THE PHOSPHORYLATION OF LIVER RIBOSOMAL PROTEIN S6 DURING THE DEVELOPMENT OF ACUTE HEPATIC CELL INJURY INDUCED BY D-GALACTOSAMINE

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1. Introduction

The administration of D-galactosamine–HCl (GalN) to rats initiates a sequence of metabolic events in the liver leading to a morphological and biochemical hepatitis-like liver injury [1]. The formation of UDP–galactosamine metabolites plays a pathogenetic key role since they reduce strongly the hepatocellular pool of uridine phosphates and UDP-sugars by a trap mechanism [2]. As a consequence, macromolecular synthesis, e.g., of RNA [3] and protein [4–9], is inhibited. It is presently unknown whether the impairment of protein biosynthesis is due to a direct effect on translation or to a secondary response to inhibition of RNA synthesis [2]. A direct influence on the translation machinery is suggested by experiments which indicate that the inhibition of protein synthesis by GalN can be reversed by uridine in the presence of actinomycin D [9]. However from present knowledge on the molecular mechanism of polypeptide synthesis, in particular on the role of uridine nucleotides, a specific action of UTP deficiency on translation is hardly to explain. Therefore it is conceivable that the effect of GalN on cellular protein synthesis is a direct one but mediated by a common, non-specific pathway.

Postsynthetic modifications of ribosomal proteins have been suggested as a regulatory mechanism of protein biosynthesis [10]. Recently presented evidence for hormone-dependent regulation of the phosphorylation of liver ribosomal proteins [11–13] which was shown to be due to the phosphorylation of the seryl-residues in 40 S subunit protein S6 [14,15] offers a possible control mechanism. We report that the phosphorylation of small subunit protein S6 is significantly enhanced in the course of GalN induced liver cell injury.

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2. Materials and methods

2.1. *Treatment of rats*

Male Sprague-Dawley rats (370 ± 20 g body weight) were injected intraperitoneally between 7:00 and 9:00 a.m. with 700 mg/kg body weight of D-galactosamine–HCl (C. Roth OHG, Karlsruhe, FRG) dissolved in 0.154 M saline. Control rats received the same volume of saline. 1 h, 3 h, and 6 h later 2.5 mCi of carrier-free [^{32}P]orthophosphoric acid (New England Nuclear, Boston, USA) were administered intraperitoneally to the animals; 30 min after receiving the isotope the rats were killed and their livers quickly excised. All experiments were performed in duplicate.

2.2. *Preparation of liver ribosomal protein*

Ribosomes [16] and ribosomal subunits [17] were prepared and their specific radioactivity (cpm/A_{260}) determined [14]. Ribosomal protein was extracted from subunits with acetic acid [18] and its concentration determined by the method of Lowry et al. using bovine serum albumin as a standard [19]. The radioactivity of the extracted subunit protein was measured as described before [14].

2.3. *Two-dimensional polyacrylamide gel electrophoresis and autoradiography of ribosomal proteins*

The ribosomal protein extracted from the 40 S subunit was separated by two-dimensional electrophoresis on small polyacrylamide gel-slabs [14]. For autoradiography the gels were dried and exposed for 4–7 days to Kodak Kodirex X-ray film. To estimate the ^{32}P-radioactivity associated with protein S6 550 μg of total 40 S ribosomal protein derived from control and treated rats were separated by two-dimensional gel electrophoresis, the area of protein S6 was excised, the gel digested and counted [14].

3. Results

The administration of GalN to rats caused a time-dependent increase in the ^{32}P-radioactivity incorporated into the 40 S liver ribosomal subunit (table 1). Maximum stimulation (15-fold) of the phosphorylation of the small ribosomal subunit was reached 3 h after GalN application, an 11-fold increase in the 40 S specific ^{32}P-radioactivity was still present 6 h after a single dose of GalN. One hour after giving the hepatotoxic compound there was only a 2-fold enhancement of the 40 S radioactivity detectable. In comparison to the small subunit very little radioactivity was in the 60 S subparticle (table 1).

To test whether GalN induced liver cell injury had actually affected the phosphorylation of ribosomal protein, the protein was extracted and the specific radioactivity determined. It is shown in table 1 that about a 10-fold increase in the specific radioactivity of the protein of the 40 S subunit was reached 3–6 h after GalN, only a minor alteration of the 40 S radioactivity was observed one hour after GalN application. A very small amount of the isotope which was not appreciably changed by treatment of the rats with GalN was associated with the protein of the 60 S subunit and is actually due to contamination with a small amount of 40 S subunits [14].

The alteration of the specific ^{32}P-radioactivity of the 40 S subunit protein by GalN is entirely due to an increased phosphorylation of the small subunit protein S6. This was shown by two-dimensional gel electrophoresis of the 40 S subunit protein derived from GalN injured liver and by autoradiographs made of the gels (fig.1). Only a single radioactive spot corresponding to protein S6 was seen on the autoradiographs (fig.1c,d). From a comparison of the electropherograms it is evident that protein S6 derived from injured liver is elongated and shifted anodically in both dimensions caused by the occurrence of higher phosphorylated derivatives of S6.

Figure 2 shows more clearly the shape and the electrophoretic position of S6 relatively to protein

Table 1
Incorporation of radioactivity from [^{32}P]orthophosphoric acid into ribosomal subunits, ribosomal protein, and 40 S subunit protein S6 from normal and GalN treated rat liver

Source of liver ribosomes	cpm/A_{260}		cpm/μg r-protein		cpm/ S6
	40 S	60 S	40 S	60 S	
Normal	83.1	15.4	2.8	1.3	240
Galactosamine treated					
1 h	187	26.1	3.6	1.1	310
3 h	1248	29.5	28.8	1.9	5500
6 h	920	43.6	23.4	2.1	4050

Rats received 1 h, 3 h, and 6 h after intraperitoneal administration of D-GalN (700 mg/kg body weight) 2.5 mCi [^{32}P]orthophospheric acid 30 min before exitus. Controls received 0.154 M saline instead of GalN. The specific radioactivity of the ribosomal subunits and of extracted subunit protein was determined. The ^{32}P-activity of protein S6 was estimated after separation of identical amounts of 40 S subunit protein by two-dimensional gel electrophoresis.

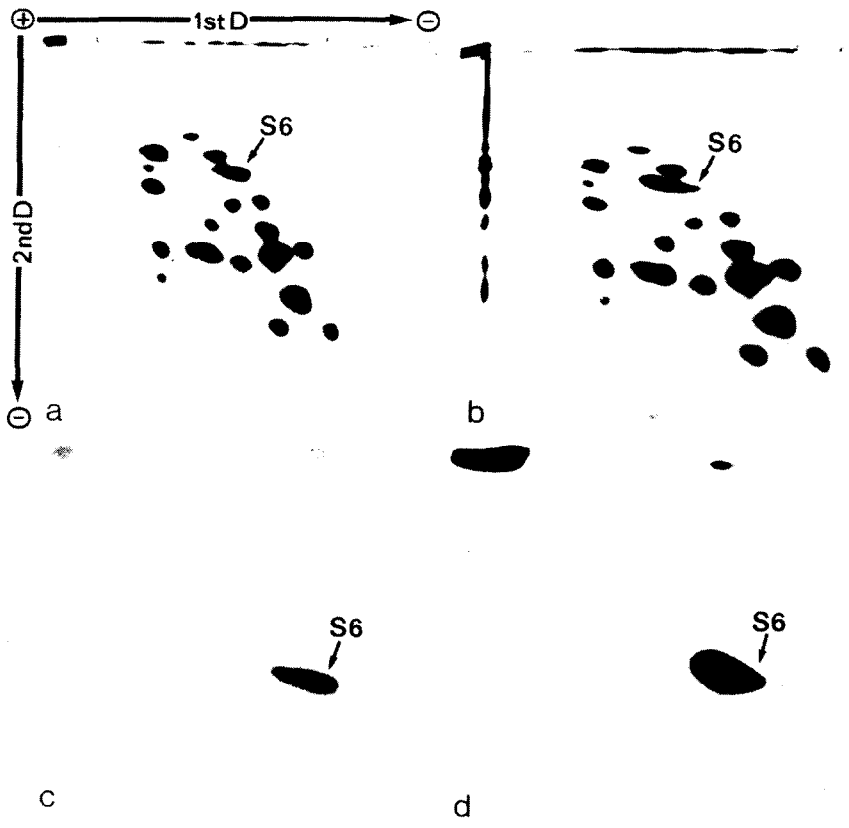

Fig.1. Two-dimensional electropherograms and autoradiographs of the liver 40 S ribosomal proteins from normal and 3 h GalN treated rats. Rats were treated as described in table 1, the extracted 40 S ribosomal protein (550 μg) separated by two-dimensional gel electrophoresis and autoradiographs were made of the gels. In (a) the electropherogram is from control, in (b) from GalN treated rat liver. In (c) the autoradiograph is from control, in (d) from GalN treated rat liver.

S2 and S4 (nomenclature of Sherton and Wool [18]) for normal liver (fig.2a), 1 h (fig.2b) and 3–6 h (fig.2c) after GalN administration. The ^{32}P-radioactivity associated with protein S6 after separation by two-dimensional electrophoresis is enhanced 22- and 16-fold 3 h and 6 h, respectively, after a single dose of GalN (table 1). No appreciable change of the radioactivity of S6 can be detected 1 h after application of the hepatotoxic agent.

4. Discussion

During development of acute liver cell injury induced by a single dose of GalN a strong stimulation of the phosphorylation of liver ribosomes occurs which is entirely due to the phosphorylation of small subunit protein S6. Similar changes occur during hepatic regeneration [14], in diabetic animals [20] and as a result of the inhibition of protein synthesis [21]. Glucagon and cyclic AMP stimulate the phosphorylation of the ribosomal protein but evidence was presented recently for a cyclic AMP-independent increased phosphorylation of S6 [22]. Our results support this finding since it was shown that GalN has no effect on the production of cyclic AMP in isolated hepatocytes [23]. Whether the change of S6 cuased by GalN is due to increased phosphorylation or decreased dephosphorylation cannot be decided. The strongest increase in ribosomal phos-

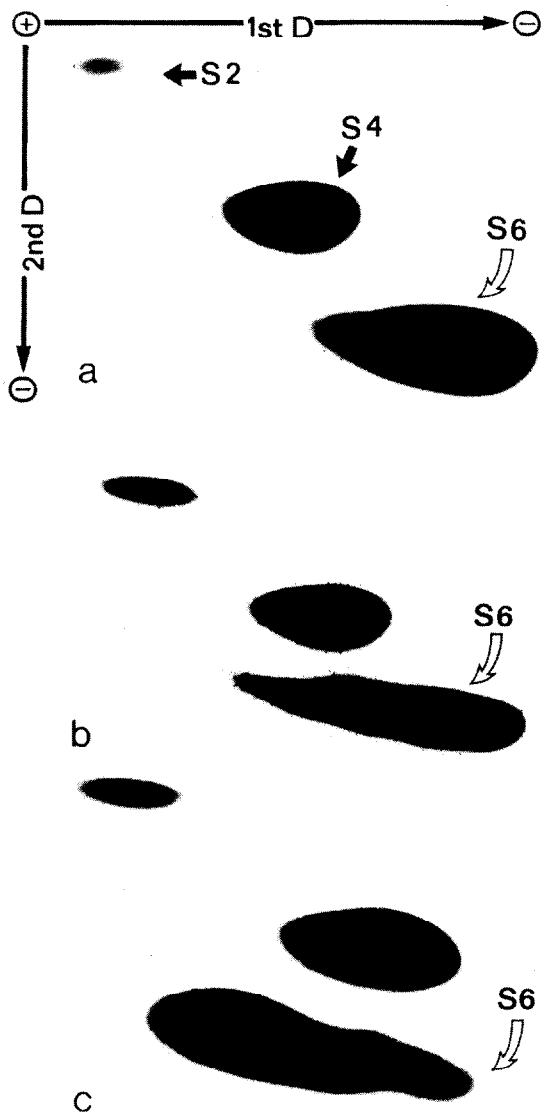

Fig.2. Electrophoretic shape and position of small subunit protein S6 relatively to S2 and S4 derived from normal and GalN treated rat liver. The experimental procedure was as described in the legends of table 1 and fig.1. The region of the two-dimensional electropherogram of the 40 S subunit protein containing the proteins S2, S4, and S6 was enlarged. In (a) the proteins are derived from control, in (b) from 1 h GalN treated rats, in (c) from 3 h GalN treated animals (identical to 6 h treated rats, not shown).

phorylation occurs between 1.5 h and 3 h after GalN application. It has been shown that 2–3 h after GalN induced hepatic UTP deficiency liver cell injury becomes irreversible [2]. It seems that the enhanced phosphorylation of S6 is correlated fortuitously or even causally with the transition of liver cell injury from the reversible to the irreversible phase.

A decision between these alternative cannot be made presently since the molecular basis for the control of protein synthesis by the phosphorylation–dephosphorylation cycle of S6 is unknown [24]. Therefore a causal relationship between the inhibition of protein synthesis [4–8], the disaggregation of polyribosomes, and the appearance of cytoplasmic aggregates probably formed by altered ribosomes [9] observed after GalN treatment and the phosphorylation of S6 cannot be established presently. However our data show clearly a direct involvement of the translational machinery in the process of GalN induced liver cell injury. This effect of GalN on the protein synthesizing apparatus is universal and nonspecific for UTP deficiency.

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