Transcriptional Regulation of the Tryptophan Oxygenase Gene in Rat Liver by Glucocorticoids*

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The enzyme tryptophan oxygenase (EC 1.13.11.11), which is synthesized in rat liver, is induced by glucocorticoids. We have used cloned tryptophan oxygenase genomic and cDNA sequences to study the mechanism of induction. Rat liver poly(A+) RNA was separated on a formaldehyde gel, blotted to nitrocellulose, and hybridized to a nick-translated tryptophan oxygenase cDNA clone to analyze the kinetics of tryptophan oxygenase mRNA accumulation. Transcription in isolated rat liver nuclei was investigated to determine the relative rate of transcription of the tryptophan oxygenase gene. Analysis of the accumulation of albumin mRNA, which is unaffected by glucocorticoids, served as an internal control. We show here that the synthetic glucocorticoid dexamethasone causes a 10-fold increase in tryptophan oxygenase and tyrosine aminotransferase in rat liver (8-10). The increased activity of tryptophan oxygenase is mediated by controlling the level of mRNA activity of tryptophan oxygenase gene subclones consisting of unique sequences: 3.2-kb EcoRI fragment, "C" (2.74 kb), "D" (1.56 kb) (1 µg of DNA of each), and (c) pBR322 (3 µg of DNA). Filter hybridization was performed at 45 °C for 48 h in a final volume of 200 µl containing 0.5 M NaCl, 50 mM Pipes, 1 M KCl, 15 mM MgCl₂, 1 mM MnCl₂, 150 mM KCl, 2.5 mM dithiothreitol, 1 mM ATP, GTP, and CTP, and 200 µCi of [α-32P]UTP (410 Ci/mmol, Amersham Corp.) at 25 °C for 30 min. The RNA was isolated according to Compere et al. (17). The RNA was hybridized to the following DNA sequences immobilized on nitrocellulose filters: (a) tryptophan oxygenase gene subclones consisting of unique sequences: 3.2-kb EcoRI fragment, 3.6-kb EcoRI fragment, and 6.0-kb XhoI-XbaI fragment of DNA. Filter hybridization was performed at 45 °C for 48 h in a final volume of 200 µl containing 0.5 M NaCl, 50 mM Pipes pH 7.0, 30% formamide, 0.4% SDS, and 2 mM EDTA. After hybridization the filters were washed in 0.3 M NaCl, 2 mM EDTA, 10 µM Tris-HCl, pH 7.5, 6.1% SDS at 45 °C, then 30 min in the same buffer without SDS. After treatment with RNase A (10 µg/ml) and T1 RNase (1 µg/ml) for 30 min at 37 °C the RNAase-resistant radioactivity on the filters was counted in Unisolve (Zinsser Analytic). To measure the efficiency of hybridization, [3H]cRNA was synthesized from pHGU 207 (20) as described (21). This plasmid contains the strong T5 promoter and amounts of tryptophan oxygenase mRNA sequences by regulation of its transcription.

MATERIALS AND METHODS

Animals—Male Wistar rats (150 g) were adrenalectomized and 5 days later injected intraperitoneally with the synthetic glucocorticoid hormone dexamethasone (Sigma: 10 µg/100 g of body weight in 0.9% sodium chloride) or only with saline as a control. The rats were killed by cervical dislocation at the times indicated (see Figs. 1 and 3), and the livers were removed and quickly frozen in liquid nitrogen.

Isolation of RNA and RNA Blotting Analysis—Poly(A) RNA was isolated by passing total liver RNA isolated as described (13) three times through a poly(U)-Sepharose column. The RNA was separated on a vertical formaldehyde gel as described elsewhere (14), transferred to nitrocellulose sheets (Schleicher & Schuell, BA 85) in 10 × SSC, and hybridized to a nick-translated 32P-labeled tryptophan oxygenase cDNA probe or albumin cDNA probe, respectively. Freezing for 4 h and hybridization for 16 h was in 3 × SSC, 0.12 M phosphate, pH 6.8, 10 mM EDTA, 0.06% bovine serum albumin, Ficoll, and polyvinylpyrrolidone, 0.1% SDS at 65 °C. The sheets were extensively washed in 2 × SSC at 65 °C, dried, and exposed to flash-activated x-ray film (Kodak XAR-5) at -70 °C using intensifier screens.

Ovalbumin Calibration Curve—Different amounts of chicken oviduct poly(A) RNA (25-0.8 ng) were separated on a denaturing formaldehyde gel (14), blotted to nitrocellulose sheets, and hybridized to a nick-translated ovalbumin cDNA clone (15) (1.5 × 10⁶ cpm/µg). The bands were scanned and compared to bands of a tryptophan oxygenase blot of the same exposure time after correction for the size and specific activities of the probes.

In Vitro Transcription and Hybridization—Liver nuclei were isolated and suspended in 25% glycerol, 60 mM KCl, 15 mM NaCl, 0.5 mM spermidine, 0.15 mM spermine, 15 mM Tris-HCl, pH 7.5, and stored at -70 °C (16). Nuclei (40-50 µg of DNA) were incubated in 100-µl reaction mixture containing 1% glycerol, 50 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 1 mM MnCl₂, 150 mM KCl, 2.5 mM dithiothreitol, 1 mM ATP, GTP, and CTP, and 200 µCi of [α-32P]UTP (410 Ci/mmol, Amersham Corp.) at 25 °C for 30 min. The RNA was hybridized for 16 h in 10 × SSC, pH 6.8, 10 mM EDTA, 0.06% bovine serum albumin, Ficoll, and polyvinylpyrrolidone, 0.1% SDS at 65 °C. The sheets were extensively washed in 2 × SSC at 65 °C, dried, and exposed to flash-activated x-ray film (Kodak XAR-5) at -70 °C using intensifier screens.

The abbreviations used are: SSC, 0.15 M NaCl, 0.015 M Na-citrate; SDS, sodium dodecyl sulfate; kb, kilobase pairs; Pipes, L-1-piperazine-N,N'-bis(2-ethanesulfonic acid).
therefore allows in vitro transcription with Escherichia coli RNA polymerase mainly from one strand (20). The H-RNA was hybridized to pGBU 207 DNA bound to filters under the conditions described above.

RESULTS

The poly(A') fraction of total RNA isolated from rat liver (13) was separated on a denaturing formaldehyde gel and blotted onto nitrocellulose filters (14). The RNA was then hybridized against a trypophan oxygenase-specific nick-translated probe to identify trypophan oxygenase mRNA sequences. As a probe, we used a trypophan oxygenase cDNA clone (pCTO-1) which contains 560 nucleotides for the 5' part of the trypophan oxygenase gene (18). As an internal control accumulation of albumin mRNA, which is not affected by glucocorticoids (22), was simultaneously analyzed using an albumin cDNA clone (23). The results of these studies are shown in Fig. 1. After injection of dexamethasone, a lag phase of 30 min is followed by an increase in trypophan oxygenase-specific mRNA reaching a 10-fold higher level 4 h after injection (see also Fig. 3). However, after administration of the hormone no major change in the amount of albumin mRNA was observed. The amounts of trypophan oxygenase and albumin mRNA sequences were quantitated by scanning autoradiograms of different exposure times to verify the course of the kinetics. Using a different method, the dot-blot procedure (data not shown) (24), we measured the hybridized DNA by scintillation counting and found similar results as those described with the blot procedures. The percentages of trypophan oxygenase mRNA in total poly(A') RNA before and 4 h after induction are 0.008 and 0.08%, respectively, as determined by a chicken ovalbumin mRNA calibration curve (see "Materials and Methods"). These values are in good agreement with previous estimates (25) and are confirmed by the frequency with which trypophan oxygenase cDNA clones are found in poly(A') RNA (18). Ovalbumin mRNA was selected because its content in chicken oviduct poly(A') RNA is known (15). Previous work, using an in vitro cell-free translation assay, has shown that the RNA of glucocorticoid-treated animals directs the synthesis of increased amounts of trypophan oxygenase protein as compared to the RNA from untreated animals (12, 25). The present study shows clearly that the hormone regulates the amounts of trypophan oxygenase mRNA and not its translational activity.

It is known that steroid hormones act at the transcriptional level (2-7) as well as on mRNA stability (26, 27). To determine whether the accumulation of trypophan oxygenase mRNA following dexamethasone stimulation is due to stabilization of the mRNA or caused by enhanced transcription of the trypophan oxygenase gene, we measured the rate of transcription in isolated nuclei. This assay measures predominantly the elongation of in vivo initiated RNA chains; it is thus a measure of the relative number of polymerases on a particular gene. Liver nuclei were isolated and the endogenous RNA polymerases were allowed to synthesize RNA in the presence of [32P]UTP. The labeled transcripts were isolated and hybridized to immobilized DNA of subclones containing unique fragments of the trypophan oxygenase gene (18) or the albumin gene (19). Transcription of the albumin gene was measured in the same way. The trypophan oxygenase mRNA represents only 0.008% of total mRNA in noninduced rat liver; it was therefore important to establish a sensitive and accurate transcription assay. Two groups of rats were injected either with dexamethasone or with 0.9% sodium chloride and killed

Fig. 1. Time course of tryptophan oxygenase mRNA (A) and albumin mRNA (B) accumulation after induction with dexamethasone. 10 μg (A) and 5 μg (B) of poly(A') RNA, isolated from animals which were treated for the indicated times as described under "Materials and Methods," were electrophoresed, blotted, and hybridized to 32P-labeled trypophan oxygenase (TO) cDNA (9.6 x 105 cpm/μg) and albumin cDNA (2.8 x 105 cpm/μg), respectively. As a size marker, 5 μg of total RNA was applied on the same gel and the ribosomal RNA was visualized by ethidium bromide staining.
2 h later. Liver nuclei were isolated and used for transcription experiments in vitro. The rate of transcription was linear for 20 min; 4–9 pmol of UMP were incorporated/1 μg of DNA (not shown). 80% of total RNA synthesis is α-amanitin-sensitive. Three different amounts of 32P-labeled RNA from induced and noninduced animals were hybridized against unique fragments of the tryptophan oxygenase and the albumin gene. Fig. 2 shows that the hybridized radioactivity is proportional to the RNA input. Nonspecific hybridization to pBR322 or to filters containing no DNA were only 2 ppm of the input RNA.

Nuclei from animals treated with dexamethasone for 2 h show a 10 times higher transcriptional rate of the tryptophan oxygenase gene compared to nuclei from animals injected with saline. The relative rates of transcription were, respectively, 1075 and 110 ppm for induced and noninduced animals (values corrected for hybridization efficiency) (Fig. 2). Surprisingly, the albumin gene also shows a 2-fold increase in the relative rate of transcription.

To determine how fast the transcription of the tryptophan oxygenase gene changes, a time course of induction was established (Fig. 3). At various times after the administration of dexamethasone, the relative transcriptional rate was measured. After only 10 min, the rate of synthesis of tryptophan oxygenase mRNA sequences starts to increase. The rate peaks at 30 min and then decreases to a plateau that is 10 times higher than the basal level of transcription. A similar higher rate of initial transcription as compared to the rate in the final plateau phase has been observed by Hager and Palmiter (6) in the case of transcription of the metallothionein gene.

**DISCUSSION**

In the experiments described, we find that dexamethasone increases the amount of tryptophan oxygenase mRNA. This change is proportional to an increase in the transcriptional rate of the tryptophan oxygenase gene 2 h after induction. Therefore, we may conclude that dexamethasone exerts its effect mainly on the rate of transcription and not on the stabilization of the tryptophan oxygenase mRNA. Similar observations have been made for other glucocorticoids-controlled genes (2, 6, 7). The accumulation studies show that after a lag phase of 30 min, the amount of tryptophan oxygenase mRNA starts to increase and reaches a 10-fold higher plateau after 3–4 h. Previously, a 4-fold increase has been observed using a mRNA translation assay (12, 25). This lower estimate may be due to the less sensitive in vitro translation assay and the fact that nonadrenal-corticalized rats have been used. The transcriptional rate of the tryptophan oxygenase gene changes very rapidly after hormone administration. This change is due to the injected steroid hormone, as rats injected only with saline show no increased transcription at 0 and 2 h after injection (Figs. 2 and 3). The increase is as rapid as the induction of the mouse mammary tumor virus RNA and argues against a protein intermediate. It is therefore likely that the hormone receptor complex interacts directly with the DNA or chromatin. This is supported by the fact that the nuclear binding sites are saturated within 5–10 min (28).

It is surprising that the rate of albumin gene transcription is increased 2 times by dexamethasone since the concentration of the mRNA is unaffected by glucocorticoid treatment. It may be possible that this increase is transient since it does not influence the accumulation rate. Transient changes of RNA polymerase II activity have been reported in many hormone controlled systems (for review, see Ref. 29). It is unclear whether this general increase of RNA synthesis is in any way related to the transient peak of tryptophan oxygenase gene transcription shown in Fig. 3.

Albumin mRNA comprises about 10% of the poly(A) mRNA from rat liver (30). The tryptophan oxygenase mRNA is present at an approximately 100-fold lower level. The transcriptional rates of these two genes are, however, very similar. This observation is best explained by the different stability of the two mRNAs. We can conclude therefore that the promoter of the tryptophan oxygenase gene is very active. The relative transcriptional rate (0.1%) is of the same order of magnitude as that observed for the chicken ovalbumin or the lysozyme gene (3). This value is calculated from the determined rate of 430 ppm in the induced state and corrected for the hybridization efficiency of 40% and the length of the probes (12.8 kb of the 19-kb tryptophan oxygenase gene was used). Based on the assumptions that 30,000 RNA polymerase II molecules are engaged in transcription in the cell nucleus (31), that the elongation rate of polymerase II is identical on all genes, and that all in vitro transcripts are conserved, we can conclude that approximately 10 molecules of RNA polymerase II are transcribing the tryptophan oxygenase gene. The tryptophan oxygenase promoter is thus favorable for an analysis of hormone-controlled expression after introduction of the tryptophan oxygenase gene into eucaryotic cells and for hormonally regulated in vitro transcription. Studies in these two directions are in progress in our laboratory.

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![Fig. 3. Time course of the tryptophan oxygenase (TO) mRNA accumulation and the relative rate of transcription of the tryptophan oxygenase gene in rat liver following dexamethasone stimulation. Livers from rats induced with dexamethasone (10 μg/100 g of body weight) for the times indicated were used to determine the mRNA concentration as in the legend to Fig. 1. For this purpose autoradiograms such as the one in Fig. 1A were scanned and the tryptophan oxygenase mRNA content was calculated by using chicken ovalbumin mRNA for calibration (see "Materials and Methods"). Nuclei from the same livers were used for the measurement of the relative rate of transcription of the tryptophan oxygenase gene as described under "Materials and Methods." The average efficiency of hybridization was 40%. The data are corrected to give an efficiency of hybridization of 100%.](http://www.jbc.org/Downloaded_from)
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U Danesch, S Hashimoto, R Renkawitz and G Schütz

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