Regulation by Androgen of mRNA Level for the Major Urinary Protein Complex in Mouse Liver

Shoji Osawa and Shiro Tomino

Department of Biology, Tokyo Metropolitan University
Setagaya-ku, Tokyo 158, Japan

Received June 13,1977

Summary: RNA extracted from mouse liver directed the synthesis of the major urinary protein (MUP) complex in a heterologous cell-free system derived from wheat germ. Livers of normal males and dihydrotestosterone-treated females showed much higher level of mRNA for the MUP complex than those of normal females, indicating that androgen controls the synthesis of the MUP complex at pretranslational level in liver.

The majority of proteins excreted in the urine of normal mice shows the electrophoretic properties as prealbumins (1, 2). The family of these prealbumins, designated the major urinary protein (MUP) complex provides a useful system for the study of regulation of gene expression in mammals, since both the quality and quantity of the MUP complex excreted are under control of sex hormone (2, 3).

When subjected to electrophoresis at pH 5.5 the MUP complex is separable into three components of identical molecular weight, designated MUP-1, MUP-2 and MUP-3, respectively (3, 4). The survey of urine fractions from a number of inbred strains concluded that a group of male mice (Group A), represented by BALB/c, excretes MUP-1 and MUP-3, whereas the other group (Group B), represented by C57BL/6, excretes all three MUPs (4). Immunochemical studies have revealed that the MUP complex is synthesized in liver (5), released into blood stream (5-7) and excreted in urine. Quantity of the MUP complex in urine is influenced by sex hormone; urine from male mice contains greater amount of the MUP complex than that from females (3, 5, 6). Moreover, most strains show phenotypic differences in the MUP complex between males and females (3, 4).

Abbreviations: SDS, Sodium dodecyl sulfate. TMV, Tobacco mosaic virus

ISSN 0006-291X

When testosterone is administered to female mice, both the level and phenotype of the MUP complex in urine become indistinguishable from those of male of the same strain. Furthermore, genetic studies have provided evidence showing the difference in androgen inducibility between the two classes of inbred strains. Both the rate and final level of excretion of the MUP complex following administration of testosterone are greater in females of Group A mice than those of Group B (4).

These biochemical and genetical informations strongly suggest that the MUP complex is a favorable system for studying mammalian gene expression. In order to expand this knowledge and to study the mechanisms underlying the genetic regulation of biosynthesis of the MUP complex, we have first attempted the assay of mRNA for the MUP complex using heterologous cell-free system. In this report we describe successful translation of mRNA for the MUP complex in vitro in wheat germ system. Our results also provide evidence that the sex hormone, dihydrotestosterone controls the level of functional mRNA for the MUP complex in mouse liver.

MATERIALS AND METHODS

Animals and androgen treatment: Six-week old ddN and C57BL/6 mice were obtained from a local breeder and maintained by feeding with laboratory mouse chow. Dihydrotestosterone suspended at 50 mg/ml in a mixture of 0.1 M Na-acetate, pH 7.2-ethylene glycol-Tween 80 (90/10/0.4) was administered by subcutaneous injection of 0.1 ml per individual every 3 days (8). Animals were sacrificed 2 weeks after first injection for RNA preparation.

Isolation of the MUP complex and preparation of antibody: The MUP complex was purified from the pooled urine of C57BL/6 male mice as described by Finlayson et al (9). Antibody was prepared by intradermal injection into rabbit of 2 mg MUP complex emulsified with Freund complete adjuvant once every week for 5 consecutive weeks. IgG was separated from serum by ammonium sulfate precipitation at 40% saturation. Antigen equivalence of IgG was determined by titration with the Fluorescein labeled MUP complex.

Isolation of RNA: Livers, which had been placed under liquid nitrogen immediately after disection, were homogenized with 10 volumes each of extraction buffer (0.1 M Tris-HCl, 0.1 M NaCl, 1 mM EDTA and 0.5% SDS*, pH 9.0) and phenol-chloroform-isoamyl alcohol (50/50/1) mixture in an Waring blender for 1 min. Total nucleic acids were isolated from the aqueous phase by the standard procedures (10). DNA was removed by washing with 3 M Na-acetate (10, 11). Final RNA preparation was dissolved in sterilized water. TMV-RNA was isolated by the same method but with omission of Na-acetate washing.

In vitro translation of RNA: A wheat germ S-30 fraction was prepared according to the method of Roberts and Patterson (12) except that the preincubation was

at 22°. Standard incubation mixture for cell-free protein synthesis contained the following in a final volume of 50 µl: 30 mM Hepes-KOH, pH 7.6; 1 mM ATP; 0.2 mM GTP; 8 mM phosphocreatine; 0.2 mg/ml creatine kinase; 5 µCi [^{3}H]leucine (sp. act. 60 Ci/mM); 0.04 mM each 19 unlabeled amino acids; 0.5 mM spermidine-HCl; 1.5 mM Mg-acetate; 86 mM KCl; 0.1 mM dithiothreitol; 15 µl wheat germ S-30 and RNA. The mixture was incubated at 22°. An aliquot (5 µl) was spotted on a filter paper disk and assayed for total protein synthesis as described (12, 13). Radioactivity was determined by a Beckman scintillation counter with toluene-PPO scintillator.

Determination of the MUP complex synthesized in vitro: Volume of incubation mixture for protein synthesis was increased to 100 µl. After 90 min incubation 10 µg RNase and 1 mM leucine were added and the mixture was incubated for 10 min at 37°. It was diluted to 0.5 ml with the buffered saline (0.02 M Tris-HCl, 0.15 M NaCl, 0.5% Triton X-100 and 0.5% Na-deoxycholate, pH 7.5) and centrifuged at 100,000 x g for 30 min. To the supernatant 100 µg of anti-MUP IgG was added and the mixture was incubated for 10 min at 37°. Carrier MUP complex (10 µg) was then added to the mixture and incubation was continued for 30 min at 37° and for 15 hours in the cold. Immunoprecipitate was collected by centrifugation, washed 3 times with the buffered saline and dissolved in 100 µl of SDS sample buffer by heating for 2 min in boiling water. SDS gel electrophoresis was performed as described by Weber and Osborn (14) using 10% acrylamide gel. Proteins were stained with Coomassie blue. Gels were cut into 1.5 mm slices and radioactivity in each slice was determined by liquid scintillation counting after solubilization of gels in hydrogen peroxide.

RESULTS AND DISCUSSION

Since earlier immunochemical studies have shown that the MUP complex is synthesized in liver, RNA extracted from mouse liver was studied for in vitro translation in wheat germ cell-free system. RNA from the livers of male ddN mice stimulated the incorporation of radioactive amino acid into acid-insoluble fraction. Total protein synthesis was proportional to the added RNA up to 50 µg per standard reaction mixture (Fig. 1, A) and the incorporation was linear with respect to time until 60 min at 22° (Fig. 1, B).

In order to identify the MUP complex in cell-free reaction products the reaction mixtures were freed of ribosomes and treated with specific anti-MUP antibody to precipitate the radioactive MUP complex selectively. The immunoprecipitates were washed extensively and analysed by SDS polyacrylamide gel electrophoresis. Liver RNA from both male and female mice directed the synthesis of a product which migrated to the same position as the authentic MUP complex in SDS gels (Fig. 2, A and B). Although RNA from livers of both sexes showed similar activity for total protein synthesis, the one from male liver was approximately 5 times as active as that from female liver in the

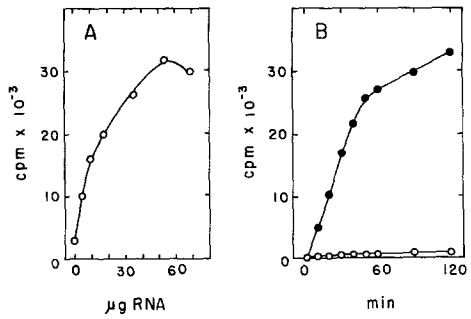
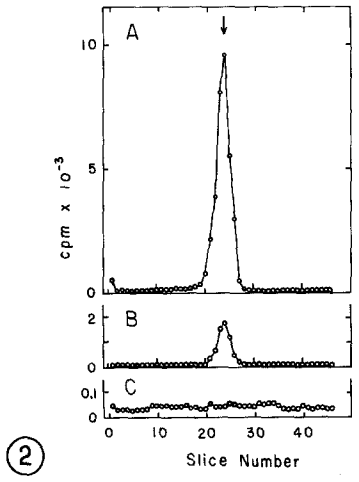


Fig. 1. RNA dependency and time course of translation system. Incorporation of [^{3}H]leucine into total protein was determined as described in "Materials and Methods". (A) Incorporation was determined after 60 min incubation with RNA from the livers of ddN males. (B) Synthesis was directed with 40 μg RNA from the livers of ddN males (●—●) or without RNA (o—o).

synthesis of immunoprecipitable product. RNA extracted from TMV, which directed total protein synthesis roughly 10 times as much as mouse liver RNA, failed to show any detectable activity for the synthesis of specific product (Fig. 2, C). The fact excluded the possibility that the product precipitated by antibody was due to non-specific contamination of radioactivity. We conclude from these results that RNA isolated from mouse liver contains the mRNA for the MUP complex and that the level of this specific mRNA in liver is higher in male than in female. Under our experimental conditions approximately 2% of total acid-insoluble radioactivity was incorporated into the MUP complex even when the system was programmed by RNA from the livers of normal females. This value is in contrast to that reported for mRNA level of α_{2u}globulin in rat liver, where, liver RNA from normal female rats failed to direct any detectable synthesis of α_{2u}globulin in cell-free system (16). Higher mRNA level for the MUP complex probably reflects proteinuria, which is a normal condition in mice in contrast to most other mammals (17).

To examine the effect of androgen on the level of mRNA for the MUP complex RNA preparations were obtained from the livers of control and dihydrotestosterone-treated females. Activity of mRNA for the MUP complex of each preparation was determined in the same way as above. As shown in Fig. 3,



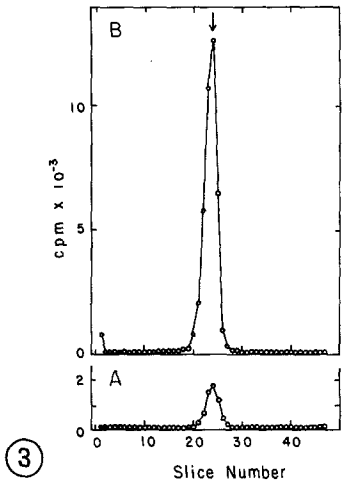


Fig. 2. SDS gel electrophoresis of immunoprecipitates. Samples were prepared and electrophoresed as described in "Materials and Methods". Protein synthesis was directed by; (A) 40 µg RNA from male ddN livers, (B) 40 µg RNA from female ddN livers and (C) 10 µg TMV-RNA. Arrow indicates the position of the MUP complex.

Fig. 3. SDS gel electrophoresis of immunoprecipitates. Procedures were the same as descrobed in "Materials and Methods" and under Fig. 2. Protein synthesis was directed by; (A) 40 µg liver RNA from control ddN females and (B) 40 µg liver RNA from ddN females treated with dihydrotestosterone for two weeks. Arrow indicates the position of the MUP complex.

administration of dihydrotestosterone greatly increased the level of mRNA for the MUP complex in female liver. At fully induced state the level of mRNA for the MUP complex of female liver exceeded that of normal male liver.

Present results clearly demonstrate that androgen controls the level of specific mRNA for the MUP complex in mouse liver. Wheat germ translation system in combination with detection of specific product by immunoprecipitation provides a useful assay system for mRNA for the MUP complex. By applying this method it would be of primary importance to identify mRNA activity for individual MUP component in the RNA preparations extracted from the livers of group A and B mice. Our preliminary experiments employing the immobilized antibody are suggesting that the liver RNA from androgen induced DBA/2 mice (Group A) predominantly directs the synthesis of MUP-1. Details of these experiments will be published elsewhere.

The findings reported on the regulation of α_{2u}globulin synthesis in rat liver (16, 18, 19) together with our present results indicate that androgen also controls the synthesis of these rodent proteins in liver via mechanisms currently accepted for the action of steroid hormones (20, 21).

Acknowledgment: We thank Drs. K. Paigen and P. Szoka for helpful discussion. We also thank Drs. J. Hashimoto and I. Takebe for supplying wheat germ and TMV.

REFERENCES

1. Thung, P. J. (1956) Experientia Suppl. 4, 195-198
2. Finlayson, J. S. and Bauman, C. A. (1958) American J. Physiol. 192, 69-72
3. Finlayson, J. S., Potter, M. and Runner, C. C. (1963) J. Nat. Cancer Inst. 31, 91-107
4. Szoka, P. (1977) Ph. D Thesis, State University of New York at Buffalo
5. Finlayson, J. S., Asofsky, R., Potter, M. and Runner, C. C. (1965) Science 149, 981-982
6. Ruemke, P. and Thung, P. J. (1964) Acta Endocrinol. 47, 156-164
7. Reuter, A. M., Hamoir, G., Marchand, R. and Kennes, F. (1968) Eur. J. Biochem. 5, 233-238
8. Swank, R. T. and Paigen, K. (1973) J. Mol. Biol. 81, 225-243
9. Finlayson, J. S., Mushinski, J. F., Hudson, D. M. and Potter, M. (1968) Biochem. Gen. 2, 127-140
10. Kirby, K. S. (1968) Methods Enzymol. 12B, 87-99
11. Shapiro, D. J., Taylor, J. M., McKnight, G. S., Palacios, R., Gonzalez, C., Kiely, M. L. and Schimke, R. T. (1974) J. Biol. Chem. 249, 3665-3671
12. Roberts, B. E. and Patterson, B. M. (1973) Proc. Nat. Acad. Sci. 68, 2303-2307
13. Bollum, F. J. (1968) Methods Enzymol. 12B, 169-173
14. Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412
15. Tzagoloff, A. and Meagher, P. (1972) J. Biol. Chem. 247, 594-603
16. Sippel, A. E., Feigelson, P. and Roy, A. K. (1975) Biochemistry 14, 825-829
17. Bernstein, S. E. (1968) in Biology of Laboratory Mouse (2nd ed.), E. L. Green ed., pp 337-372, Dover Publishing Inc. New York
18. Kurts, D. T., Sippel, A. E., Ansah-Yiadom, R. and Feigelson, P. (1976) J. Biol. Chem. 251, 3594-3598
19. Roy, A. K., Schiop, M. J., Dowbenko, D. J. (1976) FEBS Let. 70, 137-140
20. Jensen, E. V. and Desombre, E. R. (1972) Ann. Rev. Biochem. 41, 203-230
21. Chan, L. and O'Malley, B. W. (1976) New Engl. J. Med. 294, 1322-1328, 1372-1381, 1430-1437