Bursal Role in the Synthesis of γ-Chain Messenger Ribonucleic Acid in Young Chickens

TAIZABURO KANKI and YOICHI ICHIKAWA

Department of Bacteriology, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-Gun, Saitama, Japan 350-04

(Received for publication April 29, 1984)

ABSTRACT Incomplete bursectomy was performed in 3-day-old chickens by treatment with .5% testosterone propionate on the 3rd day of incubation. The purpose of this experimental approach was to determine the role of the bursa of Fabricius in the translation activity of messenger ribonucleic acids (RNA) coding immunoglobulin-isotypes.

The bursal poly(A) RNA, prepared from bursae of normal and hormone-treated chickens, directed the synthesis of 72, 43, 38, and 25-kilodalton molecules as revealed by immune precipitation and sodium dodecylsulphate acrylamide gel electrophoresis. However, unlike the messenger RNA from normal bursae, the messenger from bursae of hormonal-treated embryos failed to direct the synthesis of 68-kilodalton gamma-polypeptide.

(Key words: incomplete bursectomy, bursal ribonucleic acid, cell-free translation, μ- and γ-chain messenger ribonucleic acid)

1986 Poultry Science 65:1011–1014

INTRODUCTION

The role of the bursa of Fabricius (BF) in the synthesis of the immunoglobulin (Ig)-isotype has not been clarified. Diverse interpretations of the bursa's role have been made by different bursectomy techniques (Subba Rao *et al.*, 1978; Huang and Dreyer, 1978; Jalkanen *et al.*, 1983) and by other mechanisms generating Ig-producing cells (Lerner *et al.*, 1971; Sato and Abe, 1975).

Kanki and Ichikawa (1981) demonstrated *in vitro* synthesis of γ-chain polypeptides by normal bursal ribonucleic acid (RNA) extracted from the bursa of hatched chickens. To understand the bursa's role in the synthesis of Ig-isotypes, the activities of messenger RNA controlling the translation of μ- and γ-chain polypeptides were assayed using 1-week-old chickens in which the bursa was reduced in size by treatment with low concentrations of testosterone propionate (TP).

MATERIALS AND METHODS

Chickens. White Leghorn (GH) eggs were purchased from The Saitama Prefectural Poultry Experiment Station (Saitama, Japan). The conical end of the eggs was dipped at a depth of 2.5 cm for 5 sec on the 3rd day of incubation into a 100-ml TP solution made by ethanol (Glick and Sadler, 1961). Twenty eggs were dipped in .1, .25, .5, or 1.0% TP solutions, and ethanol was used as a control.

Ribonucleic Acid Preparation. One week after hatching, 14 to 17 bursae were collected and washed thoroughly with ice cold .85% NaCl. The method for isolation of RNA essentially followed MacDonald *et al.* (1977). Bursae were homogenized in 10 volumes of 4 M guanidine thiocyanate, .1 M Tris-HCl (pH 7.5), and 2M 2-mercaptoethanol (2-ME). The homogenate was adjusted to .1 M potassium acetate (pH 5.0), and ½ volume of ethanol was added. The precipitate was allowed to develop for 1 hr at −20 C and collected by centrifugation at 4,000 g for 10 min. The precipitate was resuspended in 6 M guanidine hydrochloride containing 10 m*M* dithiothreitol (DTT) and 25 m*M* ethylenediaminetetraacetic acid (pH 7.0). The solution was adjusted to 3 M in sodium acetate (pH 6.0) and stored overnight at −20 C to precipitate RNA. The precipitate was collected by centrifugation at 10,000 *g* for 30 min, dissolved in .2 M potassium acetate, and reprecipitated by 2.5 volumes of ethanol. The RNA precipitate was washed several times with 95% ethanol. Bursal poly(A) RNA was separated on an oligo (dT)-cellulose column (Aviv and Leder, 1972). The amounts of RNA were calculated as described previously (Kanki and Ichikawa, 1981).

Cell-Free Translation. The translation system was made by wheat-germ lysate according to Roberts and Paterson (1973). Poly(A) RNA (2 μg) from bursae was added to 50 μl of a reac-

Downloaded from http://ps.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

tion mixture which finally contained 28 m*M* HEPES (N-2-hydroxyethylpiperadine-N'-2-ethanesulphonic acid, pH 7.6), 2.5 m*M* magnesium acetate, 88 m*M* KCl, 2.4 m*M* 2-ME, 2 m*M* DTT, 1 m*M* adenosine triphosphate, .2 m*M* guanosine triphosphate, 8 m*M* creatine phosphate, 10 μg creatine phosphokinase, .1 m*M* of 19 nonradioactive amino acids omitting methionine, 20 μl wheat-germ lysate, and 60 μCi ^{35}S-methionine. After 90-min incubation at 25 C, the mixture was frozen rapidly to stop the reaction.

Immune Precipitation. For immune precipitation of synthesized Ig polypeptides, 50-μl aliquots of the reaction mixture of cell-free translation, which had been treated by 10-μg pancreatic RNase (Sigma) for 10 min at 20 C, were diluted with 90 μl of solution containing 1 μl chicken Ig (as a carrier), 15 m*M* NaCl, 2% Triton X-100, 2% sodium deoxycholate, and 10 m*M* sodium phosphate (pH 7.5) and centrifuged to remove insoluble materials. The supernatant was mixed well with 20 μl of antichicken Ig-IgG fraction (rabbit) and incubated for 2 hr at room temperature to coprecipitate synthesized Ig polypeptides. The precipitate was washed with phosphate-buffered saline.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl-Sulphate (SDS-PAGE) Containing Buffer. The immune precipitate was dissolved in a small volume of the sample buffer containing 62.5 m*M* Tris HCl (pH 6.8), 2% SDS, 10% glycerol, and 5% 2-ME and then boiled for 60 sec. The SDS-PAGE system of Laemmli (1970) was used for separation of *in vitro* product on discontinuous polyacrylamide cylindrical gels (.75 cm × 10 cm) consisting of 4.25% stacking gel and 10% running gel. After electrophoresis at 20 mA/gel, the gels were fixed in 20% trichloroacetic acid, followed by 7% acetic acid, and sliced into pieces for measuring radioactivity. Standard markers of molecular size were ^{14}C-methylated form of proteins: 30 kilodalton carbonic anhydrase, 68 kilodaltons bovine serum albumin and 46 kilodaltons ovalbumin, purchased from The Radiochemical Centre, Amersham, England.

Chicken Immunoglobulin. Chicken Ig was prepared from 3-month-old chicken serum by fractionation with three successive sodium sulphate precipitations (Dreesman and Benedict, 1965) followed by dialysis against .1 M sodium phosphate buffer.

Anti-Chicken Immunoglobulin-Rabbit Immunoglobulin G Fraction. Prepared Ig from the serum of 3-month-old chickens was emulsified with Freund's complete adjuvant and injected into New Zealand White rabbits to obtain antichicken Ig serum. The IgG fraction of rabbit serum was prepared by ammonium sulfate precipitations followed by DEAE (diethylaminoethyl)-cellulose chromatography and diluted with phosphate-buffered saline to the original volume of the serum (Livingston, (1974).

Isotopes. ^{35}S-Methionine was purchased from The Radiochemical Centre, Amersham (specific activity 1000 Ci/m mole). The ^{14}C-methylated protein markers used for estimation of molecular size of *in vitro* products were given in detail above.

RESULTS

Incomplete Bursectomy by .5% Testosterone Propionate. Bursa weight 3 days after hatching was reduced by approximately 50% in chickens hatched from eggs treated with .5% TP as embryos (Fig. 1). The bursae appeared somewhat hardened. The 1% TP treatment resulted in complete bursectomy, whereas .1 and .25% TP had no effect on bursal weight.

Translation by Bursal Poly(A) Ribonucleic Acid. The amounts of total bursal RNA yielded from 1-week chickens were 2.8 mg/17 bursae (control), 2.1 mg/14 bursae (.1% TP), 2.0 mg/17 bursae (.25% TP), and .8 mg/17 bursae (.5% TP). Poly(A) RNA prepared from the total

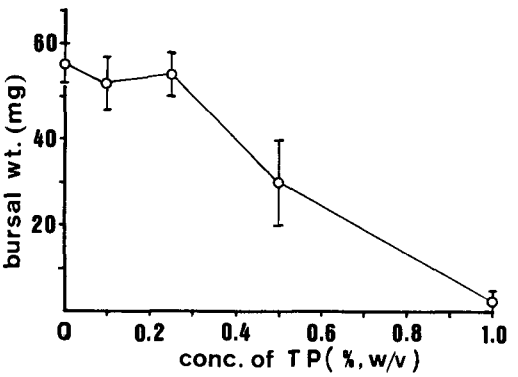

FIG. 1. Effect of testosterone propionate (TP) on bursal weight. The vertical bars are standard errors. Fertilized eggs were treated with TP solution on the 3rd day of incubation. Bursae were weighed 3 days after hatching. The control represents eggs dipped in ethanol only (0%).

Downloaded from http://ps.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

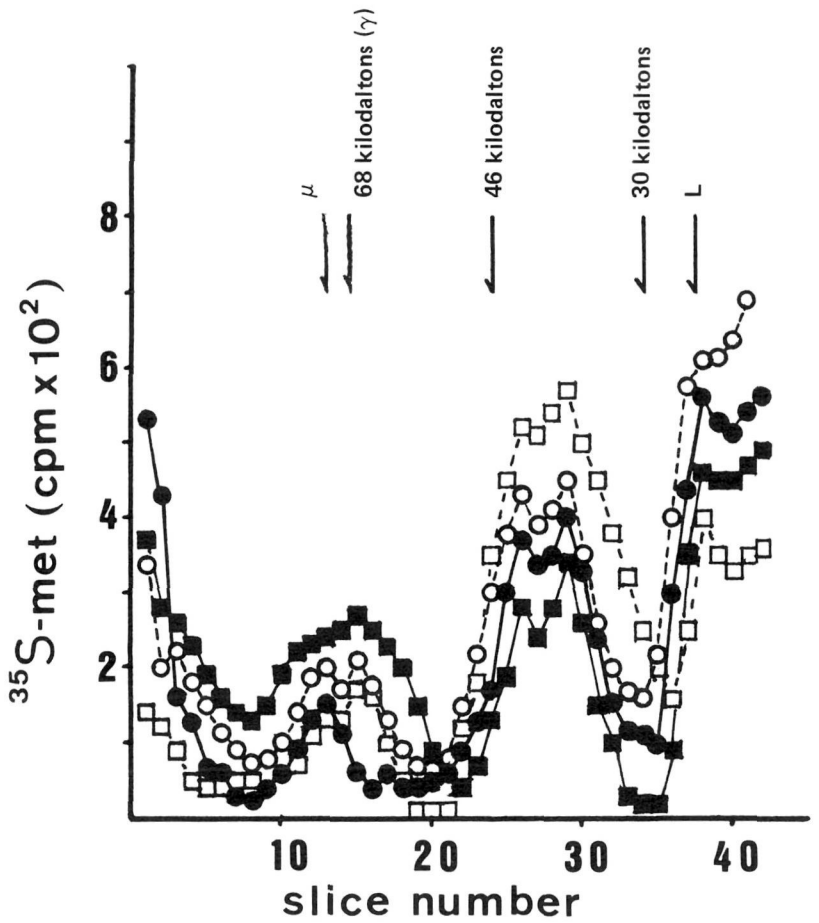

FIG. 2. Polyacrylamide gel electrophoresis in sodium dodecyl-sulphate (SDS-PAGE) of the translation products encoded by bursal poly (A) ribonucleic acids (RNA). Two μg of bursal poly (A) RNA was added to 50 μl of translation mixture with 60 μCi ^{35}S-methionine and incubated for 90 min at 25 C. Arrows indicate molecular weights (30, 46, and 68 kilodaltons) of protein standard and position of μ−, γ−, and light chains from chicken serum with molecular weights calculated from the standard. - - ○ - -, control; —●—, .5% TP; - - □ - -, .1% TP; —■—, .25% TP; L, light chain (25 kilodaltons).

bursal RNA was added in the cell-free translation system to synthesize encoded polypeptides for analysis.

By immune precipitation followed by SDS-PAGE, 2 μg of control poly(A) RNA was found to synthesize 72, 68, 43, 38, and 25-kilodalton molecules calculated by ^{14}C-labeled protein standards (Fig. 2). Heavy-(μ and γ) and light chains were obtained from serum Ig of 3-month-old chickens (GH) by reduction with 2-ME and migrated with the 72, 68, and 25-kilodalton molecules, respectively (arrows in Fig. 2). Based on analysis of the criteria of immune precipitation and their positions in the SDS gel, the 72, 68, and 25 kilodalton molecules were concluded to be identical with μ-, γ-, and light chain polypeptides, respectively. In the product translated by 2 μg poly(A) RNA from chickens hatched from .5% TP-treated eggs, the 68-kilodalton molecules were markedly decreased without loss of the other molecules, including two unknown products observed in about the 40 kilodalton region. Products synthesized by bursal poly(A) RNA from chickens hatched from eggs treated with .1- and .25% TP showed the same electrophoretic pattern as that of the controls.

DISCUSSION

Following treatment with .5% TP, the small bursa obtained had a decreased transla-

Downloaded from http://ps.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

tion activity of γ-polypeptide, whereas that encoding μ- and light chain polypeptides remained similar to the normal bursa. From these results, one role of the bursa may be the evocation of messenger RNA synthesis encoding γ-chain polypeptide in responsive cells.

When normal development of the embryonic bursa was prevented by hormone or surgical techniques, both IgG level in serum and IgG-antibody titer were markedly depressed in 2- to 9-week-old chickens (Claflin *et al.*, 1966; Lerner *et al.*, 1971; Subba Rao *et al.*, 1978). Moreover, bursectomy at 60 hr of incubation is known to cause a specific decrease in the frequency of surface IgG-positive cells in peripheral lymphoid organs (Jalkanen *et al.*, 1983), whereas normal level of circulating IgG appears to be attainable at the age of about 10 weeks (Eerola *et al.*, 1983).

ACKNOWLEDGMENT

The authors thank H. Okuyama, Chief of The Laboratory Animal Center and A. Nagata, Chief of The RI-Laboratory for use of their facilities in this study. The authors also thank the staff of Saitama Prefectural Poultry Experiment Station for supplying the animals.

REFERENCES

Aviv, H., and P. Leder, 1972. Purification of biologically active globin messenger RNA by chromatography on oligo-thymidylic acid-cellulose, Proc. Natl. Acad. Sci. USA. 69:1408–1412.

Claflin, A. J., O. Smithies, and R. K. Meyer, 1966. Antibody responses in bursa-deficient chickens. J. Immunol. 97: 693–699.

Dreesman, G. R., and A. A. Benedict, 1965. Reductive dissociation of chicken γ-G immunoglobulin in natural solvents without a dispersing agent. Proc. Natl. Acad. Sci. USA. 54:822–830.

Eerola, E., S. Jalkanen, K. Granfors, and A. Toivanen, 1983. Immune capacity of the chicken bursectomized at 60 hours of incubation: Mitogen-induced cell proliferation and immunoglobulin secretion. J. Immunol. 131:120–124.

Glick, B., and C. R. Sadler, 1961. The elimination of bursa of Fabricius and reduction of antibody production in birds from eggs dipped in hormone solutions. Poultry Sci. 40:185–189.

Huang, H. V., and W. J. Dreyer, 1978. Bursectomy *in ovo* blocks the generation of immunoglobulin diversity. J. Immunol. 121:1738–1747.

Jalkanen, S., K. Granfors, M. Jalkanen, and P. Toivanen, 1983. Immune capacity of the chicken bursectomized at 60 hr of incubation: Surface immunoglobulin and B-L (Ia-like) antigen-bearing cells. J. Immunol. 130:2038–2041.

Kanki, T., and Y. Ichikawa, 1981. Cell-free translation of immunoglobulin G polypeptides by chicken bursal RNA. Immunology 44:233–237.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) 227:680–685.

Lerner, K. G., B. Blick, and F. C. McDuffie, 1971. Role of the bursa of Fabricius in IgG and IgM production in the chicken: Evidence of the role of a non-bursal site in the development of humoral immunity. J. Immunol. 107:493–503.

Livingston, D. M., 1974. Immunoaffinity chromatography of proteins. Pages 723–731 *in* Methods in Enzymology XXXIV. Jakoby W. B. and Wilchek M., ed. Academic Press, New York, NY.

MacDonald, R. J., A. E. Przybyla, and W. J. Rutter, 1977. Isolation and *in vitro* translation of the messenger RNA coding for pancreatic amylase. J. Biol. Chem. 252:5522–5528.

Roberts, B. E., and B. M. Paterson, 1973. Efficient translation of tobacco mosaic virus RNA and rabbit globin 9S RNA in a cell-free system from commercial wheat germ. Proc. Natl. Acad. Sci. USA. 70:2330–2335.

Sato, K., and S. Abe, 1975. The possible presence of a bursa-independant, IgM-producing system in chicks. Immunology 28:293–299.

Subba Rao, D.S.V., F. C. McDuffie, and B. Glick, 1978. The regulation of IgM production in the chick: Role of the bursa of Fabricius, environmental antigens, and plasma IgG. J. Immunol. 120:783–787.

Downloaded from http://ps.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016