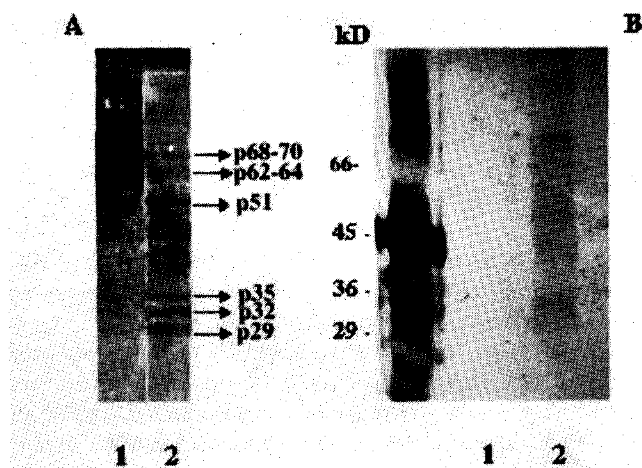

Fig. 2. WGA Western blot analyses of trans-acting nucleoproteins from the livers of control (lane 1) and turpentine treated rats (lane 2) in the absence (A) or presence (B) of 0.5 M D(+) GlcNAc as a specific competitor

Results of WGA Western blot analyses of rat liver *trans*-acting NPs (Fig. 2A) revealed that APR is accompanied by intensified GlcNAc glycosylation of p29, p32, p35 and p51 as well as deglycosylation of p62, p64, p68 and p70 (Fig 2A, lane 2) in relation to the corresponding controls (Fig.2A, lane 1). Specifity of rat liver *trans*-acting NPs-WGA interactions was confirmed by performing WGA Western blot analyses in the presence of 0.5 M D(+) GlcNAc (Fig. 2B, lanes 1 and 2).

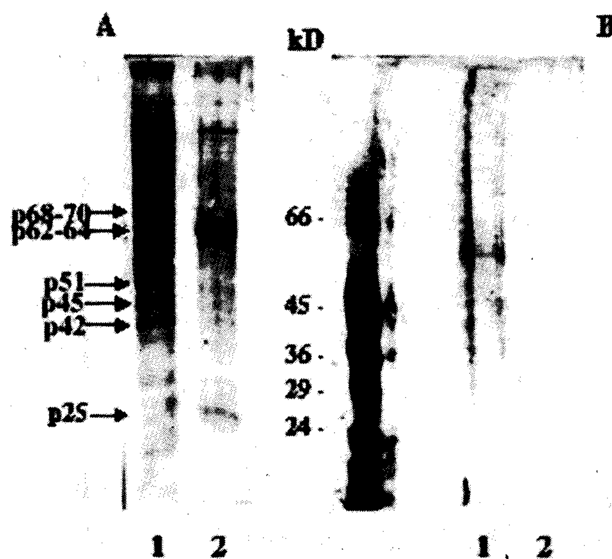

Fig. 3. ConA Western blot analyses of trans-acting nucleoproteins from the livers of control (lane 1) and turpentine treated rats (lane 2) in the absence (A) or presence (B) of 0.3 M D (+)Glc as a specific competitor.

ConA Western blot profiles of rat liver *trans*-acting proteins (Fig. 3A) showed a pronounced presence of D(+)Glc and D(+)Man sugar moieties in the control *trans*-acting p42, p45, p51, p68 and p70, while APR was followed by their deglycosylation and intensive glycosylation of p25, p62 and p64. Disappearance of coloured bands after lectin Western blot analyses in the presence of 0.3 M D(+)Glc proved the specifity of *trans*-acting NPs-ConA interactions (Fig. 3B).

The observed changes at the level of D(+)GlcNAc as well as D(+)Glc, D(+)Man glycosylation of the mentioned *trans*-acting NPs following APR induction coincide with the changes in their ability to bind ABC element. Therefore we assume that glycosylation could be one of the regulatory mechanisms involved in the regulation of the rat Hp gene transcription. This assumption is supported by the results of several authors who demonstrated the influence of glycosylation on DNA binding ability of several transcriptional factors (Jackson and Tjian 1988; Shaw *et al.* 1996; Treisman 1992). In the cases when glycosylation increases the affinity of transcriptional factors to bind DNA glycosylation sites are quite far from DNA binding domain and it is most likely that glycosylation alters protein conformation in such a way that its DNA binding ability is changed (Shaw *et al.* 1996). Besides, glycosylation can also appear within transactivation domain (Choy *et al.* 1995) or within PEST sequences (Rechsteiner *et al.* 1996) and thereby cause changes of transactivating potential or stability of transcription factor.

For these reasons, our future investigations will be aimed at mapping glycosylation sites of rat liver trans-acting NPs what would enable us to learn the exact contribution of glycosylation to the regulation of the rat Hp gene expression.

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