Unusual Charge Stabilization of NADP⁺ in 17β-Hydroxysteroid Dehydrogenase*

(Received for publication, October 3, 1997, and in revised form, December 5, 1997)

Catherine Mazza, Rock Breton‡, Dominique Housset§, and Juan Carlos Fontecilla-Camps‡

From the Laboratoire de Cristallographie et Cristallogénèse des Protéines, Institut de Biologie Structurale J.-P. Ebel, CEA-CNRS, 41, avenue des Martyrs, F-38027 Grenoble cedex, France

Type I 17β-hydroxysteroid dehydrogenase (17β-HSD1), a member of the short chain dehydrogenase reductase (SDR) family, is responsible for the synthesis of 17β-estradiol, the biologically active estrogen involved in the genesis and development of human breast cancers. Here, we report the crystal structures of the H221L 17β-HSD1 mutant complexed to NADP⁺ and estradiol and the H221L mutant/NAD⁺ and a H221Q mutant/estradiol complexes. These structures provide a complete picture of the NADP⁺-enzyme interactions involving the flexible 191–199 loop (well ordered in the H221L mutant) and suggest that the hydrophobic residues Phe192, Met192 could facilitate hydride transfer. 17β-HSD1 appears to be unique among the members of the SDR protein family in that one of the two basic residues involved in the charge compensation of the 2'-phosphate does not belong to the Rossmann-fold motif. The remarkable stabilization of the NADP⁺ 2'-phosphate by the enzyme also clearly establishes its preference for this cofactor relative to NAD⁺. Analysis of the catalytic properties of, and estradiol binding to, the two mutants suggests that the His221-steroid O3 hydrogen bond plays an important role in substrate specificity.

The 17β-estradiol (E₂) is known to promote the genesis and development of human breast cancers (1, 2). Its presence in tumor cells comes from in situ synthesis (3), and its concentration is significantly increased in malignant breast tissues (4–6). Type 1 17β-hydroxysteroid dehydrogenase (17β-HSD1) catalyzes the reversible transformation of estrone (E₁) into the biologically active estradiol (E₂) (7). Thus, preventing the formation of E₂ by a specific inhibition of 17β-HSD1 appears to be unique among the members of the SDR protein family in that one of the two basic residues involved in the charge compensation of the 2'-phosphate does not belong to the Rossmann-fold motif. The remarkable stabilization of the NADP⁺ 2'-phosphate by the enzyme also clearly establishes its preference for this cofactor relative to NAD⁺. Analysis of the catalytic properties of, and estradiol binding to, the two mutants suggests that the His221-steroid O3 hydrogen bond plays an important role in substrate specificity.

The 17β-estradiol (E₂) is known to promote the genesis and development of human breast cancers (1, 2). Its presence in tumor cells comes from in situ synthesis (3), and its concentration is significantly increased in malignant breast tissues (4–6). Type 1 17β-hydroxysteroid dehydrogenase (17β-HSD1) catalyzes the reversible transformation of estrone (E₁) into the biologically active estradiol (E₂) (7). Thus, preventing the formation of E₂ by a specific inhibition of 17β-HSD1 appears to be unique among the members of the SDR family because it lacks both the aspartic acid residue at position 36 (Leu36 in 17β-HSD1) and the His221, first identified by affinity labeling studies (27–29), was thought to be involved in the specific binding of the steroid. Indeed, the construction of an H221A mutant led to an enzyme displaying a higher Kₘ and lower Vₘₐₓ relative to the wild type (16). Furthermore, the crystal structure of the enzyme complexed with E₂ (25, 26) revealed that His221 is directly involved in the specific binding of the steroid.

Here, we report the construction of H221L and H221Q mutants, their characterization by enzymatic assays, their crystallization, and the determination of the structures of the binary complexes H221Q-E₂ and H221L-NAD⁺ and the ternary complex H221L-NAD⁺-E₂ at 2.7, 3.0, and 2.7 Å resolution, respectively. We show for the first time a well ordered conformation for the 191–199 loop and speculate about its role in cofactor binding and hydride transfer. Moreover, the specificity of the enzyme for estrogens is reassessed.

EXPERIMENTAL PROCEDURES

Materials—Spodoptera frugiperda, purified AcNPV DNA (Autographa Californica nuclear polyhedrosis virus), and transfer vector pVL1393 were purchased from Invitrogen Corporation; Grace’s insect cell culture medium, yeastolate, lactalbumin hydrolysate, fetal bovine serum, and restriction enzymes were from Life Technologies Inc.; NAD⁺, NAD⁺⁺, estradiol, and estrone were from Sigma; and Blue-Sepharose CL-6B was from Amersham Pharmacia Biotech.

Cell Culture and Virus—The TNM-FH medium was prepared from Grace’s medium by the addition of 3.3 g/liter yeastolate and 3.3 g/liter

This paper is available online at http://www.jbc.org
Unusual Charge Stabilization of NADP+ in 17β-HSD1

lactalbumin hydrolase. The SF9 cells were grown as monolayers at 28 °C in TNM-FH medium supplemented with 10% fetal bovine serum. Cells were infected with virus at a multiplicity of infection of 0.1–1 plaque-forming unit to produce virus stocks or at multiplicity of infection >10 for maximal protein expression. Cells were harvested 60 h after infection.

Site-directed Mutagenesis—The two mutants H221Q and H221L were constructed with one round of polymerase chain reaction made on the pVL/17β-HSD transfer vector previously constructed for 17β-HSD1 overexpression in baculovirus (30). These mutations use two primers. The first one is located on the cDNA, upstream of a PsaI unique site (5’-GCCGCTGCTCCGGCG-3’). The second one introduces the mutation. For H221Q and H221L, it overlaps the codon to be mutated and a NruI unique site (5’-GCCGCTGCTCCGGCG-3’). The second one introduces the mutation. Amplified fragments and pVL/17β-HSD1 were digested with NruI and PsaI. The mutated fragments were then cloned instead of the nonmutated one. Mutations were checked by dideoxynucleotide sequencing, and mutated transfer vectors were co-transfected in SF9 cells with the wild-type AcNPV virus following the plaque-forming unit to produce virus stocks or at multiplicity of infection >10 for maximal protein expression. Cells were harvested 60 h after infection.

Table I

**Data collection statistics**

| Resolution (Å) | H221L - NAD+ | H221L - NADP+ | H221Q - E2 |
|----------------|--------------|---------------|-------------|
| Number of reflections | 3.0 | 2.7 | 2.7 |
| R Intensity (%) | 13.3 | 9.2 | 11.8 |
| Rmerge (%) | 83 | 84 | 65.2 |

**Table II**

**Refinement statistics**

| Model | H221L - NAD+ | H221L - NADP+ | H221Q - E2 |
|-------|--------------|---------------|-------------|
| Number of residues | 1118 | 1127 | 279 |
| Number of atoms | 8568 | 8625 | 2127 |
| Protein | 176 | 192 | 8 |
| Estradiol | 10.0–3.0 | 10.0–2.7 | 10.0–2.7 |
| NADP | 36666 | 49542 | 4887 |
| Solvent | 0.223 | 0.22 | 0.175 |
| Free R-factor | 0.300 | 0.295 | 0.259 |

**Structure Determination**—For the H221Q mutant, a rigid body refinement with the model of the wild-type 17β-HSD1 was sufficient to place it correctly in the unit cell. The H221L mutant structure was solved by molecular replacement methods with the wild-type 17β-HSD1 as a search model using AMoRe (37). Four molecules per asymmetric unit were found. The mutants structures were refined with X-PLOR (Version 3.1) (38) and REFMAC (CCP4 Suite of Programs) (39), and model corrections were made with O (40). The refinement statistics are presented in Table II. The coordinates have been deposited with the Protein Data Bank (codes: 1FDU for the H221L-E2-NADP+ complex, 1FDV, for the H221L-NAD+ complex, and 1FDW for the H221Q-E2 complex).

**RESULTS**

Two different crystalline complexes were obtained with the H221L mutant: the first one by co-crystallizing the protein with NADP+ and diffusing E2 in the crystal, and the second one by crystallizing the protein in the presence of NAD+. Both complex crystallize in the monoclinic P21 space group (Table I) with four molecules in the asymmetric unit corresponding to two biologically active dimers (Fig. 1), here named mA/mB and mC/mD, respectively.

In the H221L-NADP+-E2 ternary complex, the electron density is very well defined along all the polypeptide chain, including the 191–199 loop that was disordered in the wild-type protein. The average B-factor is 16 Å², with solvent accessibility of 61.6% (Table II). The crystal statistics are summarized in Table I.

**Crystallization**—All recombinant mutant enzymes were concentrated to 5 mg/ml, in a buffer containing 40 mM Tris, pH 7.5, 1 mM dithiothreitol, 0.05 mM dithioreitol, and 2–2.4 M ammonium sulfate as a precipitant. Space groups and unit cell dimensions of these crystals are summarized in Table I. The two mutants H221Q and H221L, it overlaps the codon to be mutated and a NruI unique site (5’-GCCGCTGCTCCGGCG-3’). The second one introduces the mutation. Amplified fragments and pVL/17β-HSD1 were digested with NruI and PsaI. The mutated fragments were then cloned instead of the nonmutated one. Mutations were checked by dideoxynucleotide sequencing, and mutated transfer vectors were co-transfected in SF9 cells with the wild-type AcNPV virus following the plaque-forming unit to produce virus stocks or at multiplicity of infection >10 for maximal protein expression. Cells were harvested 60 h after infection.

**Sit...
structure (Fig. 2). The observed conformation for this loop is completely different from that proposed by Ghosh et al. (24) but the 190–192 segment conformation is similar to that of residues 184–186 in mouse lung carbonyl reductase (MLCR) (22) (Fig. 3). The electron density map is also very well defined for the steroid and cofactor (Fig. 4). As a result, NADP\(^+\) and estradiol have been modeled with full occupancies in the four subunits.

In the H221L-NAD\(^+\) binary complex, the electron density for NAD\(^+\) and the 191–200 loop is well defined for monomers mA and mC but discontinuous for monomers mD and mB. Consequently, the 191–199 loop was not included in the models of these two monomers. Since this site is known to have Michaelian kinetics, we do not understand the observed difference in NAD\(^+\) occupancy. It may be due to subtle packing effects difficult to characterize at this resolution.

Both NADP\(^+\) and NAD\(^+\) bind in the same extended conformation already observed for the wild-type enzyme: the cofactor points toward the active site of the enzyme with the nicotinamide ring in the syn conformation and the adenine in the anti conformation (25).

The structure of the H221Q-E\(_2\) binary complex displays two conformations for Gln221. In one of these, the amide group of Gln221 forms a hydrogen bond with the steroid O17 atom; whereas in the other, Gln221 is oriented toward the solvent. The double conformation of the Gln221 may be a consequence of the partial occupation of the steroid-binding site, as it was already suggested in the case of the wild-type enzyme (25). No electron density was found for the cofactor.

Having a complete model of the cofactor binding site allows for a full description of the NAD(P)/protein interactions. Some of these interactions are common to NADP\(^+\) and NAD\(^+\), and most of them were present in the wild-type ternary complex.
However, due to the disorder in the neighborhood of the NADP$^+$ binding site in the latter structure, two major interactions were not observed: the extensive hydrophobic contact between the nicotinamide moiety and the Phe$^{192}$ side chain, and the charge compensation of the dinucleotide 2$^\prime$-phosphate through salt bridges with Arg$^{37}$ and Lys$^{195}$ side chains (Figs. 4 and 5). In addition, the 2$^\prime$-phosphate is further stabilized by a hydrogen bond with Ser$^{11}$ O$^g$. In the NAD$^+$ complex, the binding pocket of the ribose 2$^\prime$-phosphate of monomers mA and mC is occupied by a sulfate ion, presumably coming from the crystallization solution. This ion is bound to the protein through a hydrogen bond network involving 2$^\prime$-OH adenine ribose, Lys$^{195}$, Arg$^{37}$, Ser$^{11}$, and Thr$^{11}$ side chains. This phenomenon has already been observed in glutathione reductase where an inorganic phosphate ion substitutes for the missing 2$^\prime$-phosphate group when NAD$^+$ is bound (41). In both NAD$^+$ and NADP$^+$ complexes, the NH$_3^+$ group of Lys$^{195}$ interacts with the O1A of the pyrophosphate.

The 191–199 loop, located between the $\beta$F sheet and the $\alpha$G helix, seems to be predominantly stabilized by its interactions with the dinucleotide, particularly by the salt bridge between Lys$^{195}$ and the 2$^\prime$-phosphate. In the H221L-NAD$^+$ complex, where a sulfate ion replaces the 2$^\prime$-phosphate, the electron density corresponding to the 191–199 loop is less well defined even though the NAD$^+$ site appears to be fully occupied. This implies that although the sulfate ion establishes a series of interactions with both the protein and the cofactor, these are less efficient in stabilizing the 191–199 loop than those formed by the covalently bound 2$^\prime$-phosphate. Once stabilized, the 191–199 loop appears to protect the coenzyme from solvent as the NADP$^+$ accessible surface (42) is reduced from 122 Å$^2$ when the loop is removed to 38 Å$^2$ when it is well ordered.

The E$_2$ molecule and the residues forming the steroid binding site are well superposed in the two wild-type structures (Table III). On the other hand, the rms differences values resulting from the superposition of each mutant onto the wild-type models show a significant deviation for estradiol, relative to the residues involved in the hydrophobic site (Fig. 6). In this
respective, the loss of a hydrogen bond between residue 221 and estradiol may be responsible for the increased steroid mobility observed in the H221L mutant. There is also a significant difference in substrate position between two non-equivalent monomers such as mC and mD. The slight substrate reorientation observed in the H221Q mutant is likely to be due to a shift of the hydrogen bond between $E_2\cdot\text{O}^\alpha$ and $\text{Gln}^{221}\cdot\text{N}^\alpha$.

The catalytic efficiency is strongly affected by mutations at the His$^{221}$ position (Table IV). Reduction is decreased 10-fold for the H221L mutant and 4-fold for the H221Q mutant (18-fold and 6-fold, respectively, for the oxidation). This may be partially explained by a 4.4-fold increase in the reduction reaction $K_m$ for H221L and a respective 2.3-fold increase for the H221Q mutant (4-fold and 3.4-fold, respectively, for the oxidation reaction). As the histidine-to-glutamine mutation preserves the hydrogen bond with $\text{C}^\alpha\cdot\text{OH}$, this interaction seems to be essential for the catalytic activity. As expected from the structure, the H221L mutation has a limited effect on the cofactor binding site architecture, the $K_m$ value for NADP$^+$ reduction being close to that of the wild-type enzyme (Table V).

**DISCUSSION**

**The Role of the 191–199 Loop**—The H221L mutant provides the first image of a well ordered 191–199 loop in a 17$\beta$-HSD1 structure. The conformation of this loop in the H221L-E$_2$-NADP$^+$ complex is very likely to be identical to that of the wild-type enzyme when fully complexed to the cofactor. This is supported, on the one hand, by biochemical results that indicate that the affinity of the H221L mutant enzyme for NADP$^+$ is similar to that of the wild-type protein (Table V) and, on the other, by the fact that the 191–199 loop is located at the protein surface, and it is not involved in potentially constraining crystal packing interactions in either structure. The stabilization of the 191–199 loop, that is disordered in the absence of cofactor, is mediated by the interactions of $\text{Lys}^{195}$ and Phe$^{192}$ with NADP$^+$. Furthermore, loop residues Phe$^{192}$ and Met$^{193}$ shield NADP$^+$ from solvent and contribute to the hydrophobic character of the nicotinamide binding pocket.

Other dehydrogenases also have a similarly flexible loop near their dinucleotide binding site. One example is the mobile loop comprising residues 16–20 in *Escherichia coli* dihydrofolate reductase which is involved in hydride transfer (43). This loop, which becomes well ordered only in the enzyme-NADP$^+$-folate complex, has been found to shield the nicotinamide moiety from solvent and to participate in the transition-state stabilization (44). Replacement of the Met$^{19}$-Ala$^{19}$ stretch by glycine results in a 550-fold decrease in the hydride transfer rate. Another case is lactate dehydrogenase. As in 17$\beta$-HSD1, a loop comprising residues 97–123 is stabilized by interactions with NADH and shields the cofactor from solvent (45). In the SDR family, *E. coli* 7$a$-HSD (23) exhibits a large conformational change of the 185–210 loop upon substrate binding that also results in shielding of the catalytic site. In the 3$a$,20$\beta$-HSD structure (19), a small conformational change of the 184–189 loop located close to the active site is also observed. In the MLCR-NADP$^+$ complex (22), the Met$^{186}$ interaction with the nicotinamide moiety is similar to the one observed between Phe$^{192}$ and the NADP$^+$ in 17$\beta$-HSD1 (Fig. 3). However, it is not known whether the 184–190 loop has the same conformation in the MLCR apoenzyme.

All the SDR loops described above are located between the $\beta$F sheet and the $\alpha$G helix and are flanked by two proline

---

**FIG. 5. The NADP$^+$ environment.** The 191–199 loop is represented in black, and NADP$^+$ and estradiol are in medium gray. The 2′-phosphate group is stabilized by three interactions (dashed lines): two salt bridges with Arg$^{17}$ and Lys$^{195}$ and a hydrogen bond with Ser$^{142}$. Phe$^{192}$ protects the nicotinamide moiety from solvent by an extensive hydrophobic contact. The catalytic site formed by Ser$^{142}$, Tyr$^{155}$, the steroid O$^{17}$ atom, and nicotinamide are also represented. Dashed lines represent the triangular hydrogen bond arrangement between Ser$^{142}$, Tyr$^{155}$, and O$^{17}$.

---

**Table III**

| Table III | Estradiol positional differences |
| --- | --- |
| Superpositions were made with the ALIGN program (52) on the substrate binding site residues (Ser$^{142}$, Val$^{143}$, Met$^{147}$, Leu$^{149}$, Tyr$^{155}$, Pro$^{157}$, Tyr$^{218}$, Val$^{222}$, Phe$^{228}$, Phe$^{232}$, and Met$^{270}$). Monomer mC of the H221L · E2 · NADP$^+$ complex was used for the superposition with the wild-type enzyme. 1fds and 1fdt are Protein Data Bank accession codes for 17$\beta$-HSD1 · E2 and 17$\beta$-HSD1 · E2 · NADP$^+$ complexes, respectively. |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
| |  |
residues (Pro\textsuperscript{187} and Pro\textsuperscript{200} in 17\beta-HSD1). As it was previously suggested by Tanaka\textit{et al.} (23), these two prolines may prevent conformational changes from propagating to the rest of the protein. An amino acid sequence alignment of SDR enzymes reveals that a proline residue located at position 185 (17\beta-HSD1 numbering) is highly conserved, except for 17\beta-HSD1, where this residue is found at position 187 (Fig. 7). Furthermore, a hydrophobic residue, equivalent to Phe\textsuperscript{192} of 17\beta-HSD1, is found in 38 out of the 46 amino acid sequences analyzed (Fig. 7). These loops, that are stabilized by both substrate or cofactor, may be characteristic of enzymes belonging to the SDR family. In turn, their protection of the nicotinamide moiety and contribution of hydrophobic residues to the active site structure suggest they are involved in catalytic hydride transfer.

The Coenzyme Specificity—As indicated by the different x-ray complex structures, and by previous biochemical results (7), 17\beta-HSD1 is able to bind both NADP\textsuperscript{+} and NAD\textsuperscript{+} cofactors. The 17\beta-HSD1-NADP\textsuperscript{+} complex has been shown to be stabilized through hydrogen bonds established between the dinucleotide 2'-phosphate moiety and the main chain NHs of residues Cys\textsuperscript{10}, Ser\textsuperscript{11}, and Arg\textsuperscript{37} (25). In most known structures of NADP\textsuperscript{+}-preferring enzymes, the two negative charges of the 2'-phosphate group are compensated by one to three residues located in the variable loop. Moreover, in most of the NADP\textsuperscript{+} enzyme complex structures having a Rossman-fold binding motif (47), the 2'-phosphate group interacts with one to three residues located in either the \(\beta\)A\(\alpha\)B or the \(\beta\)BaC turn (Fig. 1). Thus 17\beta-HSD1 is the first SDR structure for which such an atypical charge compensation is observed. This may be relevant to the understanding of the evolution of the NADP(H) binding motif.

A second basic residue, located in the 4th position of the Gly-Xaa-Xaa-Xaa-Gly-Xaa-Gly consensus sequence of the dinucleotide binding motif, often further compensates the 2'-phosphate charge. Indeed, sequence alignment studies reveal that this residue is conserved in 54\% of the NADP\textsuperscript{+}-preferring SDR proteins (Fig. 7). In 17\beta-HSD1, there is no basic residue in the 4th position. Instead, the 2'-phosphate group interacts with Ser\textsuperscript{142}, which is located in the third position of the consensus sequence. In fact, among the 21 NADP\textsuperscript{+}-preferring enzymes of the SDR family that do not have a basic residue at this position, 15 are found to have either serine or threonine instead (Fig. 7).

Several authors have suggested that the 2'-phosphate group is involved in the catalytic mechanism (48), but it is not clear which residues may be involved in the charge compensation. The 2'-phosphate group is also involved in the catalytic reaction. Significant deviations are observed for the steroid, especially for its C and D rings.

The Role of His\textsuperscript{227} in Substrate Specificity—In the wild-type structure, His\textsuperscript{227} was found to hydrogen bond to the C\textsuperscript{3}OH of the estradiol moiety. This hydrogen bond is preserved in the H221Q mutant. In the H221L mutant, however, the mutated

![Image](http://www.jbc.org/)

**FIG. 6.** Stereoscopic view of the steroid binding site of wild-type (1fdt model in yellow, and 1fds model in orange), H221L (monomers C in blue, MD in light blue), and H221Q (pink). Phe\textsuperscript{226} of the 1fdt model and Gln\textsuperscript{225} of the H221Q mutant are shown with their two modeled conformations. The steroid environment is very well conserved, especially Phe\textsuperscript{155} and Ser\textsuperscript{142} which are the residues involved in the catalytic reaction. Significant deviations are observed for the steroid, especially for its C and D rings.

| Table IV | Catalytic activity linked to the steroid binding site |
|---------|-----------------------------------------------------|
|         | Reduction | Oxidation  |
|         | \(K_m\) (\(\mu\)M) | \(V_{max}\) (units/mg) | \(V_{max}/K_m\) | \(K_m\) (\(\mu\)M) | \(V_{max}\) (units/mg) | \(V_{max}/K_m\) |
| Wild type | 1.8 ± 0.2 | 5.4 ± 0.2 | 3.1 ± 0.5 | 1.5 ± 0.2 | 3.9 ± 0.4 | 2.6 ± 0.7 |
| H221L    | 8.1 ± 0.2 | 2.4 ± 0.1 | 0.3 ± 0.03 | 5.3 ± 0.6 | 0.79 ± 0.05 | 0.15 ± 0.03 |
| H221Q    | 4.2 ± 0.6 | 3.5 ± 0.2 | 0.8 ± 0.2 | 5.0 ± 0.8 | 2.6 ± 0.2 | 0.52 ± 0.16 |

**TABLE V**

| NADP\textsuperscript{+} reduction | Wild type | H221L |
|-----------------------------------|-----------|-------|
| \(K_m\) (\(\mu\)M) | 0.81 ± 0.05 | 0.62 ± 0.04 |
| \(V_{max}\) (units/mg) | 1.83 ± 0.04 | 1.13 ± 0.02 |
leucine residue can only participate in the formation of the hydrophobic pocket of the steroid binding site. Consequently, it can be concluded that this hydrogen bond is not essential to the catalytic efficiency. However, comparison of all the available protein interactions of O17, Tyr 155 O, and Ser 142 O, these results suggest that the hydrogen bond between His 221 and the O3 atom would place the O17 in a position which is unfavorable for catalysis. This can be compared with a 4- to 5-fold reduction in the H221Q mutant (Table IV). Taken together, these results suggest that the hydrogen bond between His\textsuperscript{221} and the C3-OH group is important for enzyme specificity between 17β-HSD1 and 18β-GT. Therefore, these observations may explain the specificity of 17β-HSD1 for aromatic A ring-containing substrates. With substrates like testosterone or 4-androstenedione, the orientation of the C3–O3 bond relative to the C17–O17 bond is different. Accordingly, we suggest that for these substrates, the interaction of His\textsuperscript{221} with the O3 atom would place the O17 in a position which is unfavorable for catalysis. This can be compared with a 4- to 5-fold reduction in the H221Q mutant (Table IV). Taken together, these results suggest that the hydrogen bond between His\textsuperscript{221} and the C3–O3 bond relative to the C17–O17 bond is different. Accordingly, we suggest that for these substrates, the interaction of His\textsuperscript{221} with the O3 atom would place the O17 in a position which is unfavorable for catalysis.
Acknowledgments—We thank J.-L. Ferrer and M. Roth (IBS, Grenoble) for help using the D2AM ESRF beam line.
Unusual Charge Stabilization of NADP$^+$ in 17β-Hydroxysteroid Dehydrogenase
Catherine Mazza, Rock Breton, Dominique Housset and Juan Carlos Fontecilla-Camps

J. Biol. Chem. 1998, 273:8145-8152.
doi: 10.1074/jbc.273.14.8145

Access the most updated version of this article at http://www.jbc.org/content/273/14/8145

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 49 references, 8 of which can be accessed free at http://www.jbc.org/content/273/14/8145.full.html#ref-list-1