Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 9499–9502, December 1988
Biochemistry

Structure and regulation of the rat 1,25-dihydroxyvitamin D_3 receptor

(vitamin D/steroid receptor/calcium transport/steroid receptor cDNA)

JAMES K. BURMESTER*, RUSSELL J. WIESE*, NOBUYO MAEDA†‡, AND HECTOR F. DELUCA*

Departments of *Biochemistry and †Genetics, University of Wisconsin–Madison, Madison, WI 53706

Contributed by Hector F. DeLuca, September 26, 1988

ABSTRACT **Complementary DNA clones encoding the entire rat 1,25-dihydroxyvitamin D_3 receptor were isolated, and the total 423-amino acid sequence was deduced. The amino acid sequence of the rat receptor is similar but not identical to the reported human receptor sequence. The cysteine-rich DNA-binding domain is completely conserved and the steroid-binding domain is >93% conserved between rat and human. The cDNA was used as a probe in blot analysis of polyadenylylated RNA to show that the 1,25-dihydroxyvitamin D_3 receptor mRNA is a single 4.4-kilobase mRNA that is expressed in intestine and kidney, slightly expressed in heart, and not detectable in liver and spleen. The receptor mRNA concentration is markedly increased during development of the rat intestine between day 14 and day 21, in accord with previous results obtained by measurement of receptor concentration by ligand binding or immunoblotting.**

The primary function of 1,25-dihydroxyvitamin D_3, the active form of vitamin D, is to increase serum calcium and phosphorus (1, 2). The action of 1,25-dihydroxyvitamin D_3 is mediated through an intracellular receptor protein that is present in target tissues at a very low concentration, ≈0.001% (3, 4). This receptor binds 1,25-dihydroxyvitamin D_3 and then interacts with specific sites in chromatin, resulting in specific gene transcription. These gene products then effect calcium transport in the intestine and cause calcium mobilization from bone and calcium reabsorption in kidney (1, 2).

The importance of a functional receptor is clearly demonstrated through the disease vitamin D-dependent rickets type II (5, 6). Patients with this disease suffer impaired calcium absorption despite high circulating levels of 1,25-dihydroxyvitamin D_3. A defective receptor has been reported to be the cause in at least some kindrids (7).

Monoclonal antibodies against the receptor have been isolated (8). The receptor has a molecular weight of 55,000 when identified by these antibodies on an immunoblot (9, 10). A partial cDNA for the rat receptor was isolated by screening a kidney λgt11 expression library with these antibodies (11). When expressed in *Escherichia coli*, this cDNA produced a region of the receptor capable of specifically binding 1,25-dihydroxyvitamin D_3, confirming the authenticity of the clone. In addition, a partial cDNA for the avian receptor (12) and the complete cDNA for the human receptor (13) have been sequenced.

We report here the nucleotide sequence and the predicted amino acid sequence for the complete rat receptor.§ Comparison of the rat sequence to the human sequence shows that the receptor has remained highly conserved between the two species. The cloned cDNA was used to study the regulation of receptor gene transcription. A single 4.4-kilobase (kb) receptor mRNA is expressed in the intestine, kidney, and heart of adult rats. This mRNA is not detectable in preparations isolated from intestine of 14-day-old rat, but by day 21, it has increased substantially.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

RNA Isolation. RNA was prepared by the procedure of Kessler *et al.* (14) from the intestine, kidney, heart, liver, and spleen of 7-week-old Sprague–Dawley rats maintained on a diet of Purina Rat Chow. Similarly, RNA was isolated from a 10% length of the small intestine, immediately distal to the pyloric sphincter, of 14- and 21-day-old rats obtained from Harlan Sprague-Dawley. mRNA was purified by oligo(dT)-cellulose chromatography in all cases.

Northern Analysis. mRNA was electrophoresed in a 1% agarose/2.2 M formaldehyde gel and then transferred to a nylon membrane. A 1784-base-pair (bp) receptor cDNA in the plasmid pUC18 was radiolabeled by nick-translation with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham; 1 Ci = 37 GBq) and then used as a hybridization probe according to the conditions described (14). Similarly, a nick-translated cDNA coding for lactate dehydrogenase (the generous gift of R. Jungmann, Northwestern University Medical School) was used to standardize the amounts of mRNA loaded in each lane. Following washing, filters were autoradiographed for 72 hr.

Isolation of the Full-Length Receptor Clone. To enrich for the receptor mRNA, 200 μg of rat intestinal mRNA was loaded onto an 11.5-ml 5–20% (wt/vol) sucrose gradient in 10 mM Tris/1 mM EDTA, pH 7.4, and centrifuged for 6 hr at 40,000 rpm in a Beckman SW40 Ti rotor. Twenty-eight fractions were collected and an aliquot of each was used for Northern analysis. The fractions enriched for the receptor mRNA were pooled and used as a source of mRNA for primer-extended cDNA cloning. An oligonucleotide having the sequence GCCGATGTCCACACAGCGTT was used as a primer. This sequence is complementary to the receptor mRNA and begins 80 bp from the 5′ end of the previously known sequence. Blunt-ended cDNA was made by using this oligonucleotide as a specific primer according to published methods (15). This cDNA was ligated to pUC18 plasmid DNA that had been cut with *Sma* I and dephosphorylated. *E. coli* JM109 cells were transformed with the ligated DNA. One receptor-positive clone was detected from among the resulting colonies by using an oligonucleotide having the sequence CAATCTCCATTGAAGGGACA as a probe. This oligonucleotide begins 12 bp from the 5′ end of the previous sequence. Hybridization conditions for oligonucleotide screening have been described (16). The cDNA was sequenced by the dideoxy method of Sanger *et al.* (17).

No reprints will be available from the authors.

‡Present address: University of North Carolina, Chapel Hill, NC 27514.

§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04147).

RESULTS

Cloning of the Full-Length Receptor cDNA. A partial cDNA encoding a major portion of the rat receptor was described previously (11). A synthetic oligonucleotide complementary to the receptor mRNA was made according to the sequence near the 5′ end of this cDNA and was used as a primer for cDNA synthesis. One positive clone was obtained. The cDNA from this clone codes for the remainder of the receptor protein and has a 5′ nontranslated sequence of 94 bp.

The deduced amino acid sequence is shown in Fig. 1. The first in-frame AUG has been assigned as the initiation codon. The amino-terminal sequence of the rat receptor is nearly identical to the amino-terminal sequence deduced for the human protein (Fig. 2). In addition, the rat sequence exactly matches the sequence of the porcine receptor, which was obtained by protein-sequencing techniques (18). The presence of matching sequences both near the amino terminus and near the carboxyl terminus confirms the deduced open reading frame.

The rat and human amino acid sequences are very similar (Fig. 2). A cysteine-rich region at the amino terminus most probably represents the DNA-binding domain of the receptor. This region is 100% conserved between the two species. The steroid-binding domain is located at the carboxyl terminus and is >93% conserved. Between these two domains is a region that is less well conserved, having ≈52% identical

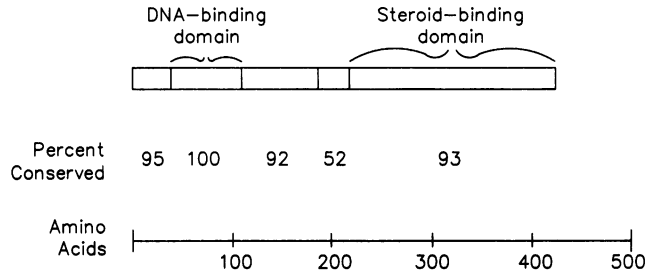

FIG. 2. Comparison of the rat amino acid sequence to the human amino acid sequence as deduced by Baker *et al.* (13). The rat and human amino acid sequences were aligned and the percentage of identical amino acids for different regions of the protein have been determined. The likely positions of the DNA-binding domain and the steroid-binding domain are shown.

```
-94  CGTCCACCGCCAGACCAGAGTTCTTTTGGTCGGACAGATCTGTGAGACTTCCAGGAGAGCACCCTTGGGCTCTACTCACCCTGCTCCTTC

 -4  AGGGATGGAGGCAACAGCGGCCAGCACCTCCCTGCCCGACCCTGGTGACTTTGACCGGAACGTGCCCCGGATCTGTGGAGTGTGTGGAGA
         M  E  A  T  A  A  S  T  S  L  P  D  P  G  D  F  D  R  N  V  P  R  I  C  G  V  C  G  D
                                    10                                  20
 87  CCGAGCCACAGGCTTCCACTTCAATGCTATGACCTGTGAAGGCTGCAAAGGTTTCTTCAGGCGGAGCATGAAGCGGAAGGCCCTGTTCAC
      R  A  T  G  F  H  F  N  A  M  T  C  E  G  C  K  G  F  F  R  R  S  M  K  R  K  A  L  F  T
      30                            40                                  50
177  CTGTCCCTTCAATGGAGATTGCCGCATCACCAAGGACAACCGGCGACACTGCCAGGCCTGCCGGCTCAAACGCTGTGTGGACATCGGCAT
      C  P  F  N  G  D  C  R  I  T  K  D  N  R  R  H  C  Q  A  C  R  L  K  R  C  V  D  I  G  M
      60                            70                                  80
267  GATGAAGGAGTTCATCCTGACAGATGAGGAGGTACAGCGTAAGAGGGAGATGATAATGAAGAGAAAAGAGGAAGAGGCCTTGAAGGACAG
      M  K  E  F  I  L  T  D  E  E  V  Q  R  K  R  E  M  I  M  K  R  K  E  E  E  A  L  K  D  S
      90                            100                                 110
357  TCTGAGGCCCAAGCTATCTGAAGAACAACAGCACATCATAGCCATCCTGCTGGACGCCCACCACAAGACCTATGACCCCACCTACGCTGA
      L  R  P  K  L  S  E  E  Q  Q  H  I  I  A  I  L  L  D  A  H  H  K  T  Y  D  P  T  Y  A  D
      120                           130                                 140
447  CTTCAGGGACTTCCGGCCTCCAGTTCGTATGGACGGAAGTACAGGGAGCTATTCTCCAAGGCCCACACTCAGCTTCTCCGGGAACTCCTC
      F  R  D  F  R  P  P  V  R  M  D  G  S  T  G  S  Y  S  P  R  P  T  L  S  F  S  G  N  S  S
      150                           160                                 170
537  CTCCTCCAGCTCTGACCTGTACACCACCTCACTAGACATGATGGAACCATCCGGCTTTTCCAACCTGGATCTGAACGGAGAGGATTCTGA
      S  S  S  S  D  L  Y  T  T  S  L  D  M  M  E  P  S  G  F  S  N  L  D  L  N  G  E  D  S  D
      180                           190                                 200
627  TGACCCGTCTGTGACTCTGGACCTGTCTCCTCTCTCCATGCTGCCCCACCTGGCTGACCTTGTCAGTTACAGCATCCAAAAGGTCATCGG
      D  P  S  V  T  L  D  L  S  P  L  S  M  L  P  H  L  A  D  L  V  S  Y  S  I  Q  K  V  I  G
      210                           220                                 230
717  CTTTGCCAAGATGATCCCAGGATTCAGGGATCTCACCTCCGATGACCAGATTGTCCTGCTTAAGTCAAGCGCCATTGAGGTGATCATGTT
      F  A  K  M  I  P  G  F  R  D  L  T  S  D  D  Q  I  V  L  L  K  S  S  A  I  E  V  I  M  L
      240                           250                                 260
807  ACGCTCCAACCAGTCTTTCACCATGGATGATATGTCCTGGGACTGTGGCAGCCAGGACTACAAGTACGACGTCACCGATGTCTCCAAAGC
      R  S  N  Q  S  F  T  M  D  D  M  S  W  D  C  G  S  Q  D  Y  K  Y  D  V  T  D  V  S  K  A
      270                           280                                 290
897  TGGGCACACCCTGGAGCTGATCGAGCCCCTCATAAAGTTCCAGGTGGGGCTGAAGAAGCTGAACTTACATGAGGAAGAGCATGTCCTTCT
      G  H  T  L  E  L  I  E  P  L  I  K  F  Q  V  G  L  K  K  L  N  L  H  E  E  E  H  V  L  L
      300                           310                                 320
987  CATGGCCATCTGCATTGTCTCCCCGGACCGACCTGGGGTCCAGGACGCCAAGCTGGTGGAAGCCATTCAGGACCGCCTATCCAACACGCT
      M  A  I  C  I  V  S  P  D  R  P  G  V  Q  D  A  K  L  V  E  A  I  Q  D  R  L  S  N  T  L
      330                           340                                 350
1077 GCAGACCTACATCCGCTGCCGCCACCCGCCCCCAGGCAGCCACCAGCTCTATGCCAAGATGATCCAGAAACTGGCCGACCTGCGGAGCCT
      Q  T  Y  I  R  C  R  H  P  P  P  G  S  H  Q  L  Y  A  K  M  I  Q  K  L  A  D  L  R  S  L
      360                           370                                 380
1167 CAACGAGGAACACTCCAAACAATACCGCTCCCTCTCCTTCCAGCCCGAGAATAGCATGAAGCTCACACCCCTTGTGCTGGAGGTGTTCGG
      N  E  E  H  S  K  Q  Y  R  S  L  S  F  Q  P  E  N  S  M  K  L  T  P  L  V  L  E  V  F  G
      390                           400                                 410
1257 CAATGAGATCTCCTGACCAGGGTGGCCCACAGTGGTGCCTGGGTAGGGCCGCTCCTCCAGAGCCCTGTGCCCAGGCCCTGGGCTTGGTTG
      N  E  I  S
      420
1347 CAGCCCAGCAGTGCCTCCTGCCCTTTCTGGAGTTCAGTCCTTCCTCTGCCATGGCCTCTGTCTGTCTGCCTCATCCTTTCTCCTGCCCAG  1436
1437 CCTAACACCTGGTCTCCCTTTCCTGTAGACCTCGAGTTGCTCCTGTCTCTTGAGACCTCAGTTAGGAGAGGCTGCTGTTTATCTGACAAA  1526
1527 GGAACTCAATTGGGGATAGAGGGCAGGGGCTGAAGGCGGAACTCTGCCTAGGGGATGCCTCCACCACAAGGGGCTGCTGCTTGTGTCAAG  1616
1617 GGAGGCAGGCAGAAGAGACGCATTCACTCCTCAGGGACAGGTACCTGCACCTCCCCTCACTCCAGCCCTACCTGCCCAAAGCCTAGTGAG  1706
1707 AAATCTGGCCCCTGCCTGCGAAGGGTACACAACCTACCCATCATCCCTACTGTGTCCCGTCTCGTCCTGCCGCCTGTCTGTGTTATTCTG  1796
1797 ACCCGGGGGAGTAGGTCACTGAGGGGCCTCCTTCCTCTGCCTTTATACTCACGGGGCTCACTCACTGCCAAGATGACCAAATACACTACC  1886
1887 ACACGAACCAAGGAGCACTCACCCAGCCCTGCAGTTCCCACCTTTGAGGTTTTGCCATGGGAA  1949
```

FIG. 1. Nucleotide sequence and deduced amino acid sequence (one-letter notation) of the rat 1,25-dihydroxyvitamin D_3 receptor. The underlined amino acids have also been determined by protein sequencing of receptor purified from porcine intestine (18).

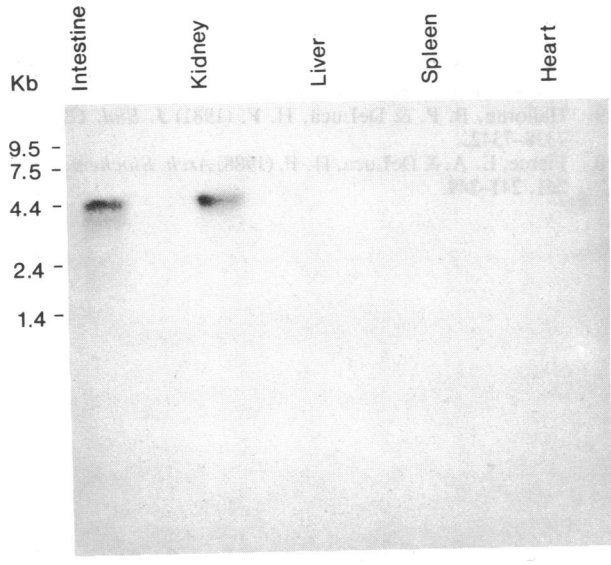

FIG. 3. Tissue distribution of the rat 1,25-dihydroxyvitamin D_3 receptor mRNA. Polyadenylylated RNA (4 μg per lane) from rat intestine, kidney, liver, spleen, and heart was electrophoresed in a 1% agarose/2.2 M formaldehyde gel and then transferred to a nylon membrane. The membrane was probed with a nick-translated piece of DNA containing the majority of the protein-coding region. Autoradiography was for 72 hr. Positions of molecular size markers are shown at left.

amino acids. The rat receptor is 4 amino acids shorter than the human receptor in this region. Further understanding of these domains awaits specific site-directed mutation and analysis of function.

Tissue Distribution of Receptor Message. The cDNA for the receptor was used to investigate the tissue distribution of receptor message. Polyadenylylated RNA from intestine, kidney, heart, liver, and spleen was electrophoresed in a 1% agarose/2.2 M formaldehyde gel, transferred to a nylon membrane, and then probed with radiolabeled receptor cDNA. A single mRNA of 4.4 kb was identified in intestine and kidney and was present at a much lower concentration in heart (Fig. 3). No receptor mRNA was detected in liver or spleen. These results are consistent with the known distribution of the receptor protein (3, 4).

Regulated Expression of Receptor Message During Development. The 1,25-dihydroxyvitamin D_3 receptor is uniquely regulated during intestinal development. Little or no receptor is detectable in 14-day rat intestine. A marked increase in receptor can be detected by day 21 (19, 20). Northern analysis of 14-day and 21-day mRNA was done by using a nick-translated cDNA probe for the receptor message. This analysis showed no detectable receptor mRNA at day 14, whereas at day 21, receptor message was clearly detected (Fig. 4). As a control, the filter was probed with a nick-translated cDNA for lactate dehydrogenase. This control shows that equal amounts of polyadenylylated RNA were loaded in each of the lanes.

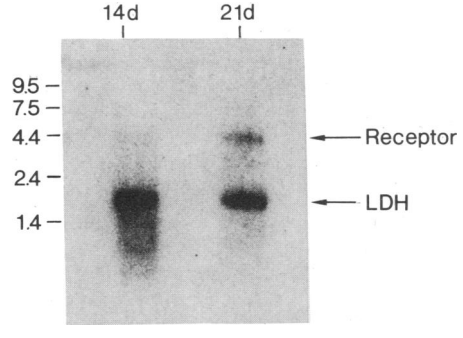

FIG. 4. Northern analysis of polyadenylylated RNA isolated from neonatal rat intestine. Polyadenylylated RNA from 14- and 21-day rat intestine was electrophoresed and transferred to nylon as in Fig. 3. The membrane was probed with a nick-translated fragment of the receptor cDNA and with a nick-translated cDNA for lactate dehydrogenase (LDH). Autoradiography was for 72 hr.

DISCUSSION

1,25-Dihydroxyvitamin D_3 is thought to act through its receptor to modulate specific gene expression by interacting directly with DNA. This receptor is found at very low concentrations in target tissues and has proven very difficult to study. To further understand how the receptor mediates the actions of 1,25-dihydroxyvitamin D_3, we isolated a cDNA coding for the rat receptor. From this cDNA, the amino acid sequence for the protein was deduced. The rat receptor is 423 amino acids long with a large steroid-binding domain at the carboxyl terminus and a conserved DNA-binding domain near the amino terminus. A comparison of the rat and human sequences shows that there are regions of extensive homology between the two receptors.

The cDNA was also used to study the regulation of receptor mRNA expression. A single mRNA of 4.4-kb was found in rat intestine and kidney and, at a much lower concentration, in heart. No receptor mRNA was detected in liver or spleen. These results are consistent with the known distribution of receptor protein.

The receptor is uniquely regulated during rat intestinal development. Little or no receptor is detectable in intestinal tissue of 14-day-old rat pups. A marked increase in receptor is detected by day 21. The appearance of the receptor during neonatal development has been characterized as an increase in steroid-binding activity (19) resulting from an increased synthesis of receptor protein (20). We have further characterized this event by demonstrating that an increase in the amount of the receptor mRNA occurs during this time. These results suggest that a change in transcription of the gene encoding the receptor occurs between days 14 and 21 of rat neonatal life.

This work was supported by Program Project Grant DK-14881 from the National Institutes of Health and by the Steenbock Research Fund of the Wisconsin Alumni Research Foundation.

1. DeLuca, H. F. & Schnoes, H. K. (1983) *Annu. Rev. Biochem.* **52,** 411–439.
2. DeLuca, H. F. (1988) *FASEB J.* **2,** 224–236.
3. Link, R. & DeLuca, H. F. (1985) in *The Receptors*, ed. Conn, P. M. (Academic, New York), Vol. 2, pp. 1–35.
4. Simpson, R. U. & DeLuca, H. F. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 16–20.
5. Brooks, M. H., Bell, N. H., Love, L., Stern, P. H., Orfei, E., Queener, S. F., Hamstra, A. J. & DeLuca, H. F. (1978) *N. Engl. J. Med.* **298,** 996–999.
6. Rosen, J. F., Fleischman, A. R., Finberg, L., Hamstra, A. & DeLuca, H. F. (1979) *J. Pediatr.* **94,** 729–735.
7. Eil, C., Liberman, U. A., Rosen, J. F. & Marx, S. J. (1981) *N. Engl. J. Med.* **304,** 1588–1591.
8. Dame, M. C., Pierce, E. A., Prahl, J. M., Hayes, C. E. & DeLuca, H. F. (1986) *Biochemistry* **25,** 4523–4534.
9. Dame, M. C., Pierce, E. A. & DeLuca, H. F. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7825–7829.
10. Pierce, E. A., Dame, M. C. & DeLuca, H. F. (1987) *J. Biol. Chem.* **262,** 17092–17099.
11. Burmester, J. K., Maeda, N. & DeLuca, H. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1005–1009.
12. McDonnell, D. P., Mangelsdorf, D. J., Pike, J. W., Haussler, M. R. & O'Malley, B. W. (1987) *Science* **235,** 1214–1217.

13. Baker, A. R., McDonnell, D. P., Hughes, M., Crisp, T. M., Mangelsdorf, D. J., Haussler, M. R., Pike, J. W., Shine, J. & O'Malley, B. W. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3294–3298.
14. Kessler, M. A., Lamm, L., Jarnagin, K. & DeLuca, H. F. (1986) *Arch. Biochem. Biophys.* **215,** 403–412.
15. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25,** 263–269.
16. Darwish, H. M., Krisinger, J., Strom, M. & DeLuca, H. F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6108–6111.
17. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
18. Brown, T. A., Prahl, J. M. & DeLuca, H. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2454–2458.
19. Halloran, B. P. & DeLuca, H. F. (1981) *J. Biol. Chem.* **256,** 7338–7342.
20. Pierce, E. A. & DeLuca, H. F. (1988) *Arch. Biochem. Biophys.* **261,** 241–249.