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Structures Important in NAD(P)(H) Specificity for Mammalian Retinol and 11-*Cis*-Retinol Dehydrogenases

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Mammalian retinol and 11-*cis* retinol dehydrogenases catalyze the formation of retinaldehyde from retinol and 11-*cis* retinaldehyde from 11-*cis* retinol, respectively. Although their amino acid sequences are 54% identical, these enzymes have different cofactor specificities: rat retinol dehydrogenase uses $NADP^+$, while cow 11-*cis* retinol dehydrogenase uses NAD^+. We used their close sequence similarity and the availability of 3D structures of their homologs to construct a 3D model of the two retinol dehydrogenases to investigate the determinants for cofactor specificity. The structure of rat retinol dehydrogenase shows that lysine-64 is important in stabilizing binding of 2′-phosphate on $NADP^+$ in two ways: lysine's positively charged side chain has a coulombic attraction to the 2′-phosphate and partially compensates for the negative charge of aspartic acid-38. Cow 11-*cis* retinol dehydrogenase has threonine-61 at the position homologous to lysine-64. Threonine-61 does not have a stabilizing coulombic interaction with $NADP^+$, nor can threonine-61 counteract the repulsive interaction between $NADP^+$ and aspartic acid-37 in 11-*cis* retinol dehydrogenase. This suggests that aspartic acid-37 and threonine-61 are important in the specificity of 11-*cis* retinol dehydrogenase for NAD^+.

11-*cis* Retinol dehydrogenase catalyzes the oxidation of 11-*cis* retinol to 11-*cis* retinaldehyde, which is the universal chromophore in visual pigments in higher animals (1,2). Retinol dehydrogenase catalyzes the oxidation of retinol to retinaldehyde, which is further oxidized to retinoic acid, a compound that regulates gene transcription during development and postnatally in a wide range of organs (3-6). The central roles that these two retinol dehydrogenases play in vision and development, respectively, has stimulated interest in understanding their regulation and mechanism of action. An important advance was the recent cloning and sequencing of cow 11-*cis* retinol dehydrogenase (7) and rat retinol dehydrogenase (8). The two retinol dehydrogenase amino acid sequences are about 54% identical (9) and belong to a large group of oxidoreductases - over 50 different enzymes are present in release 32 of the Swiss Protein database (10-14) - that constitute the short chain alcohol dehydrogenase family. These oxidoreductases metabolize a diverse group of secondary alcohols or ketones (10-15), and based on this substrate specificity they have been called *sec*-alcohol dehydrogenases (15). The substrates metabolized by this enzyme family include androgens, estrogens, glucocorticoids, and prostaglandins E_2 and $F_{2\alpha}$ in humans. Moreover, organisms that cause diseases in humans contain dehydrogenase homologs, which are sites for drugs to control these organisms. Two examples are: the protozoan parasite *Leishmania* contains a homolog that is important in resistance to methotrexate (16,17), and *Mycobacterium tuberculosis* contains a homolog that is a site for the action of isoniazid and ethionamide, drugs that control *M. tuberculosis* (18). Thus, information about catalysis in 11-*cis* retinol and retinol dehydrogenases is likely to be useful understanding catalysis in steroid and prostaglandin dehydrogenases and other homologs of medical importance (14-19).

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Despite the 54% amino acid sequence identity between the two retinol dehydrogenases, they have different preferences for cofactors: 11-*cis* retinol dehydrogenase uses NAD^+; retinol dehydrogenase uses $NADP^+$. Their close sequence similarity suggested to us that a comparison of their 3-dimensional structures could elucidate the basis for the cofactor preferences. Although the tertiary structure for either retinol dehydrogenase has not yet been determined, the structures for four homologs, *Streptomyces hydrogenans* 20β-hydroxysteroid dehydrogenase (20,21), rat dihydropteridine reductase (22,23), human 17β-hydroxysteroid dehydrogenase-type 1 (24) and enoyl-acyl carrier protein reductase (25,26) have been determined. The first two structures are in the Brookhaven database and can be used as templates for modeling the retinol dehydrogenases. Important for confidence in the models is that the tertiary structures of 20β-hydroxysteroid dehydrogenase and rat dihydropteridine reductase show good superposition (21,23), although their sequences are less than 20% identical. Moreover, these two tertiary structures superimpose on the tertiary structures of human 17β-hydroxysteroid dehydrogenase-type 1 (24) and enoyl-acyl carrier protein reductase (26) although these enzymes have less than 20% sequence identity with each other. With this in mind, we modeled the tertiary structure of cow 11-*cis* retinol dehydrogenase and rat retinol dehydrogenase and identified residues important in their cofactor specificity. Our analysis also revealed a surprising discovery that phenylalanine-40 in 11-*cis* retinol dehydrogenase and phenylalanine-41 in retinol dehydrogenase have the aromatic face of their side chain close to the nicotinamide ring of the cofactor. This indicates that the amino terminal domain in these dehydrogenases has important stabilizing interactions with the entire cofactor molecule and not just with the adenosine monophosphate (AMP) moiety (27-29).

METHODS

Molecular modeling. The structure *S. hydrogenans* 20β-hydroxysteroid dehydrogenase (20,21) was taken from the Brookhaven database. The 3D structure of this protein is similar to homologs: dihydropteridine reductase (23) and human 17β-hydroxysteroid dehydrogenase-type 1 (24), although the protein sequences have less than 20% identity.

We aligned *S. hydrogenans* 20β-hydroxysteroid dehydrogenase with rat retinol dehydrogenase and cow 11-*cis* retinol dehydrogenase using the GAP program in the Wisconsin GCG package (30). The multiple sequence alignment and the phylogenetic tree were constructed with the method of Feng and Doolittle (31). The 3D structures of rat retinol dehydrogenase and cow 11-*cis* retinol dehydrogenase were obtained with the Homology program (Biosym Inc.). This program substitutes the template's amino acids with the corresponding amino acids from the aligned sequence and then relaxes possible steric overlap by minimization for a specified number (1000 in our analysis) of steps. Insertions in modeled proteins were found only in the loop regions, which indicates that the aligned structure is close to the template. The insertion loops were built using loop generator in the Homology program. Then the structure was minimized for 1000 steps using the steepest descent method with the Discover program (Biosym Inc.). The coordinates of the X-ray structure of $NADP^+$ were determined by Tanaka et al. (32).

RESULTS AND DISCUSSION

Figure 1 shows the alignment of rat retinol dehydrogenase with cow 11-*cis* retinol dehydrogenase. Boxed residues are discussed in analysis of the structures.

The structure of the amino terminal and substrate binding domains of cow 11-*cis* retinol dehydrogenase with NAD^+ and rat retinol dehydrogenase with $NADP^+$ are shown in Figure 2. As with other *sec*-alcohol dehydrogenases, the two retinol dehydrogenases contain a motif at the amino terminus consisting of β-strand-A, α-helix-B, β-strand-B ($\beta\alpha\beta$), which interacts with the adenosine monophosphate (AMP) moiety (27-29). β-strand-A and α-helix-B are joined by a glycine-rich turn with the sequence: Gly_1-Cys-Asp-Ser-Gly_2-Phe-Gly_3 (Figure 1). The region at the end of β-strand-B and at the beginning of α-helix-C is thought to be important in selectivity for NAD^+ versus $NADP^+$ (27-29).

Binding of $NADP^+$ to rat retinol dehydrogenase. The structure shows the adenosine monophosphate moiety of NAD^+ and $NADP^+$ close to the $\beta\alpha\beta$ fold in agreement with that reported for bacterial 20β-hydroxysteroid dehydrogenase (21), rat dihydropteridine reductase (23), human 17β-hydroxysteroid dehydrogenase-type 1 (24) and bacterial and plant enoyl acyl carrier

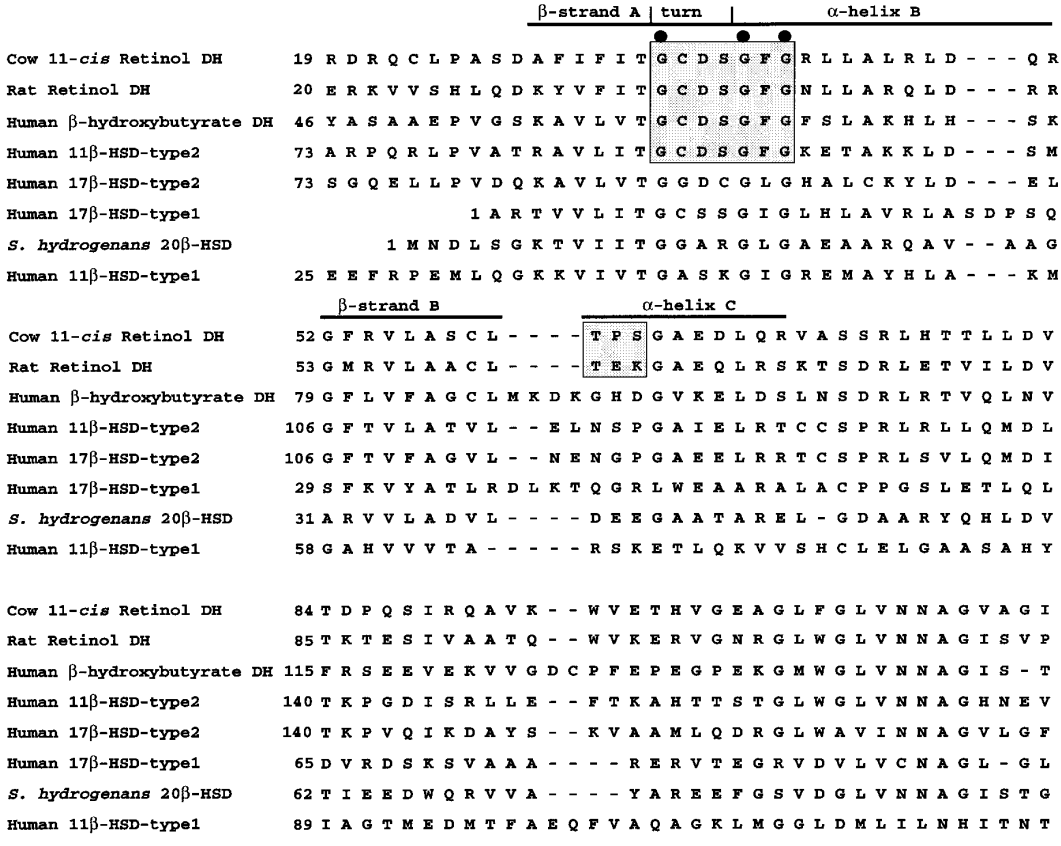

FIG. 1. A multiple alignment of mammalian retinol and 11-*cis* retinol dehydrogenases, 11β- and 17β-hydroxysteroid dehydrogenases, β-hydroxybutyrate dehydrogenase and *Streptomyces hydrogenans* 20β-hydroxysteroid dehydrogenase. The alignment was constructed with the algorithm of Feng and Doolittle (31). Amino acids that are important in cofactor binding and specificity are shown in shaded boxes. Also shown is the glycine rich turn in the $\beta\alpha\beta$ fold and the locations of β-strand-A through α-helix-C.

protein reductases (25,26). Our analysis identifies a segment in rat retinol dehydrogenase beginning with cysteine-60 through lysine-64 as important in specificity for $NADP^+$. The key residue appears to be lysine-64, which is about 6.5 Å from the 2′-phosphate on $NADP^+$. The attractive coulombic interaction between the positively charged side chain of lysine and the negatively charged 2′-phosphate stabilizes $NADP^+$ binding. Also important for the stabilizing effect of lysine-64 is the interaction between the negatively charged side chain of glutamic acid-63 and Oγ of threonine-62, which are about 5 Å apart. This orients glutamic acid's negatively charged side chain away from the positively charged lysine-64 side chain, minimizing any canceling of the coulombic attraction between of lysine and $NADP^+$, as well as minimizing a destabilizing coulombic interaction between glutamic acid and the 2′-phosphate of $NADP^+$.

Examination of Figure 2 reveals that the negatively charged side chain of aspartic acid-38 is about 6 Å from the 2′-phosphate of $NADP^+$. Such a strong coulombic repulsion would destabilize binding of $NADP^+$. Binding of $NADP^+$ to rat retinol dehydrogenase is possible because the positively charged side chain of lysine-64 diminishes the repulsion between 2′-phosphate and the negative charge on aspartic acid-38.

Binding of NAD^+ to cow 11-cis retinol dehydrogenase. In contrast, 11-*cis* retinol dehydroge-

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Cow 11-cis Retinol DH                118 I G P T P W Q T R E D F Q R V L N V N T L G P I G V T L A L L P - L L L
Rat Retinol DH                       119 V G P N E W M R K K D F A S V L D V N L L G V I E V T L N M L P - L V R
Human β-hydroxybutyrate DH           150 F G E V E F T S L E T Y K Q V A E V N L W G T V R M T K S F L P - L I R
Human 11β-HSD-type2                  174 V A D A E L S P V A T F R S C M E V N F F G A L E L T K G L L P - L L R
Human 17β-HSD-type2                  174 P T D G E L L L M T D Y K Q C M A V N F F G T V E V T K T F L P - L L R
Human 17β-HSD-type1                   96 L G P L E A L G E D A V A S V L D V N V V G T V R M L Q A F L P D M K R
S. hydrogenans 20β-HSD                94 M F - L E T E S V E R F R K V V D I N L T G V F I G M K T V I P A M K D
Human 11β-HSD-type1                  125 S L N L F H D D I H H V R K S M E V N F L S Y V V L T V A A L P - M L K

Cow 11-cis Retinol DH                153 Q A R G R V I N I T S V L G R L A A N G - G G Y C V S K F G L E A F S D
Rat Retinol DH                       154 K A R G R V V N I A S T M G R M S L V G - G G Y C I S K Y G V E A F S D
Human β-hydroxybutyrate DH           185 R A K G R V V N I S S M L G R M A N P A R S P Y C I T K F G V E A F S D
Human 11β-HSD-type2                  209 S S R G R I V T V G S P A G D M P Y P C L G A Y G T S K A A V A L L M D
Human 17β-HSD-type2                  209 K S K G R L V N V S S M G G G A P M E R L A S Y G S S K A A V T M F S S
Human 17β-HSD-type1                  132 R G S G R V L V T G S V G G L M G L P F N D V Y C A S K F A L E G L C E
S. hydrogenans 20β-HSD               129 A G G G S I V N I S S A A G L M G L A L T S S Y G A S K W G V R G L S K
Human 11β-HSD-type1                  160 Q S N G S I V V V S S L A G K V A Y P M V A A Y S A S K F A L D G F F S

Cow 11-cis Retinol DH                188 S L R R D V A P F G V R V S I V E P G - - - - F F - - R T P V T N L E T
Rat Retinol DH                       189 S L R R E L T Y F G V K V A I I E P G - - - - G F - - K T N V T N M E R
Human β-hydroxybutyrate DH           221 C L R Y E M Y P L G V K V S V V E P G - - - - N F I A A T S V L Y N P E
Human 11β-HSD-type2                  245 T F S C E L L P W G V K V S I I Q P G - - - - C F - K T E S V R N V G Q
Human 17β-HSD-type2                  245 V M R L E L S K W G I K V A S I Q P G - - - - G F - L T N I A G T S D K
Human 17β-HSD-type1                  168 S L A V L L L P F G V H L S L I E C G P V H T A F M E K V L G S P E E V
S. hydrogenans 20β-HSD               165 L A A V E L G T D R I R V N S V H P G - - - - - - - - - - M T Y T P M T
Human 11β-HSD-type1                  196 S I R K E Y S V S R V N V S I T L C V - - - - - L G L I D T E T A M K A

Cow 11-cis Retinol DH        218 L E D T L Q A C W A R L - - - - - - - - - - - - - - P P A T Q A L Y G E A F L T K
Rat Retinol DH               219 L S D N L K K L W D Q T - - - - - - - - - - - - - - T E E V K E I Y G E K F Q D S
Human β-hydroxybutyrate DH   253 I Q A I A K K M W E E L - - - - - - - - - - - - - - P E V V R K D Y G K K Y F D E
Human 11β-HSD-type2          276 W E K R K Q L L L A N L - - - - - - - - - - - - - - P Q E L L Q A Y G K D Y I E H
Human 17β-HSD-type2          276 W E K L E K D I L D H L - - - - - - - - - - - - - - P A E V Q E D Y G Q D Y I L A
Human 17β-HSD-type1          204 L D R T D I H T F H R F Y Q Y L A H S K Q V F R E A A Q D P E E V A E V F L T A
S. hydrogenans 20β-HSD       191 A E T G I R Q G E G N Y - - - - - - - - - - - - - - P N T P M G R V G N E - P G E
Human 11β-HSD-type1          227 V S G I V H M Q A A P K - - - - - - - - - - - - - - E E C A L E I I K G G A L R Q

Cow 11-cis Retinol DH        245 Y L R V Q Q R I M N M I C D P D L A K V S R C L E H A L T A R H P R T R Y S P G W
Rat Retinol DH               246 Y M K A M E S L V N - T C S G D L S L V T D C M E H A L T S C H P R T R Y S P G W
Human β-hydroxybutyrate DH   280 K I A K M E T Y C S - S G S T D T S P V I D A V T H A L T A T T P Y T R Y H P M D
Human 11β-HSD-type2          303 L H G Q F L H S L R - L A M S D L T P V V D A I T D A L L A A R P R R R Y Y P G Q
Human 17β-HSD-type2          303 - Q R N F L L L I N S L A S K D F S P V L R D I Q H A I L A K S P F A Y Y T P G K
Human 17β-HSD-type1          244 L R A P K P T L R Y F T T E R F L P L L R M R L D D P S G S N Y V T A M H R E V F
S. hydrogenans 20β-HSD       217 I A G A V V K L L S - D T S S Y V T G A E L A V D G G W T T G P - T V K Y V M G Q
Human 11β-HSD-type1          254 E E V Y Y D S S L W - T T L L I R N P C R K I L E F L Y S T S Y N M D R F I N K
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FIG. 1—*Continued*

nase has threonine-61 at the position of lysine-64 in retinol dehydrogenase (Figure 2). The lack of a positively charged group in this region in 11-*cis* retinol dehydrogenase results in a coulombic repulsion between $NADP^+$ and aspartic acid-37. The energetics are different when NAD^+ occupies this site in 11-*cis* retinol dehydrogenase because the hydroxyls on the AMP ribose group have stabilizing interactions with aspartic acid-37, serine-38, cysteine-59 and threonine-61. Threonine-61 also has a stabilizing interaction with aspartic acid-37.

Interplay of positive and negative charges in cofactor specificity. Based on the above analy-

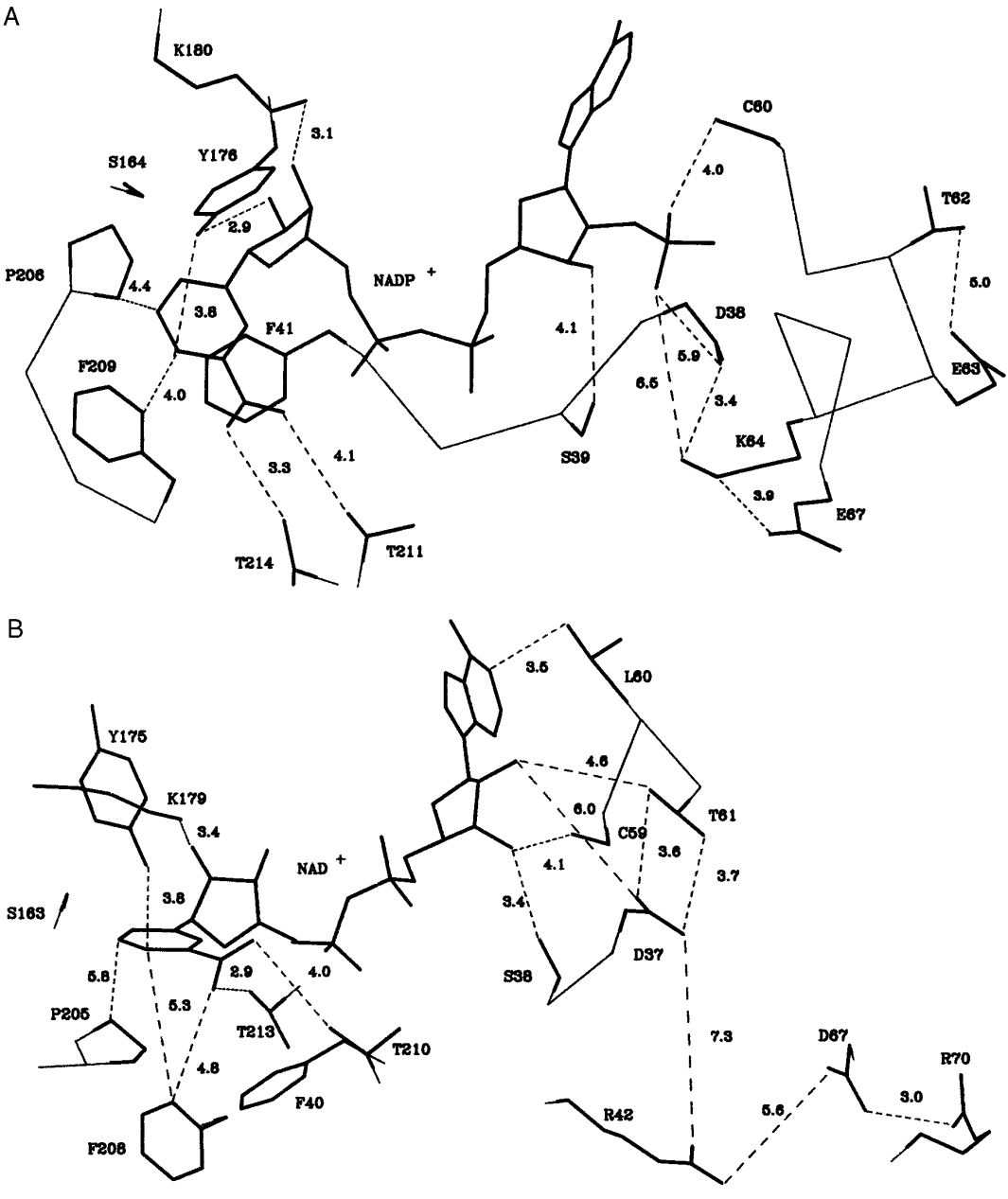

FIG. 2. 3-dimensional model of the nucleotide cofactor binding sites in rat retinol dehydrogenase and cow 11-*cis* retinol dehydrogenase. (A) Amino acids in rat retinol dehydrogenase that interact with $NADP^+$. The region around the 2′-phosphate has lysine-64 coordinated with several negatively charged groups and serine-39. Lysine-64 is about 3.4 Å from aspartic acid-38, 6.5 Å from 2′-phosphate, 3.9 Å from glutamic acid-67, and 3.2 Å from serine 39. Aspartic acid-38 is 5.9 Å from the 2′-phosphate. Also, threonine-62 is about 5 Å from glutamic acid-63. Cysteine-60 is about 4 Å from the 2′-phosphate. Phenylalanine-41, proline-206, and phenylalanine-209 are about 7.2 Å, 4.4 Å, and 4 Å, respectively, from the nicotinamide ring. Threonines-211 and 214 are about 4.1 Å and 3.3 Å, respectively, from the carboxamide moiety. These interactions may stabilize the N-glycosidic bond in $NADP^+$ during catalysis. (B) Amino acids in cow 11-*cis* retinol dehydrogenase that interact with NAD^+. Aspartic acid-37 has a stabilizing interaction with the hydroxyls on the AMP ribose, threonine-61 and serine-38. Serine-38 has a stabilizing interaction with the ribose's 3′-hydroxyl. Insertion of $NADP^+$ into this site reveals strong repulsive forces between the 2′-phosphate and aspartic acid-37. Cow 11-*cis* retinol dehydrogenase lacks a positively charged residue that can compensate for

sis, we propose that there are two key factors in the preference for NAD^+ by 11-*cis* retinol dehydrogenase. First, binding of $NADP^+$ is energetically unfavorable due to the destabilizing negative coulombic interaction between its 2′-phosphate group and aspartic acid-37. Second, 11-*cis* retinol dehydrogenase lacks a positively charged residue that can stabilize binding of $NADP^+$ and compensate for the repulsion of negatively charged aspartic acid-37. For rat retinol dehydrogenase, binding of $NADP^+$ depends on the presence of lysine-64. Its positively charged side chain has a coulombic attraction to $NADP^+$ and diminishes the effect on the cofactor of the negatively charged aspartic acid-38.

Rat retinol dehydrogenase's lysine-64 is at the beginning of α-helix-C, which was identified by Wierenga et al. (27,28) in binding to the adenosine ribose in a variety of oxidoreductases. A negative charge in this region would favor binding of NAD^+ and disfavor binding of $NADP^+$. This is consistent with studies near the c-terminus of β-strand-B and the beginning of α-helix-C in *Drosophila* alcohol dehydrogenase (33,34) in which mutations that either reduce the negative charge or add a positive charge to this region improve binding of $NADP^+$. It also is consistent with mutagenesis studies in two other unrelated enzymes: *E. coli* glutathione reductase (35) and yeast alcohol dehydrogenase (36). Thus, the role of lysine-64 in stabilizing $NADP^+$ binding to rat retinol dehydrogenase is not surprising.

Importance of electrostatic properties in the turn between β-strand-A and α-helix-B. However, a role for aspartic acid-37 in cofactor specificity is unexpected as this residue is in the glycine-rich motif that comprises the turn between β-strand-A and α-helix-B. The function of this segment has usually be assumed to be mainly structural: to permit close association of the AMP moiety so that residues elsewhere, such as the c-terminus of β-strand-B can stabilize cofactor binding. Indeed, mutations that replace glycine residues with alanine in this part of *Drosophila* alcohol dehydrogenase reduce its affinity for NAD^+, presumably by sterically hindering close binding of AMP (37,38).

Electrostatic interactions in the turn between β-strand-A and α-helix-B in homologs of retinol dehydrogenase. Close homologs of retinol dehydrogenase: 11β- and 17β-hydroxysteroid dehydrogenases-type 2 and β-hydroxybutyrate dehydrogenase also contain an homologous aspartic acid (Figure 1) indicating conservation of this residue during their divergence from a common ancestor. All of these homologs use NAD^+ as a cofactor.

While we were completing this manuscript, Tanaka et al. (32) reported the 3-dimensional structure of mouse lung carbonyl reductase, which uses NADPH as a cofactor. In carbonyl reductase, the glycine rich motif is Gly_1-Ala-Gly-Lys-Gly_2-Ile-Gly_3. Tanaka et al. (32) found that lysine-17, the residue adjacent to Gly_2 in the motif, stabilizes binding of the 2′-phosphate of NADPH. They proposed that the absence of this lysine is important in preference for NAD^+. Their and our model of the role of the residues between Gly_1 and Gly_2 in cofactor specificity are in general agreement. However, comparison of retinol and 11-*cis* retinol dehydrogenases indicates that a negatively charged amino acid between Gly_1 and Gly_2 also influences cofactor specificity. Moreover, examination of homologs (Figure 1, (10-14)) reveals that glutamic acid, arginine or lysine or amino acids with uncharged side chains can be at the position homologous to retinol dehydrogenase's aspartic acid-37. Thus, the important general insight is that positive, negative and uncharged residues between Gly_1 and Gly_2 are important in specificity for NAD^+ or $NADP^+$. The absence of charged residues between Gly_1 and Gly_2 may allow some enzymes to use either cofactor for catalysis, depending on the charge at the c-terminus of β-strand B.

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this repulsive interaction. Arginine-42 has a coulombic interaction with aspartic acid 67, which is also bonded to arginine-70. The region around the nicotinamide group contains phenylalanine-40, proline-205, and phenylalanine-208 are 7.2 Å, 5.8 Å, and 5.3 Å, respectively, from the nicotinamide ring. Threonines-210 and 213 are 4 Å and 2.9 Å, respectively, from the carboxamide moiety. These interactions may stabilize the N-glycosidic bond in NAD^+ during catalysis.

Phenylalanine between Gly_2 and Gly_3 interacts with the nicotinamide ring. We uncovered a surprising aromatic-aromatic interaction between the nicotinamide ring and the aromatic side chain of phenylalanine-41 in retinol dehydrogenase and phenylalanine-40 in 11-*cis* retinol dehydrogenase. These phenylalanines are between Gly_2 and Gly_3 in β-strand-A and α-helix-B, which is usually thought of interacting mainly with the AMP moiety (29). Gly_2 and Gly_3 provide flexibility to orient Phe-40 and Phe-41 to interact with the nicotinamide moiety.

Based on various models (21,23-26,32, 38-42) for the structure of the active site of homologs of rat retinol dehydrogenase, we expect that retinol is between the nicotinamide ring and the catalytically active tyrosine-176. The other side of the nicotinamide ring interacts with phenylalanine-40, which provides a hydrophobic cushion for $NADP^+$. Blanke et al. (43) found a stabilizing aromatic-aromatic interaction between tyrosine-65 of diphtheria toxin and nicotinamide ring of NAD^+. They suggested that this could stabilize the N-glycosidic bond of NAD^+ during catalysis. A similar role may hold for Phe-40 and Phe-41 in retinol dehydrogenases, as well as other residues that are discussed below.

The nicotinamide binding site in the two retinol dehydrogenases contains an interesting configuration of hydrophobic residues. In rat retinol dehydrogenase, this consists of proline-206, phenylalanine-209, as well as phenylalanine-41 (Figure 2). In 11-*cis* retinol dehydrogenase this consists of proline-205, phenylalanine-208, as well as phenylalanine-40. The sequence alignment (Figure 1) shows that all four residues are conserved in β-hydroxybutyrate dehydrogenase and 11β-hydroxysteroid dehydrogenase; 17β-hydroxysteroid dehydrogenase has three of the four residues, with leucine-94 present instead of phenylalanine (Figure 1).

Examination of many homologs of retinol dehydrogenase indicates that a phenylalanine residue between Gly_2 and Gly_3 is an unusual. Most *sec*-alcohol dehydrogenases have either leucine or isoleucine at this position ((10-14), Figure 1). Figure 3 shows the 3D structures of *S. hydrogenans* 20β-hydroxysteroid dehydrogenase and rat dihydropteridine reductase with NAD^+. In *S. hydrogenans* 20β-hydroxysteroid dehydrogenase, leucine-18 is close to the nicotinamide moiety, although with a smaller interacting surface that can occur with phenylalanine. In contrast, leucine-18 in dihydropteridine reductase is distant.

Another interesting feature in the nicotinamide binding site of rat retinol dehydrogenase is the stabilizing interaction between the carboxamide group of $NADP^+$ and threonines-211 and 214; in cow 11-*cis* retinol dehydrogenase, threonines 210 and 213 have a similar interaction with NAD^+. A threonine homologous to threonine-211 has been suggested to be important in binding of nicotinamide moiety in rat dihydropteridine reductase (11), mouse carbonyl reductase (32), *Drosophila* alcohol dehydrogenase (34,39), human 11β-hydroxysteroid dehydrogenase-type 1 (40) and human 17β-hydroxysteroid dehydrogenase-type 1 (41).

Evolutionary implications. The phylogenetic tree for the two retinol dehydrogenase and their homologs (Figure 4), shows the close relationship of the retinol dehydrogenases, mammalian β-hydroxybutyrate dehydrogenase and the type 2 11β-hydroxysteroid and 17β-hydroxysteroid dehydrogenases. These latter three enzymes are NAD^+ dependent, suggesting that the $NADP^+$ dependence in rat retinol dehydrogenase is a more recent evolutionary event. A parsimonious model is that the ancestral enzyme at node C had preference for NAD^+ and contained an aspartic acid between Gly_1 and Gly_2 that destabilized binding of $NADP^+$. A gene duplication at or after node E, followed by mutation at the sites corresponding to proline-62 and serine-63 to glutamic acid and lysine, respectively, led to a retinol dehydrogenase with preference for $NADP^+$. The phylogenetic tree also suggests that leucine-94 in 17β-hydroxysteroid dehydrogenases-type 2 is a recent mutation from a phenylalanine residue.

This phylogenetic analysis combined with structures of retinol dehydrogenases suggests that mutating residues such as the aspartic acid and phenylalanine in the glycine-rich turn could lead to a better understanding of catalysis in these enzymes and in homologs, such as 11β- and 17β-hydroxysteroid dehydrogenases-types 1 and 2.

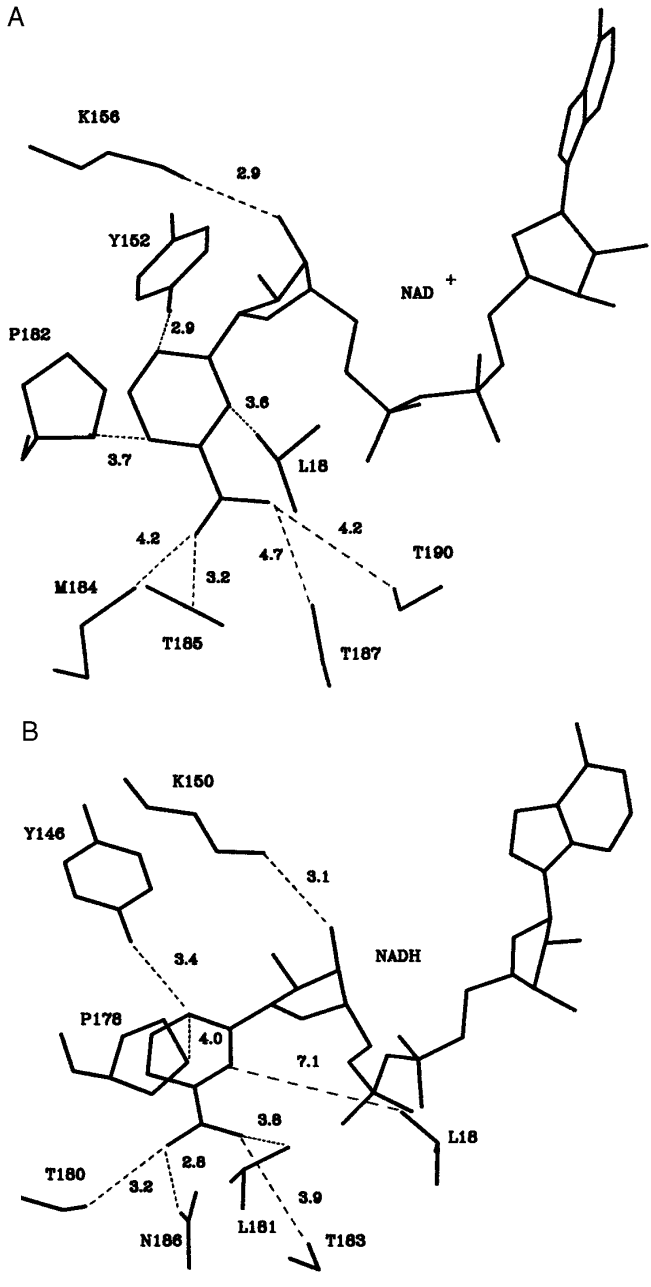

FIG. 3. Interaction of leucine-18 in *Streptomyces hydrogenans* 20β-hydroxysteroid dehydrogenase and rat dihydropteridine reductase with the nicotinamide ring. (A) *S. hydrogenans* 20β-hydroxysteroid dehydrogenase. (B) Rat dihydropteridine reductase. Leucine-18 in 20β-hydroxysteroid dehydrogenase is close to the nicotinamide moiety. In contrast, leucine-18 in dihydropteridine reductase is over 7 Å from the nicotinamide moiety. Also shown are residues that stabilize the nicotinamide ring. Proline and threonine are at homologous sites to residues in the two retinol dehydrogenases.

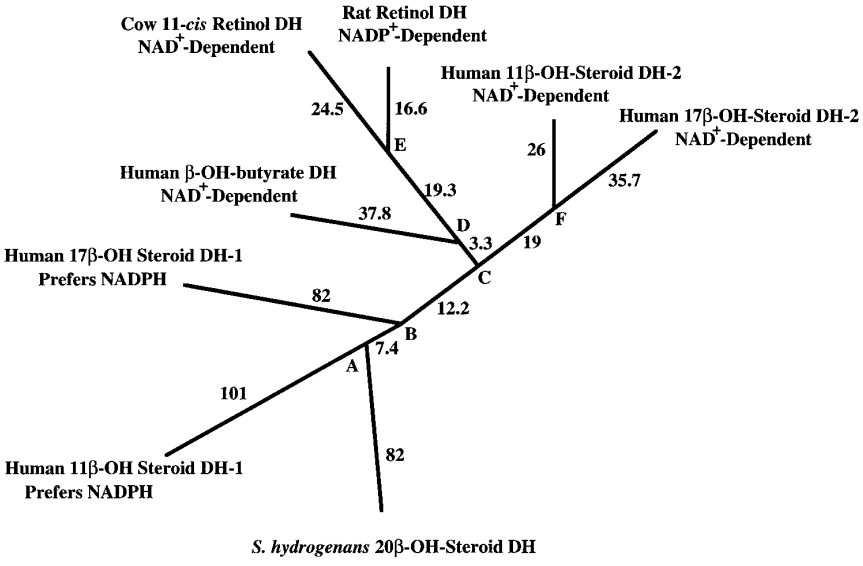

FIG. 4. Phylogenetic tree for dehydrogenases in retinol dehydrogenase Phylogenetic tree was constructed using the Feng and Doolittle algorithm (31). In this method, the proteins are progressively aligned using the Dayhoff PAM-250 scoring matrix to assess pairwise similarity of each protein with the others, and the scores are assembled into a distance matrix. Then the method of Fitch and Margoliash (44) is used to obtain the best branching order for the sequences. Branch lengths are calculated by a linear regression analysis of the best fit of the pairwise distances and the branching order. The lengths of the branches are proportional to the relative distance between the sequences.

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REFERENCES

1. Wald, G. (1968) *Science* **162,** 230–239.
2. Stryer, L. (1991) *J. Biol. Chem.* **266,** 10711–10714.
3. Petkovich, M., Brand, N. J., Krust, A., and Chambon, P. (1987) *Nature* **330,** 444–450.
4. Giguere, V., Ong, E. S., Segui, P., and Evans, R. M. (1987) *Nature* **330,** 624–629.
5. Tabin, C. J. (1991) *Cell* **66,** 199–217.
6. Chambon, P. (1994) *Seminars in Cell Biology* **5,** 115–125.
7. Simon, A., Hellman, U., Wernstedt, C., and Eriksson, U. (1995) *J. Biol. Chem.* **270,** 1107–1112.
8. Chai, X., Boerman, M. H. E. M., Zhai, Y., and Napoli, J. L. (1995) *J. Biol. Chem.* **270,** 3900–3904.
9. Baker, M. E. (1996) *Bioessays* **18,** 63–70.
10. Baker, M. E. (1991) *Steroids* **56,** 354–360.
11. Persson, B., Krook, M., and Jornvall, H. (1991) *Eur. J. Biochem.* **200,** 537–543.
12. Tannin, G. M., Agarwal, A. K., Monder, C., New, M. I., and White, P. C. (1991) *J. Biol. Chem.* **266,** 16653–16658.
13. Krozowski, Z. (1992) *Mol. Cell. Endocrinol.* **84,** C25–C31.
14. Jornvall, H., Persson, B., Krook, M., Atrian, S., Gonzalez-Duarte, R., Jeffery, J., and Ghosh, D. (1995) *Biochemistry* **34,** 6003–6013.
15. Baker, M. E. (1994) *Biochem. J.* **300,** 605–607.
16. Callahan, H. L., and Beverley, S. M. (1992) *J. Biol. Chem.* **267,** 24165–24168.
17. Papadopoulou, B., Roy, G., and Ouellette, M. (1992) *EMBO J.* **11,** 3601–3608.
18. Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Um, K. S., Wilson, T., Collins, D., de Lise, G., and Jacobs, W. R. (1994) *Science* **263,** 227–230.
19. Hopwood, D. A., and Sherman, D. H. (1990) *Annu. Rev. Genet.* **24,** 37–66.

20. Ghosh, D., Weeks, C. M., Groschulski, P., Duax, W. L., Erman, M., Rimsay, R. L., and Orr, J. C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10064–1006.
21. Ghosh, D., Wawrzak, Z., Weeks, C. M., Duax, W. L., and Erman, M. (1994) *Structure* **2,** 629–640.
22. Varughese, K. I., Skinner, M. M., Whitely, J. M., Matthews, D. A., and Xuong, N. H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6080–6084.
23. Varughese, K. I., Xuong, N. H., Kiefer, P. M., Matthews, D. A., and Whiteley, J. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5582–5586.
24. Ghosh, D., Pletnev, V. Z., Zhu, D.-W., Wawrkak, Z., Duax, W. L., Pangborn, W., Labrie, F., and Lin, S.-X. (1995) *Structure* **3,** 503–513.
25. Dessen, A., Quemard, A., Blanchard, J. S., Jacobs, Jr., W. R., and Sacchettini, J. C. (1995) *Science* **267,** 1638–1641.
26. Rafferty, J. B., Simon, J. W., Baldock, C., Artymiuk, P. J., Stuitje, A. R., Slabas, A. R., and Rice, D. W. (1995) *Structure* **3,** 927–938.
27. Wierenga, R. K., De Maeyer, M. C., and Hol, W. G. J. (1985) *Biochemistry* **24,** 1346–1357.
28. Wierenga, R. K., Terpstra, P. P., and Hol, W. G. J. (1986) *J. Mol. Biol.* **187,** 101–107.
29. Branden, C., and Tooze, J. (1991) Introduction to Protein Structure, Garland Publishing New York, pp. 33–35.
30. Smith, D. W. (1988) *Comput. Appl. Biosci.* **4,** 212.
31. Feng, D., and Doolittle, R. F. (1990) *Methods Enzymol.* **183,** 375–387.
32. Tanaka, N., Nonaka, T., Nakanishi, M., Deyashiki, Y., Hara, A., and Mitsui, Y. (1996) *Structure* **4,** 33–45.
33. Chen, Z., Lin, Z-G., Lee, W. R., and Chang, S. H. (1991) *Eur. J. Biochem.* **202,** 263–267.
34. Chen, Z., Tsigelny, I., Lee, W. R., Baker, M. E., and Chang, S. H. (1994) *FEBS Lett.* **356,** 81–85.
35. Scrutton, N. S., Berry, A., and Perham, R. N. (1990) *Nature* **343,** 38–43.
36. Fan, F., Lorenzen, J. A., and Plapp, B. V. (1991) *Biochemistry* **30,** 6397–6401.
37. Chen, Z., Lu, L., Shirley, M., Lee, W. R., and Chang, S. H. (1990) *Biochemistry* **29,** 1112–1118.
38. Ribas dePoplana, L., and Fothergill-Gilmore, L. A. (1994) *Biochemistry* **33,** 7047–7055.
39. Chenevert, S., Fossett, N., Lee, W. R., Tsigelny, I., Baker, M. E., and Chang, S. H. (1995) *Biochem. J.* **308,** 419–423.
40. Chen, Z., Jiang, J. C., Lin, Z. G., Lee, W. R., Baker, M. E., and Chang, S. H. (1993) *Biochemistry* **32,** 3342–3346.
41. Tsigelny, I., and Baker, M. E. (1995) *Biochem. Biophys. Res. Commun.* **217,** 859–868.
42. Tsigelny, I., and Baker, M. E. (1995) *J. Ster. Biochem. & Molec. Biol.* **55,** 589–600.
43. Blanke, S. R., Huang, K., and Collier, R. J. (1994) *Biochemistry* **33,** 15494–15500.
44. Fitch, W. M., and Margoliash, E. (1967) *Science* **155,** 279–284.