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AFRIKANDER CATTLE CONGENITAL GOITER: SIZE HETEROGENEITY IN THYROGLOBULIN mRNA

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The mRNA coding for thyroglobulin in cattle homozygous for an autosomal recessive defect of thyroglobulin synthesis was investigated using a recombinant plasmid containing bovine mRNA coding sequence. Total RNA preparations from goiter contained one third of the thyroglobulin mRNA sequences found in normal thyroid tissue. This mRNA was not translated into thyroglobulin by Xenopus oocytes. Northern transfer analysis revealed both a normal sized and a smaller thyroglobulin mRNA in the goiter.

Thyroblobulin is a dimer consisting of identical 12S sedimenting 300 kilodalton subunits. Some tyrosines of thyroglobulin are converted into hormones by sequencial iodination and coupling (1). Hereditary defects of thyroglobulin synthesis have been described in both man (2-5) and animals (6-8). These include an inbred herd of Afrikander cattle from South Africa with an autosomal recessive defect (9), Merino sheep from Australia and goats from Holland. Although reduced amounts and an abnormal subcellular distribution of thyroglobulin mRNA were found in Dutch goats (10) no directly detectable abnormality in

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thyroglobulin mRNA has been reported. In this communication we show that the thyroglobulin mRNA in Afrikander cattle with congenital goiter is not translated into thyroglobulin in Xenopus oocytes and consists of two different-sized species.

METHODS

Thyroid Glands: The goiter thyroid glands (weighing between 203 g and 1060 g) were obtained from an inbred herd of cattle with hereditary goiter selectively bred for this defect. Normal bovine thyroid glands (mass about 14 g each) were obtained from freshly slaughtered cattle at a nearby abbatoir. The glands were transported to the laboratory on ice and stored at -80°C.

Thyroglobulin Isolation and Labelling: Thyroglobulin was isolated from normal bovine thyroid glands as previously described (6). Radioactive labelled thyroglobulin was prepared by incubating slices of normal bovine thyroid glands in the presence of 100 μCi[^{3}H]amino-acid mixture (New England Nuclear) as previously described (6).

RNA Isolation: Total RNA was isolated from goiter and normal bovine thyroid glands by a guanidine extraction method (11). High molecular weight RNA was obtained by centrifugation of total RNA in 32 mℓ 10-35% linear sucrose gradients (in 0.1 M NaCℓ, 1 mM EDTA, 10 mM sodium acetate pH 5.0) for 24 hours at 80000 x g(av) and collecting the RNA sedimenting faster than the 28S ribosomal RNA peak. Poly(A)$^+$ RNA was prepared from total RNA by affinity chromatography on oligo-dT cellulose (Collaborative Research) (12).

RNA Translation in Xenopus Oocytes: Freshly obtained Xenopus laevis oocytes were injected with RNA according to Gurdon et al. (13). Batches of 40-60 oocytes were injected with 22.5 or 37.5 ng poly (A)$^+$ RNA in 50 nl water per oocyte. Controls were injected with 50 nl water. The injected oocytes were incubated in 150 μl modified Barth's medium (14) containing 75 μCi[^{3}H]Leucine at 20°C for 22 hours. After incubation the cells were washed twice with modified Barth's medium and homogenized in 2 mℓ immunoprecipitation buffer (0.15 M NaCl, 1% (v/v) Triton X-100, 1% (w/v) sodium deoxycholate, 10 mM sodium phosphate (pH7.4). The homogenate was centrifuged for 1 hour at 100 000 x g(av) and the supernatants were stored at -80°C. Rabbit antiserum directed against bovine 19S thyroglobulin was prepared as previously described (15). Immunoprecipitation of the oocyte translation products was as described by Rhoads et al. (16). In some control experiments normal rabbit serum was used.

Polyacrylamide Gel Electrophoresis: Polyacrylamide gel electrophoresis was performed in slab gels according to Laemmli (17), using a 6% separating gel and 4% stacking gel. Following electrophoresis the polyacrylamide gels were impregnated with 2,5-diphenyloxazole (18), dried and exposed to Kodak X-Omat AR-2 film.

RNA Analysis: RNA was electrophoresed in 3 mm thick vertical 1.5% agarose slab gels in a Tris-borate EDTA buffer system (19). Before application to the gel the RNA, in water, was heated to 70°C for 1 minute, cooled and a half-volume of a solution containing 0.25% bromophenol blue, 25% glycerol, 5% SDS and 0.02 M Tris-HCl (pH 7.4 at 20°C) was added. After electrophoresis the gels were stained with ethidium bromide (1 μg/mℓ) and photographed. The activation of aminophenylthioether-cellulose ("Ultrablot", Collaborative Research) to the diazo derivative (DPT-cellulose[2]), Northern transfer and hybridisation

[2]DPT-cellulose: diazophenylthioether cellulose.

was as described (20). Thyroglobulin mRNA in total RNA was quantified relative to thyroglobulin mRNA in the total RNA from normal thyroid by dot-hybridization (21) on DPT-cellulose. The RNA samples were lyophilized and dissolved in 5 µl of 0.2 M sodium acetate (pH 4.0). The RNA was spotted onto predetermined positions and the DPT-cellulose with bound RNA was then treated and hybridized as for Northern transfer. After autoradiography the spots were cut out and quantified by counting in a liquid scintillation counter.

cDNA Probe: The bovine thyroglobulin cDNA-containing recombinant plasmid, pbTg2.5, was used (22). Plasmid amplification in E. coli HB101 was carried out in a P2 laboratory, classified by the South African Committee on Genetic Experimentation (SAGENE) and conforming to the guideliness of the National Institutes of Health, Bethesda, USA. The plasmid was labelled with [^{32}P]dCTP by nick translation to a specific activity of about 2 x 10^8 dpm/µg DNA.

RESULTS AND DISCUSSION

Quantification of thyroglobulin RNA sequences: The amount of RNA coding for thyroglobulin in the total RNA preparations from normal bovine thyroid and three goiters was determined by dot-hybridisation. Decreasing amounts of total RNA from normal thyroid glands (Fig. 1, positions 1-5) established that the relationship between the amount of thyroid RNA spotted and the [^{32}P]cDNA hybridised was linear. Each of the three goiter total RNA preparations contained one third as much thyroglobulin mRNA sequence as the normal thyroid total RNA preparation (36.0% $\pm$ 3.5% for the 6 determinations). This indicated that there is considerably less thyroglobulin-related RNA relative to the total RNA in the goiter tissue. However, as the goiters are 10 to 50 fold larger

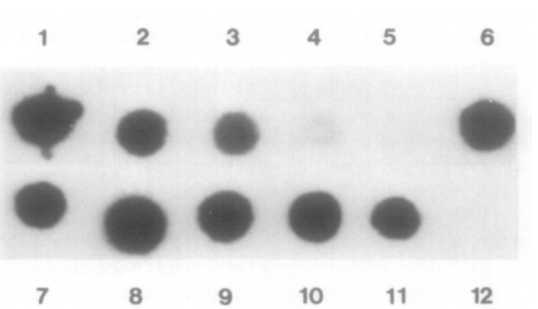

Fig. 1. Quantification of thyroglobulin mRNA in normal and goiter thyroid total RNA preparations by dot-hybridization. Positions 1-5 contained 5 µg, 1 µg, 0.5 µg, 0.1 µg and 0 µg normal bovine thyroid total RNA. Spots 6, 8, 10 and 7, 9, 11 contained 10 µg and 5 µg, respectively, of total RNA prepared from 3 different goiters. All spots contained a total of 10 µg RNA by inclusion of rabbit reticulocyte polysomal RNA. Position 12 had no RNA.

than normal thyroids, and the yield of RNA from goiter tissue was consistently 5 fold greater than from an equivalent mass of normal thyroid, there is in total considerably more thyroglobulin mRNA in the goiters. This contrasts with the absence of normal thyroglobulin in the goiter tissue (6). The translation of the goiter RNA was therefore examined in Xenopus oocytes.

Translation of RNA: Poly $(A)^+$ RNA was injected into Xenopus oocytes and the translation products were precipitated by rabbit anti-thyroglobulin antiserum and analysed by SDS polyacrylamide gel electrophoresis (Fig. 2). Oocytes injected with poly$(A)^+$ RNA from normal bovine thyroid glands synthesised a specifically immunoprecipitable protein which migrated slightly slower than the standard [^{3}H]thyroglobulin (Fig. 2 lanes 3 and 8). The slower migration of the oocyte produced thyroglobulin is possibly due to differences in post-translational modifications such as glycosylation, iodination and signal peptide cleavage. When the poly$(A)^+$ RNA from two goiters was translated in the oocyte system no such protein was detected; nor were there any smaller translation products specifically precipitated by the antithyroglobulin antiserum (Fig. 2 lanes 4, 5 and 6), even on prolonged exposure. The lower band visible in lane 3 which

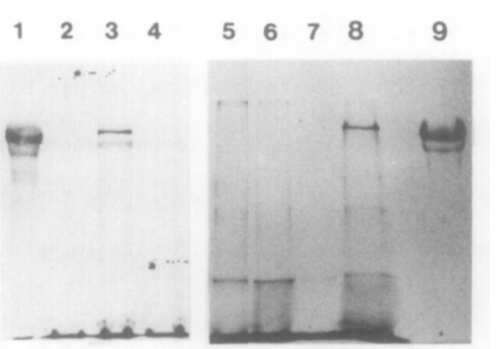

Fig. 2. SDS polyacrylamide gel separation of Xenopus oocyte translation products. Lanes 1 and 9: standard [^{3}H]thyroglobulin (30 µg). Lane 2: control (water-injected) oocytes. Lanes 3 and 8: oocytes injected with normal bovine thyroid poly$(A)^+$ RNA. Lanes 4 and 6: oocytes injected with poly$(A)^+$ RNA from goiter 55. Lane 5: oocytes injected with goiter 54 poly$(A)^+$ RNA. Lane 7: oocytes injected with normal thyroid poly$(A)^+$ RNA and immunoprecipitated with control rabbit serum.

comigrates with the standard thyroglobulin is an endogenous oocyte product visible in all controls on longer exposure. Although this result appears to be at variance with a previous report using a reticulocyte lysate cell-free system, the absence of thyroglobulin synthesis in an in vivo system such as Xenopus oocytes more truly reflects the situation in goiter tissue where no normal thyroglobulin is found (6). In addition an independant study has found no normal thyroglobulin synthesised in a reticulocyte lysate cell-free system in response to goiter mRNA (23).

Analysis of RNA by Northern transfer: To analyze the goiter RNA in terms of size, agarose gel electrophoresis followed by Northern transfer and hybridisation was performed. Staining of the RNA following electrophoresis showed the positions of the size markers and established that the RNA preparations were intact. Hybridisation of the labelled probe to RNA isolated from normal bovine thyroid showed a single mRNA for thyroglobulin (8000 bases) slightly larger than the marker tobacco mosaic virus RNA (6500 bases). A considerably weaker signal was obtained with the goiter RNA preparations where two bands of hybridisation were clearly seen with RNA from 2 goiters (Fig. 3 lanes 1 and 5); the upper band comigrates with the normal thyroglobulin mRNA. Hybridisation to an exceptionally large goiter (number 55) showed the smaller RNA to be predominant (lanes 2 and 6). The faster migration rate of the smaller mRNA indicates that it is about 1000 bases shorter than the normal thyroglobulin mRNA.

The presence of two mRNAs related to thyroglobulin in goiter tissue provides evidence that the defect resides in the thyroglobulin gene. Although the results seem compatible with animals heterozygous for the defect, breeding studies and Sothern blot analysis have clearly established that they are homozygous (9). The presence of two or more mRNAs coding for a particular protein could arise either from different sites of initiation or termination of transcription or alternative splicing of the precursor mRNA. The latter possibility is considered most likely to be operative in the bovine goiter. The results presented in this communica-

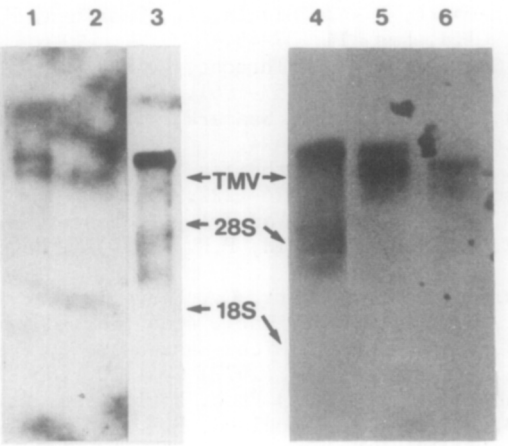

Fig. 3. Analysis of RNA from normal thyroid and goiter by electrophoresis, transfer to DPT-cellulose and hybridization with [^{32}P]pbTg2.5. The RNA was isolated and electrophoresed as described in the "Methods". Lane 1: goiter 54 poly(A)$^+$ RNA (20 µg). Lane 2: goiter 55 poly(A)$^+$ RNA (10 µg). Lane 3: normal thyroid poly(A)$^+$ RNA (10 µg). Lane 4: normal thyroid poly(A)$^+$ RNA (5 µg). Lane 5: goiter 57 high molecular weight poly(A)$^+$ RNA (5 µg) and Lane 6: goiter 55 high molecular weight poly(A)$^+$ RNA (5 µg). The positions of migration of the 28S ($\pm$ 5000 nucleotides) and 18S ($\pm$ 2000 nucleotides) ribosomal RNA and tobacco mosaic virus RNA ($\pm$ 6500 nucleotides) are indicated.

tion, in agreement with the in vivo protein profile (6), indicate that both the goiter thyroglobulin mRNAs are malfunctional. Further investigations are being directed at elucidating the precise molecular basis of this disease.

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