Messenger RNA activities of four acute phase proteins during inflammation

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Poly(A)+ RNA isolated from the livers of normal rats and of rats suffering from an acute inflammation was translated in a cell-free translation system from rabbit reticulocytes. The translation products were immunoprecipitated with specific antisera against α1-acid glycoprotein, α2-macroglobulin, transferrin, α1-proteinase inhibitor and albumin. 15 to 21 h after intramuscular injection of turpentine 73-, 66-, 2.8-, and 2-fold increases in translatable mRNAs for α1-acid glycoprotein, α2-macroglobulin, transferrin and α1-proteinase inhibitor, respectively, were observed. For albumin a decrease in translatable mRNA to about 30% of controls was measured.

Acute phase protein *Cell-free translation* *α2-Macroglobulin* *α1-Acid glycoprotein* *α1-Proteinase inhibitor* *Transferrin* *Rat serum albumin*

1. INTRODUCTION

There is a group of plasma proteins, designated as acute phase proteins, which increase during inflammation caused by infections, necrosis, vaccination, burning and neoplastic growth [1–3]. The majority of those acute phase proteins studied are glycoproteins, which are synthesized in the liver and secreted into the blood.

Thus far, in most of the studies on acute phase proteins during experimental inflammation [4–11] plasma concentrations or synthesis rates [11] have been determined. These experiments, however, give little information on the mechanisms, which underlie the increased synthesis. To learn more about the possible mechanisms involved, we have measured the levels of translatable mRNA for the following acute phase proteins from rat liver: α1-acid glycoprotein, α1-proteinase inhibitor, transferrin, and α2-macroglobulin during acute inflammation.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

L-[^{35}S]Methionine (>600 Ci/mmol) was purchased from the Radiochemical Centre, Amersham; Protosol was from New England Nuclear (Boston MA); Protein A Sepharose CL-4B from Pharmacia (Freiburg).

2.2. *Animals*

Male Wistar rats of 250 g body wt were generously supplied by Professor Dr H. Ueberberg (Thomae GmbH, Biberach). The animals had free access to water and a carbohydrate-rich 20% protein diet (Altromin, Lage).

2.3. *Preparation of specific antisera*

The preparation of antisera against rat α1-acid glycoprotein [12], transferrin [13], α1-proteinase inhibitor [14] and α2-macroglobulin [15] has been described previously.

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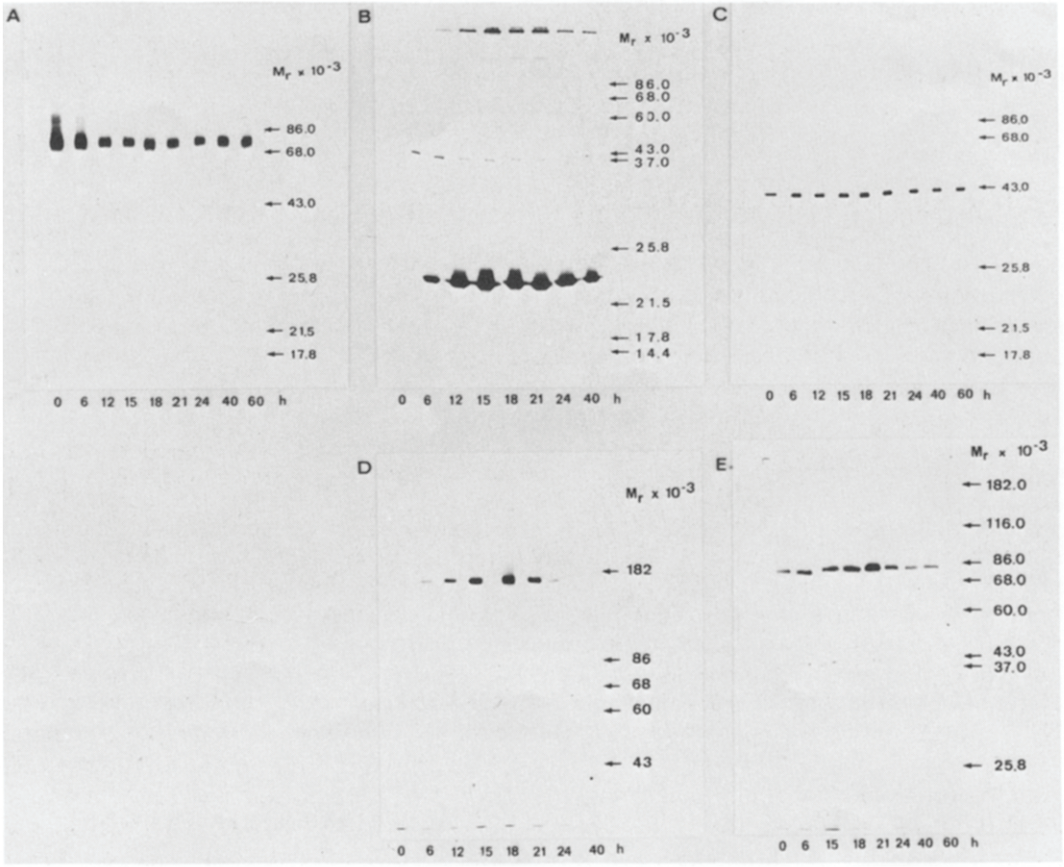

Fig.1. Cell-free translation of mRNAs of acute phase proteins and albumin at different times after turpentine injection. Poly(A)$^+$ RNA was isolated from the livers of male rats of 250 g body wt at various times after intramuscular injection of 1 ml turpentine/animal. Comparable amounts of ^{35}S-radioactivity (15×10^6 cpm) were used for the immunoprecipitation of albumin (A), α1-acid glycoprotein (B), α1-proteinase inhibitor (C), α2-macroglobulin (D) and transferrin (E) synthesized in a rabbit reticulocyte system from 8 μg poly(A)$^+$ RNA. The immunoprecipitated proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and fluorography as described in section 2. α2-Macroglobulin from rat plasma (182000), β-galactosidase from *Escherichia coli* (116000), conalbumin (86000), bovine serum albumin (68000), catalase (60000), ovalbumin (43000), yeast alcohol dehydrogenase (37000), porcine elastase (25800), soy bean trypsin inhibitor (21500), horse myoglobin (17800), and lysozyme (14400) were used as M_r standards.

2.4. *Isolation of poly(A)$^+$ RNA from rat liver*

Total RNA was extracted with guanidinium HCl from livers removed from rats at various times after intramuscular injection of 4 ml turpentine/kg body wt as in [16]. Oligo(dT)-cellulose chromatography was used for the isolation of poly(A)$^+$ RNA.

2.5. *In vitro translation of poly(A)$^+$ RNA and immunoprecipitation of α2-macroglobulin*

5–6 μg of poly(A)$^+$ RNA were translated in a total volume of 0.1 ml in the presence of a nuclease-treated rabbit reticulocyte lysate [17], 50 μCi of [^{35}S]methionine and 100 units of RNase inhibitor purified from human placenta as in [18]. The newly-synthesized proteins were then immunoprecipitated with specific antisera and protein A Sepharose [19]. The immunoprecipitates were solubilized and analyzed by sodium dodecyl sulfate–polyacrylamide slab gel (8%) electrophoresis [20] and fluorography [21]. The radioactive bands were excised from the gel, solubilized

with 90% Protosol in water at 40°C overnight and the radioactivity was determined in a liquid scintillation spectrometer.

3. RESULTS

Poly(A)$^+$RNA was isolated from livers of rats at different times after turpentine administration and translated in a cell-free system from rabbit reticulocytes. By use of specific antisera, albumin, α1-acid glycoprotein, α1-proteinase inhibitor, α2-macroglobulin and transferrin were immunoprecipitated and subjected to electrophoretic separation on sodium dodecyl sulfate–polyacrylamide slab gels and fluorography. The levels of translatable mRNAs for the acute phase proteins α1-acid glycoprotein, α1-proteinase inhibitor, α2-macroglobulin and transferrin increased 6 h after turpentine injection, reached a maximum between 15 and 21 h, and declined thereafter (fig.1). On the other hand, in the case of albumin a continuous decrease in mRNA translatability was observed, the rate of the decline being fastened during the first 12 h after turpentine administration.

For quantitation the individual protein bands were excised from the gels and their radioactivity was determined. As shown in table 1 the levels of α1-acid glycoprotein, α1-proteinase inhibitor, α2-macroglobulin and transferrin mRNAs reached maxima 15 h, 18 h, 18 h and 21 h, respectively, after turpentine injection. The data clearly show that the most dramatic increases in mRNA activities were found for α1-acid glycoprotein (72.9-fold) and α2-macroglobulin (65.9-fold). Only small increases were observed for α1-proteinase inhibitor and transferrin mRNA activities. Moreover, these returned relatively soon to normal values.

4. DISCUSSION

In the experiments presented, it was found that the translatable mRNAs for α1-acid glycoprotein, α1-proteinase inhibitor, transferrin and α2-macroglobulin showed increases of very different extent after turpentine injection. Whereas the mRNAs for α1-acid glycoprotein and α2-macroglobulin increased 73- and 66-fold, respectively, the levels of mRNA for α1-proteinase inhibitor and transferrin were increased only 2- and 3-fold and returned quickly to normal (table 1). In the case of rat α1-acid glycoprotein a 90-fold increase in the relative amount of α1-acid glycoprotein mRNA was found [22] 36 h after the onset of inflammation using an α1-acid

Table 1

Translatability of mRNA for albumin and acute phase proteins (synthesis) experimental inflamation

Days after turpentine	Albumin		α1-Acid glycoprotein		α1-Proteinase inhibitor		α2-Macroglobulin		Transferrin	
	(%)	-fold change	(%)	-fold change	(% × 10	-fold) change	(% × 10^2	-fold) change	(% × 10^2	-fold) change
1	2.66	1	0.03	1	1.91	1	0.07	1	1.27	1
6	1.94	0.73	0.24	7.1	2.62	1.37	0.81	11.2	1.73	1.36
12	1.14	0.43	1.55	45.5	2.91	1.52	1.83	25.0	–	–
15	0.98	0.37	2.48	72.9	3.30	1.73	3.24	45.0	2.32	1.83
18	0.96	0.36	1.82	53.5	3.72	1.95	4.74	65.9	2.50	1.97
21	0.95	0.36	1.50	44.2	3.19	1.67	2.31	32.1	3.56	2.8
24	0.86	0.32	1.19	35.0	2.27	1.19	0.93	13.0	1.73	1.36
40	0.83	0.31	0.85	25.0	2.10	1.10	0.19	2.7	1.23	0.97
60	0.78	0.29	–	–	2.01	1.05	–	–	1.13	0.89

The data are given in percent of total trichloroacetic acid-insoluble radioactivity obtained after cell-free translation of poly(A)$^+$ RNA in a reticulocyte lysate. For each immunoprecipitation 15 × 10^6 cpm of the total trichloroacetic acid-insoluble radioactivity were used

glycoprotein-specific mRNA cDNA probe. In our experiments the maximum of translatable mRNA for α1-acid glycoprotein was found already 15 h after the injection of turpentine. Using a cDNA probe specific for baboon α1-acid glycoprotein authors in [23] have found increased α1-acid glycoprotein levels after turpentine administration. However, no detailed data were given by these authors.

Maximum translatability is reached at different times for the various proteins after the onset of inflammation. Furthermore, it is remarkable that in the case of α2-macroglobulin the mRNA activity declines very rapidly. The mRNA for albumin shows a rapid initial decrease during the first 15 h of inflammation. This finding is in agreement with experiments in [24].

It can be concluded from the experiments presented in this paper that the increase of α1-acid glycoprotein, α1-proteinase inhibitor, α2-macroglobulin and transferrin observed in plasma after turpentine administration is due to increased mRNA activities.

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