INCREASED LEVELS OF MICROSOMAL ALBUMIN-mRNA IN THE LIVER OF NEPHROTIC RATS

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1. Introduction

Experimental nephrosis produced in rats by the administration of either the aminonucleoside of puromycin [1,2] or nephrotoxic serum [3,4] is accompanied by albuminuria, hypoalbuminemia [2], hyperlipemia, ascites [2,3], liver hypertrophy [3], and a rise in the tissue content of RNA and DNA [3,4], as well as by increases in the synthesis of plasma proteins [4] and the rate of labeling of albumin in the serum [5]. An increase in the synthesis of albumin has been demonstrated in vivo [6,7], in the isolated rat liver [4,8], in liver slices [9], and in cell-free preparations containing liver microsomes and liver cell sap [10,11]. Synthesis of total liver protein remains unchanged or is enhanced only slightly [11,12].

To investigate this situation in more detail, we isolated rat liver polysomes and poly(A)-containing mRNA from normal and nephrotic rats and translated them in cell-free systems. The studies reported here show that the albumin mRNA found in liver polysomes from nephrotic rats increases by approximately 50% and suggest that this increase is at least partially responsible for the elevation in albumin synthesis observed in nephrotic rats.

2. Materials and methods

Male Sprague-Dawley rats (Charles River Breeding Laboratory, Wilmington, MA) were used in all experiments. All chemicals used were of analytical grade: L-leucine (4,5-^{3}H[N]), specific activity 30–50 Ci/mmol, was obtained from New England Nuclear, Boston, MA. Oligo-(dt)-cellulose was purchased from Collaborative Research, Inc., Waltham, MA. Wheat germ was kindly provided by Dr Bryan Roberts (Massachusetts Institute of Technology). Rat serum albumin was obtained from Sigma Chemical Co., St. Louis, MO and rat serum albumin antibody from ICN Pharmaceuticals, Cleveland, OH. Nephrosis was produced by the administration to 200–250 gram rats of the aminonucleoside of puromycin (obtained from ICN Pharmaceuticals) [7].

Rat liver polysomes were prepared from normal or nephrotic rats that had been fasted overnight. The incubation conditions for polysomes in cell-free systems containing [^{3}H]leucine and pH 5 enzymes have been described [13]. Poly(A)-containing mRNA was isolated from microsomes of normal and nephrotic rats by phenol extraction [14] followed by affinity chromatography on oligo-(dt)-cellulose [14], except that 20 mM Hepes buffer, pH 7.4, was used instead of Tris buffer. The poly(A)-containing mRNA was eluted from the oligo-(dt)-cellulose with 1 mM Hepes buffer, pH 7.4. The peak fractions were combined and frozen at −80°C in 50-μl aliquots. Liver mRNA was translated in a wheat germ S30 extract [15].

Albumin synthesized in these cell-free systems was identified by SDS-polyacrylamide gel electrophoresis after immunoprecipitation. Albumin synthesized in vitro (released and nascent chains) was precipitated by excess albumin antibody after the addition of 5 μg carrier albumin. Incubation was done for 60 min at 30°C and overnight at 4°C. The immunoprecipitate was purified as described [16]

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and analyzed on a SDS-polyacrylamide gradient (10–15%) gel, which was prepared and run essentially as described by Maizel [17]. The gel was stained to show protein bands, then sliced and analyzed for radioactivity distribution [16]. Total incorporation of [^{3}H]leucine was measured according to the method of Mans and Novelli [18].

The concentration of RNA solutions was determined, assuming $E^{1\%}_{260\,nm}$ to be 250. Protein was determined according to the method of Lowry et al. [19].

3. Results

Liver polysomes from normal and nephrotic rats were incubated in vitro in the presence of pH 5 enzymes and [^{3}H]leucine [13]. The incubation products were tested for specific albumin synthesis by immunoprecipitation followed by SDS-polyacrylamide gel electrophoresis (fig.1). Protein staining of the gel showed the carrier albumin band and the light and heavy chains of the antibody. The major radioactivity peak co-migrated with the carrier albumin peak, thus demonstrating that our method of immunoprecipitation and SDS-gel analysis is efficient for obtaining pure albumin and also showing that both polysome preparations synthesize authentic albumin. In control experiments in which brain polysomes were used instead of rat liver polysomes, no radioactivity co-migrating with albumin could be detected. In some experiments, antigenic nascent albumin chains that migrated faster than finished

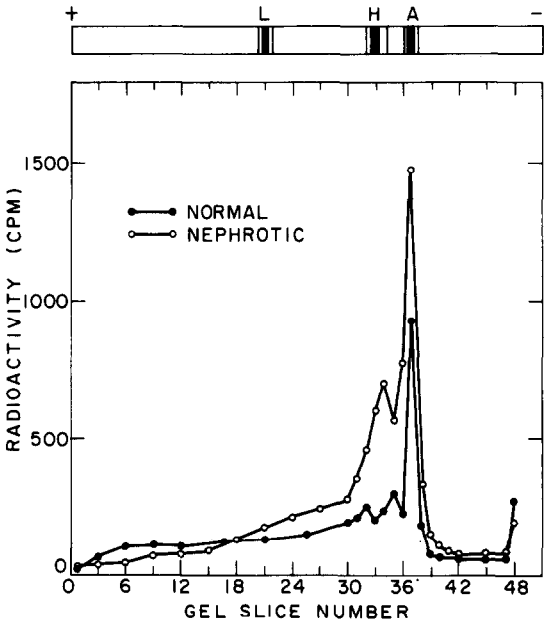

Fig.1. SDS-polyacrylamide gradient (10–15%) gel analysis of nascent and released albumin chains recovered from immunoprecipitated cell-free incubations containing liver polysomes and pH 5 enzymes. Liver polysomes from normal and nephrotic rats were incubated in the presence of [^{3}H]leucine as described [13]. Five micrograms of rat serum albumin were added to 50 μl of the incubation mixture, which contained approximately 200 000 cpm of total TCA-precipitable radioactivity. Incubation for immunoprecipitation was described in Materials and methods. SDS-gel analysis of the immunoprecipitate (containing nascent and released albumin chains) was performed as described [16]. (•——•) Normal polysomes (total radioactivity: 204 000 cpm); (○——○) Nephrotic polysomes (total radioactivity: 225 000 cpm). *Abbreviations:* L = L-chain, H = H-chain of immunoglobulin, A = Albumin.

Table 1
Albumin synthesis in a cell-free system containing liver polysomes from normal and nephrotic rats

	Incorporation/Incubation			
	Radioactivity in total protein (cpm)	Radioactivity in albumin (cpm)	Ratio: $\frac{\text{Albumin}}{\text{Total protein}} \times 100$	
Normal	67 820	1560	2.3 ± 0.17	
Nephrotic	75 000	2550	3.4 ± 0.26	

Albumin was purified by immunoprecipitation from the 105 000 *g* supernatant of the incubated polysomes and incorporation into albumin was determined by measuring the radioactivity under the albumin peak (see fig.1). The results are the mean of 8 experiments ± SE.

Table 2
Albumin synthesis in a cell-free system containing wheat germ S30 and liver mRNA from normal and nephrotic rats

	Incubation/Incorporation		
	Radioactivity in total protein (cpm)	Radioactivity in albumin (cpm)	Ratio: $\frac{\text{Albumin}}{\text{Total protein}} \times 100$
Normal	94 660	1420	1.5 ± 0.09
Nephrotic	99 170	2380	2.4 ± 0.20

Albumin was purified by immunoprecipitation. Incorporation into albumin was determined by two methods: (1) by measuring the radioactivity under the albumin peak (see fig.1) when incorporation into released albumin chains was determined (after the ribosomes had been spun down, see text), or (2) by measuring the radioactivity both under the albumin peak and in the antigenic nascent chains when ribosomes were not spun down before the immunoprecipitation. The results are the mean of 7 experiments ± SE from 3 different mRNA preparations.

albumin (fig.1) were removed by centrifuging the incubation mixture at 105 000 *g* for 90 min to pellet down the ribosomes carrying nascent chains.

Uptake of radioactivity into albumin averaged 2.3% of the total protein radioactivity for polysomes from normal rats; this value increased to 3.4% for polysomes from nephrotic rats (table 1). This observation clearly demonstrates that the number of polysomes synthesizing albumin increases in nephrotic animals, presumably because of an increase in the polysomal albumin mRNA content.

To test for this increase in mRNA, our experiments were extended to a cell-free system consisting of wheat germ S30 and liver poly(A)-containing mRNA. Liver mRNA was extracted from microsomes from normal or nephrotic animals and was subsequently assessed for its albumin mRNA content in the wheat germ cell-free system. As can be seen in table 2, both mRNA preparations stimulate the incorporation of [^{3}H]leucine into total protein to about the same extent above the background incorporation of 2980 cpm (= no mRNA added). Liver mRNA from nephrotic animals directed the incorporation of about 50% more radioactivity into albumin than liver mRNA from normal animals. In control experiments in which either hemoglobin mRNA or brain mRNA was used, less than 0.1% of the total radioactivity co-precipitated with carrier albumin after the addition of albumin antiserum. This result shows that liver polysomes from nephrotic animals contain approximately 50% more albumin mRNA than those from normal animals.

4. Discussion

Whereas most [4–11, 20], although not all [20,21], reports have indicated that albumin synthesis increases in rats with experimental nephrosis, attempts to elucidate the precise mechanism by which this increase occurs have been hampered by a lack of methods to determine accurately the level of albumin mRNA in the liver cytoplasm, which has been suspected of being one of several possible regulatory factors. In this report we demonstrate the efficient use of a method to assess quantitatively the level of albumin mRNA in the liver and show that liver polysomes of nephrotic animals contain approximately 50% more albumin mRNA than those of normal animals. Other results [Zähringer, J., Baliga, B. S., and Munro, H. N., in preparation] suggest that the post-microsomal supernatant of rat liver contains mRNA capable of synthesizing albumin. Further experiments are required to establish whether this pool of post-microsomal albumin mRNA can be used in nephrosis to increase the level of polysomal albumin mRNA.

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