# Activity of human 11-*cis*-retinol dehydrogenase (Rdh5) with steroids and retinoids and expression of its mRNA in extra-ocular human tissue

Jin WANG*, Xiyun CHAI*, Ulf ERIKSSON† and Joseph L. NAPOLI*[1]

*Department of Biochemistry, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY 14214, U.S.A., and †Ludwig Institute for Cancer Research, Stockholm Branch, Box 240, S-171 77 Stockholm, Sweden

This report describes the activity of recombinant human Rdh5 (11-*cis*-retinol dehydrogenase) with steroids and retinoids and expression of the *Rdh5* mRNA in extra-ocular human tissue. The data show that Rdh5 catalyses 9-*cis*-retinol metabolism equally efficiently as 11-*cis*-retinol metabolism and recognizes 5$\alpha$-androstan-3$\alpha$,17$\beta$-diol and androsterone as substrates (3$\alpha$-hydroxysteroid dehydrogenase activity), but not testosterone, dihydrotestosterone, oestradiol and corticosterone (lack of 17$\beta$-hydroxysteroid and 11$\beta$-hydroxysteroid dehydrogenase activities). *Rdh5* mRNA expression was widespread in extra-ocular tissues with human liver (100 % relative expression in extra-ocular tissues only) and mammary gland (97 % relative to liver) showing the most intense signals. Other noteworthy relatively intense expression sites included colon (45 %), thymus (43 %), small intestine (39 %), kidney (37 %), bladder (29 %), pancreas and spleen (28 % each), heart (26 %), uterus and ovary (25 % each), testis (22 %) and spinal cord (24 %). Human fetal tissues also expressed *Rdh5* with fetal liver showing the most intense expression among the fetal tissues (20 %). Considered along with the identical nucleotide sequences in the untranslated regions of human *Rdh5* and human 9-*cis*-retinol dehydrogenase cDNAs and the nearly identical nucleotide sequences overall (99 % identity), the current results suggest that the two cDNAs represent a single gene product.

Key words: 5$\alpha$-androstan-3$\alpha$,17$\beta$-diol, androsterone, 9-*cis*-retinol, 11-*cis*-retinol, short-chain dehydrogenase.

## INTRODUCTION

all-*trans*-Retinol (vitamin A) supports diverse physiological functions in vertebrates, including acting as a cofactor in vision and regulating the differentiation programme of numerous cell types and the functions of many systems [1–4]. These actions are not mediated by all-*trans*-retinol itself; rather, several different metabolites of the vitamin serve either as the cofactor in vision (e.g. 11-*cis*-retinal) or as hormones that control differentiation and other biological processes (e.g. all-*trans*- and 9-*cis*-retinoic acid) [5,6]. Biosynthesis of 11-*cis*-retinal relies on 11-*cis*-retinol, generated by concerted hydrolysis–isomerization of all-*trans*-retinyl palmitate [7]. Biosynthesis of all-*trans*-retinoic acid from all-*trans*-retinol also proceeds through a retinal intermediate, all-*trans*-retinal [8]. Paths of 9-*cis*-retinoic acid biosynthesis are less well understood, but recent work from several laboratories suggests a path that involves conversion of 9-*cis*-retinol into 9-*cis*-retinal [9–11].

Within the past 3 years several members of the short-chain dehydrogenase/reductase (SDR) superfamily have been identified as retinol dehydrogenases and their cDNAs have been cloned [9–18]. SDRs are oxido-reductases that range in mass from 25 to 35 kDa, do not require Zn for catalysis, and have their cofactor-binding residues, GXXXGXG, in their N-termini and their catalytic residues, YXXXK, downstream of their cofactor-binding sites [19]. In these aspects SDRs, which are important to steroid and prostaglandin metabolism, differ from perhaps the better-known medium-chain or classical alcohol dehydrogenases, i.e. ADHs, which metabolize ethanol and other xenobiotics.

A cDNA encoding an SDR with 11-*cis*-retinol dehydrogenase (Rdh5) activity has been cloned from bovine and human retinal pigment epithelium (RPE) [14,17]. Because of *Rdh5*'s major site of mRNA expression in RPE and Rdh5's catalysis of 11-*cis*-retinal biosynthesis, it most likely contributes to the production of the retinoid ligand that binds with opsin to form rhodopsin. More recently, a cDNA has been cloned encoding an SDR that catalyses the conversion of 9-*cis*-retinol into 9-*cis*-retinal and has the mammary gland as a major site of mRNA expression [18]. This SDR has been referred to as 9-*cis*-retinol dehydrogenase (9cRDH) and has been proposed as being different from Rdh5. Despite the apparently different substrate preferences and mRNA expression loci, the two cDNAs differ by only 15 nucleotides, although the *9cRDH* cDNA includes somewhat more extensive 5′- and 3′-untranslated regions. The two deduced amino acid sequences differ by only eight residues, which have not been associated with cofactor binding, substrate binding or catalysis [19].

This report describes the enzymic characteristics of Rdh5 expressed from its cDNA with respect to both steroids and all-*trans*-retinol and three of its isomers, and the mRNA expression pattern of *Rdh5* in extra-ocular human tissue. The data show that Rdh5 catalyses 9-*cis*-retinol dehydrogenation as well as 11-*cis*-retinol dehydrogenation, and has mRNA expressed in the

Abbreviations used: 3$\alpha$-adiol, 5$\alpha$-androstan-3$\alpha$,17$\beta$-diol; 9cRDH, 9-*cis*-retinol dehydrogenase; RPE, retinal pigment epithelium; SDR, short-chain dehydrogenase/reductase; CHO, Chinese hamster ovary.

[1] To whom correspondence should be addressed (e-mail jlnapoli@acsu.buffalo.edu).

The HUGO/GDB nomenclature committee has recommended that the 11-*cis*-retinol dehydrogenase gene be designated as *Rdh5*. Accordingly, the enzyme 11-*cis*-retinol dehydrogenase will be referred to as Rdh5 in this report.

**Table 1 mRNA expression of *Rdh5* in human tissues**

Indices refer to co-ordinates on the commercial multi-dot-blot shown in Figure 1.

| Index | Tissue | Expression (%) |
|---|---|---|
| A1 | Whole brain | 8 |
| A2 | Amygdala | 5 |
| A3 | Caudate nucleus | 9 |
| A4 | Cerebellum | 6 |
| A5 | Cerebral cortex | 5 |
| A6 | Frontal lobe | 3 |
| A7 | Hippocampus | 7 |
| A8 | Medulla oblongata | 7 |
| B1 | Occipital lobe | 8 |
| B2 | Putamen | 9 |
| B3 | Substantia nigra | 11 |
| B4 | Temporal lobe | 6 |
| B5 | Thalamus | 7 |
| B6 | Sub-thalamic nucleus | 9 |
| B7 | Spinal cord | 24 |
| B8 | No RNA | 0 |
| C1 | Heart | 26 |
| C2 | Aorta | 26 |
| C3 | Skeletal muscle | 5 |
| C4 | Colon | 45 |
| C5 | Bladder | 29 |
| C6 | Uterus | 25 |
| C7 | Prostate | 21 |
| C8 | Stomach | 21 |
| D1 | Testis | 22 |
| D2 | Ovary | 25 |
| D3 | Pancreas | 28 |
| D4 | Pituitary | 20 |
| D5 | Adrenal | 17 |
| D6 | Thyroid | 9 |
| D7 | Salivary | 10 |
| D8 | Mammary | 97 |
| E1 | Kidney | 37 |
| E2 | Liver | 100 |
| E3 | Small intestine | 39 |
| E4 | Spleen | 28 |
| E5 | Thymus | 43 |
| E6 | Peripheral leucocyte | 9 |
| E7 | Lymph node | 21 |
| E8 | Bone marrow | 11 |
| F1 | Appendix | 24 |
| F2 | Lung | 10 |
| F3 | Trachea | 17 |
| F4 | Placenta | 9 |
| F5–F8 | No RNA | 0 |
| G1 | Fetal brain | 5 |
| G2 | Fetal heart | 6 |
| G3 | Fetal kidney | 17 |
| G4 | Fetal liver | 20 |
| G5 | Fetal spleen | 5 |
| G6 | Fetal thymus | 7 |
| G7 | Fetal lung | 14 |
| G8 | No RNA | 0 |
| H1 | Yeast total RNA | 0 |
| H2 | Yeast tRNA | 0 |
| H3 | *Escherichia coli* rRNA | 0 |
| H4 | *Escherichia coli* DNA | 24 |
| H5 | Poly r($A^+$) | 0 |
| H6 | Human $C_0t$ 1 DNA | 0 |
| H7 | Human DNA (1 ×) | 6 |
| H8 | Human DNA (5 ×) | 16 |

same tissues that express *9cRDH*. These results expand insight into Rdh5 and, along with the nucleotide and amino acid sequence data, are consistent with co-identity of the two dehydrogenases.

## EXPERIMENTAL

### *Rdh5* cDNA

Human kidney RNA (1.5 $\mu$g; Clontech, Palo Alto, CA, U.S.A.) was allowed to react with 0.5 $\mu$l of random primer hexamer, 0.5 $\mu$l of rRNase inhibitor and 12.5 units of avian myeloblastosis virus (AMV) reverse transcriptase (Promega, Madison, WI, U.S.A.) in a total volume of 20 $\mu$l at room temperature for 10 min, followed by 42 °C for 30 min. An aliquot (3 $\mu$l) was added to a PCR mixture (50 $\mu$l) consisting of (final concentrations): 1 $\mu$M of each primer (5′-GCGGATCCTCACTTGGGCTCCAGCTA-3′ and 5′-GCGAATTCTGCTGGAAGGCTGGA-3′), 2.0 mM $MgCl_2$, 0.2 mM each dNTP and 2.5 units of *Taq* DNA polymerase (Promega) in 50 $\mu$l of 10 mM Tris/HCl, pH 9.0/50mM KCl/0.1 % Triton X-100. PCR was done for 35 cycles (45 s/94 °C; 45 s/50 °C; 1.5 min/72 °C), with a final cycle for 10 min/72 °C. An aliquot (5 $\mu$l) of this reaction mixture was added to a second PCR containing the same reagents, except that 1 $\mu$M each of 5′-GCGGATCCATGTGGCTGCCTCTT-3′ and 5′-GCGAATTCTCAGTAGACTGCTTGGG-3′ were used as primers. The second PCR was done for 35 cycles of 45 s/94 °C, 45 s/55 °C, 1.5 min/72 °C and 10 min/72 °C after the final cycle. The major PCR product (970 bp) was digested with *Bam*HI and *Eco*RI and ligated into pBluescript II $SK^{+/-}$ to produce pBSK-/hRDH5. Six clones were isolated and 230 bp sequenced starting from the ATG codon. One contained no recognizable sequence; the other five were identical to *Rdh5*. One of these was then sequenced in both directions by automated DNA sequencing.

### RNA blotting

A sequence-specific 255 bp probe (nucleotides 649–904 in the Simon et al. [17] *Rdh5* cDNA) was amplified from pBSK/hRDH5 by PCR with the primers: 5′-GCGGATCCGGATGTAGCTCATT-3′ (sense) and 5′-GCGAATTCGTGTCGAGCAGTCA-3′ (antisense; the added restriction-enzyme sites are shown underlined) and inserted into pBluescript II $SK^{+/-}$. The probe was labelled with $^{32}P$ by random priming. mRNA was measured on a human Master Blot (Clontech) according to the manufacturer's instructions. In brief, prehybridization was done in 10 ml of ExpressHyb solution at 37 °C for 30 min. Hybridization was done in the same solution at 37 °C for 1 h. The blot was rinsed and washed in 2 × SSC (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate)/0.05 % SDS for 30 min at room temperature followed by 0.1 × SSC/0.1 % SDS for 40 min at 50 °C. *Rdh5* mRNA signals were normalized to the glyceraldehyde-3-phosphate dehydrogenase mRNA signal, i.e. each value in Table 1 represents ([*Rdh5* signal/glyceraldehyde 3-phosphate signal] × 100).

Also, both a human multiple tissue Northern blot and human mammary gland mRNA (2 $\mu$g) were purchased from Clontech. The latter was fractionated on a 1.2 % formamide/agarose gel. Samples were transferred to a nylon membrane and fixed at 80 °C for 2 h. The samples were hybridized under the conditions and with the probe described above.

### Expression of *Rdh5*

The coding region was digested from pBSK/hRDH5 with *Bam*HI and *Eco*RI and ligated into pcDNA3 to produce pcDNA3/hRDH5. CHO (Chinese hamster ovary)-K1 cells were transfected using LipofectAMINE with pcDNA3/hRDH5 or with pcDNA3 (mock) as described [9]. Cell pellets were suspended in

10 mM Hepes/10 % sucrose, pH 7.5, and sonicated. The lysate was centrifuged at 830 ***g*** for 20 min at 4 °C. The supernatant was used for enzyme assays. Protein concentrations were determined by the method of Bradford [20].

### Enzyme assays

Incubations and analyses of products have been described previously in detail [9]. Briefly, retinoid and steroid dehydrogenase assays were done at 37 °C in 0.25 ml of 50 mM Hepes/150 mM KCl/1 mM EDTA/2 mM $NAD^+$, pH 8, with the 830 ***g*** supernatant of mock- or pcDNA3/hRDH5-transfected CHO cells. Generally, 1 or 2 $\mu$g of protein was used, except for testosterone, dihydrotestosterone, oestradiol and corticosterone, where 100 $\mu$g of protein was used. Reactions were conducted between 5 and 7.5 min and with 2–4 replicates per value. Retinoids were analysed by HPLC, with a detection limit of $\sim$ 1 pmol. 11-*cis*-Retinal isomerizes into more stable isomers during incubation and extraction; therefore, the sums of the retinal isomers recovered from 11-*cis*-retinol incubations were used to determine the rate of 11-*cis*-retinal synthesis. Steroid dehydrogenase assays were done with $^3$H-labelled steroids (40–101 Ci/mmol, 20000 d.p.m./reaction). Steroids were separated by TLC and detected by autoradiography. The radioactive products were excised and counted with a liquid scintillation counter. Kinetic data were obtained under initial velocity conditions and were fitted with the non-linear regression analysis program *Enzfitter* using simple weighing [21].

## RESULTS AND DISCUSSION

We used reverse transcriptase PCR with oligonucleotide primers identical to both human RPE *Rdh5* and human *9cRDH* to clone a cDNA from human kidney RNA. Five of six clones identified with recognizable sequences were identical with *Rdh5* in their first 230 coding-region bp. This area contains 9 of the 15 nucleotide differences between *Rdh5* and *9cRDH*. The nucleotide sequence in the coding region and the deduced amino acid sequence of the clone totally sequenced were identical with those of the *Rdh5* cDNA reported by Simon et al. [17].

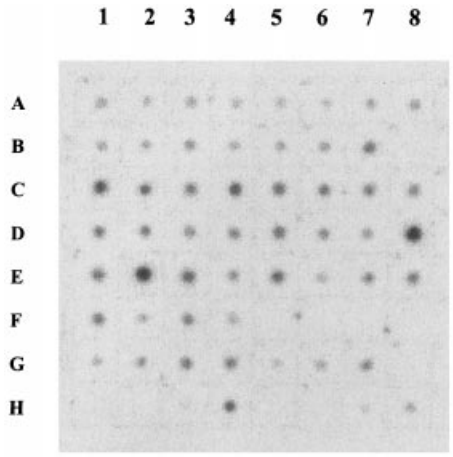


**Figure 1 mRNA expression of *Rdh5* in human tissues**

The Figure shows the results of the spot-blot obtained with the *Rdh5* probe and a commercially available multi-dot-blot of human tissue mRNA. The identities of individual dot-blots are given in Table 1.

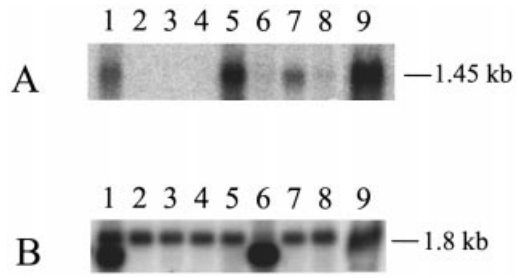


**Figure 2 Northern-blot analysis of *Rdh5* mRNA**

A human multiple tissue Northern blot (Clontech) was used that included mRNA from: 1, heart; 2, brain, 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas. Lanes 9 show mRNA from human mammary gland analysed independently. mRNA (2 $\mu$g) was loaded on to each lane: (**A**) *Rdh5* probe; (**B**) human $\beta$-actin probe. The larger, faster-migrating bands in lanes 1 and 6 of (**B**) represent $\alpha$-actin mRNA.

A commercial blot containing mRNA from diverse human tissues was used to determine the extra-ocular expression of *Rdh5* (Figure 1, Table 1). Outside of the eye *Rdh5* expression was widespread, with liver (100 % relative expression, grid reference E2 in Figure 1) and mammary gland (97 % relative to liver, D8) showing the most intense signals. Other noteworthy relatively intense expression sites included heart (C1), colon (C4), spinal cord (B7), kidney (E1), small intestine (E3), thymus (E5) and several endocrine glands (row D). Human fetal tissues (row G) also expressed *Rdh5*. Previously, bovine *Rdh5* mRNA was detected by Northern blot only in bovine RPE and not in several extra-ocular tissues, because of the overwhelming intensity of the signal in RPE relative to extra-ocular tissue [14,16]. Comparison of the mRNA signals observed under the conditions used in this work to the signal previously detected in RPE [14] indicates that the RPE signal is $\sim$ 100–500 fold greater in intensity than the liver signal. In comparison, strong signals for *9cRDH* have been detected in human mammary gland, testis and kidney, with lung, liver, heart, adrenal and skeletal muscle showing much weaker signals [18]. mRNA expression of the human *9cRDH* was not determined in RPE.

The current data show widespread expression of human *Rdh5* outside of the RPE in all of the tissues in which *9cRDH* expression was detected and in many additional tissues not assayed previously. With the exception of mammary gland, the relative intensities of *Rdh5* mRNA reported here and those of *9cRDH* are not similar. The largest discrepancy occurs with liver. Expression of human liver *9cRDH* was reported to be low relative to mammary gland [18], but in this work expression of *Rdh5* in liver was as intense as expression in mammary gland. These intensity differences may have been influenced by the genetic diversity of humans, mRNA degradation of autopsy-sample RNA and possible environmental factors (especially with liver mRNA).

To verify the size of the *Rdh5* mRNA Northern blots were done (Figure 2). Single signals were observed at 1.45 kb, consistent within experimental limitations with the 1.2 kb of *Rdh5* mRNA reported for bovine RPE [16], the 1.4 kb mRNA reported for bovine RPE *Rdh5* [14], and the 1.5 kb mRNA reported for human *9cRDH* [18].

The previous reports of bovine Rdh5 and human 9cRDH used single concentrations of substrates in the absence of $K_{0.5}$ values to determine activity and did not include assays with both 9-*cis*-

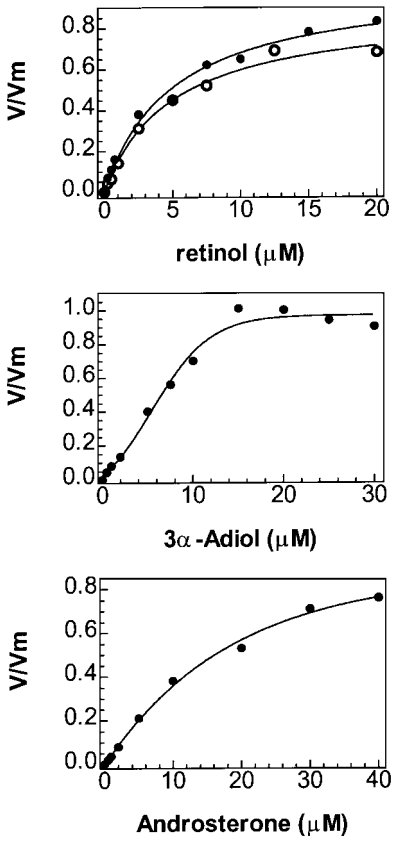


**Figure 3 Substrate concentration versus rates of product formation catalysed by recombinant Rdh5**

(Top panel) Activity with 11-*cis*-retinol (○) and 9-*cis*-retinol (●). (Middle panel) Activity with 3α-adiol; (bottom panel) activity with androsterone. Each point represents the average of duplicates. Each curve represents one of either two (retinoids) or three (steroids) independent determinations.

**Table 2 Kinetic values of Rdh5 with retinoid and steroid substrates**

The terms *v* and *V* represent velocity at the substrate concentration of 15 μM (determined experimentally) and the limiting velocity (calculated from *v* and $K_{0.5}$), respectively, in units of nmol/min per mg of protein. Data represent the means ± S.E.M. of three replicates for *v* and three independent determinations for $K_{0.5}$, except where noted.

| Substrate | *v* | $K_{0.5}$ (μM) | *V* | $V/K_{0.5}$ |
|---|---|---|---|---|
| 9-*cis*-Retinol | 15.1 ± 0.2 | 6.3† | 21 | 3.4 |
| 11-*cis*-Retinol | 15.7* | 6.6‡ | 23 | 3.5 |
| 13-*cis*-Retinol | 6.9 ± 0.7 | – | – | – |
| all-*trans*-Retinol | Not significant | – | – | – |
| 3α-Adiol§ | 6.9 ± 0.8 | 6.4 ± 1.5 | 8.7 | 1.4 |
| Androsterone§ | 12.8 ± 0.7 | 18 ± 2.4 | 29 | 1.6 |

* Average of duplicates: 16.9 and 14.5 nmol/min/mg.
† Average of duplicates: 7.7 ± 2.3 and 4.8 ± 0.2 (± S.E.M.).
‡ Average of duplicates: 8.2 ± 0.01 and 4.9 ± 0.06 (± S. E.M).
§ These two substrates showed co-operative kinetics with Hill coefficients of 1.6 ± 0.2 and 1.2 ± 0.08, respectively (mean ± S.E.M., $n = 3$ independent determinations).

retinol and 11-*cis*-retinol. Only one or the other was assayed, nor were steroid substrates assayed. Here we show that human Rdh5 displays typical Michaelis–Menten kinetics with both 9-*cis*- and 11-*cis*-retinol (Figure 3). Within experimental error human Rdh5 was equally efficient with 9-*cis*- and 11-*cis*-retinol, had less activity with 13-*cis*-retinol, and was about an order of magnitude less efficient with all-*trans*-retinol. These results, reported here in Table 2 with single retinoid concentrations, are similar qualitatively and quantitatively to the results with the 9cRDH, with the exception of 11-*cis*-retinol, which was not assayed with 9cRDH.

Of the steroids assayed with human Rdh5, testosterone, dihydrotestosterone, oestradiol and corticosterone produced no detectable products with 100 μg of protein, in contrast to the relatively rapid rates of 5α-androstan-3α,17β-diol (3α-adiol) and androsterone metabolism with 2 μg of protein. Rdh5 was nearly equally efficient with both 3α-adiol and androsterone, despite the rather high $K_{0.5}$ with the latter. Moreover, unlike the retinoids, the steroids showed co-operative kinetics.

The apparent lack of expression of bovine *Rdh5* outside of the RPE and the ability of 9cRDH to catalyse metabolism of 9-*cis*-retinol contributed considerably to the conclusion that the *9cRDH* cDNA was not the human homologue of the bovine cDNA. But *9cRDH* mRNA was not measured in the RPE and, as mentioned above, 9cRDH was not assayed with 11-*cis*-retinol. Also, the nucleotide and amino acid sequences of bovine *Rdh5* and human *9cRDH* were compared. Bovine *Rdh5* and human *9cRDH* share only 87% and 89% nucleotide and amino acid identities, respectively [14,18]. On the other hand, human *Rdh5* and human *9cRDH* share 99% and 97% nucleotide and amino acid identities, respectively [17]. Of 1128 overlapping nucleotides in the cDNAs of human *Rdh5* and human *9cRDH*, only 15 differ. Significantly, there are no nucleotide sequence differences in the 5′- or 3′-untranslated regions. The *9cRDH* cDNA, however, extends 37 nucleotides longer into the 5′-untranslated region and 65 nucleotides into the 3′-untranslated region.

In summary, this report shows that: (i) Rdh5 catalyses 9-*cis*-retinol metabolism equally efficiently as 11-*cis*-retinol metabolism; (ii) Rdh5 recognizes 3α-adiol and androsterone as substrates; and (iii) many tissues express *Rdh5* mRNA. In addition, a single locus for the *Rdh5* gene has been established on human chromosome 12q13–q14, despite the extensive nucleotide identity between the *Rdh5* and *9cRDH* cDNAs [17]. These data indicate that there is no compelling rationale for distinguishing between Rdh5 and 9cRDH based on substrate recognition, mRNA expression or chromosomal localization. Considered with the identical nucleotide sequences in the untranslated regions of the two human cDNAs and the nearly identical nucleotide sequences in the coding regions, the current results suggest that the two cDNAs represent a single gene product. Because 11-*cis*-retinoids are not known outside of the eye, the substrate specificity and expression locus of Rdh5 suggest that it could serve as both an 11-*cis*-retinol dehydrogenase in the RPE and a 9-*cis*-retinol dehydrogenase and/or an androgen dehydrogenase outside of the RPE. Its function will probably depend on expression loci, substrate availability and humoral influences. The large difference in *Rdh5* mRNA expression in the RPE compared with its extraocular expression makes sense in view of the widely differing quantitative requirements for 11-*cis*-retinal and 9-*cis*-retinal (as a precursor for 9-*cis*-retinoic acid). The eye consumes the majority of vitamin A to support vision; in contrast, quantitative needs for 9-*cis*-retinoic acid are miniscule, owing to its action as a hormone.

This work was supported in part by the United States National Institutes of Health (J. L. N., grant DK47839) and the Swedish Medical Research Council (U. E., grant K97-03P-12070-01A).

## REFERENCES


1 Maden, M. (1993) Acta Biotheor. **41**, 425–445
2 Ross, A. C. and Gardner, E. M. (1994) Adv. Exp. Med. Biol. **352**, 187–200
3 Lohnes, D., Mark, M., Mendelsohn, C., Dolle, P., LeMeur, M., Dierich, A., Gorry, P. and Chambon, P. (1995) J. Steroid Biochem. Mol. Biol. **53**, 475–486
4 Sucov, H. M. and Evans, R. M. (1995) Mol. Neurobiol. **10**, 169–184
5 Napoli, J. L. (1996) Clin. Immunol. Immunopathol. **80**, S52–S62
6 Napoli, J. L. (1997) Semin. Cell Dev. Biol. **8**, 403–415
7 Bernstein, P. S., Law, W. C. and Rando, R. R. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 1849–1853
8 Napoli, J. L. (1996) FASEB J. **10**, 993–1001
9 Chai, X., Zhai, Y. and Napoli, J. L. (1997) J. Biol. Chem. **272**, 33125–33131
10 Romert, A., Tuvendal, P., Simon, A., Dencker, L. and Eriksson, U. (1998) Proc. Natl. Acad. Sci. U.S.A. **95**, 4404–4409
11 Su, J., Chai, X., Kahn, B. and Napoli, J. L. (1998) J. Biol. Chem. **273**, 17910–17916
12 Chai, X., Boerman, M. H. E. M., Zhai, Y. and Napoli, J. L. (1995) J. Biol. Chem. **270**, 3900–3904
13 Chai, X., Zhai, Y., Popescu, G. and Napoli, J. L. (1995) J. Biol. Chem. **270**, 28408–28412
14 Simon, A., Hellman, U., Wernstedt, C. and Eriksson, U. (1995) J. Biol. Chem. **270**, 1107–1112
15 Chai, X., Zhai, Y. and Napoli, J. L. (1996) Gene **169**, 219–222
16 Driessen, C. A. G. G., Janssen, B. P. M., Winkens, H. J., van Vugt, A. H. M., de Leeuw, T. L. M. and Janssen, J. J. M. (1995) Invest. Opthalmol. Vis. Sci. **36**, 1988–1996
17 Simon, A., Lagercrantz, J., Bajalica-Lagercrantz, S. and Eriksson, U. (1996) Genomics **36**, 424–430
18 Mertz, J. R., Shang, E., Piantedosi, R., Wei, S., Wolgemuth, D. J. and Blaner, W. S. (1997) J. Biol. Chem. **272**, 11744–11749
19 Jörnvall, H., Persson, B., Krook, M., Atrian, S., Gonzàlez-Durate, R., Jeffry, J. and Ghosh, D. (1995) Biochemistry **34**, 6003–6013
20 Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
21 Leatherbarrow, R. J. (1987) Enzfitter: A non-linear regression data analysis program for the IBM PC, Elsevier-Biosoft, Cambridge


Received 13 July 1998/29 October 1998; accepted 2 December 1998