Tyrosine 343 in human sulfite oxidase (SO) is conserved in all SOs sequenced to date. Intramolecular electron oxidized enzyme (IET) rates between reduced heme (Fe(I)) and oxidized molybdenum (Mo(VI)) in the recombinant wild-type and Y343F human SO were measured for the first time by flash photolysis. The IET rate in wild-type human SO at pH 7.4 is about 37% of that in chicken SO with a similar decrease in $k_{cat}$. Steady-state kinetic analysis of the Y343F mutant showed an increase in $K_m$ for sulfite and a decrease in $k_{cat}$ resulting in a 23-fold attenuation in the specificity constant $k_{cat}/K_m$ for sulfite at the optimum pH value of 8.25. This indicates that Tyr-343 is involved in the binding of the substrate and catalysis within the molybdenum active site. Furthermore, the IET rate constant in the mutant at pH 6.0 is only about one-tenth that of the wild-type enzyme, suggesting that the OH group of Tyr-343 is vital for efficient IET in SO. The pH dependences of IET rate constants in the wild-type and mutant SO are consistent with the previously proposed coupled electron-proton transfer mechanism.

In vertebrates the molybdenum cofactor-containing enzyme sulfite oxidase (SO), EC 1.8.3.1 catalyzes the oxidation of sulfite to sulfate with the reduction of two equivalents of ferri-protoporphyrin (dRFH), electron paramagnetic resonance (EPR) spectroscopy. One-electron transfer to exogenous cyt $c_{ox}$, leaving the enzyme in the one-electron-reduced state Mo(V)/Fe(III), accomplishes re-oxidation of the Fe(III) center. A second Mo → Fe IET step (giving Mo(V)/Fe(III)), followed by reduction of a second equivalent of cyt $c_{ox}$ returns the enzyme to the fully oxidized resting state. We have previously shown that exogenous flavin radicals generated in situ with a laser pulse will rapidly reduce the heme center of SO by one electron (Fig. 2) and have used this unique technique to investigate IET between the molybdenum and iron centers as a function of pH values (10–12). Note that this process corresponds to the second IET step noted above. Combining this kinetic information with the crystal structure (4) and the pulsed EPR results (13–17) for the Mo(V) form of the two-electron-reduced state of SO has led to a plausible proposal for a coupled electron-proton transfer (CEPT) reaction at the molybdenum center (18) as shown in Scheme 1. In the crystal structure of chicken SO (4), Tyr-322 is within hydrogen-bonding distance of the equatorial Mo–O group, is also accessible to water molecules (Fig. 1A), and was proposed to serve as a proton shuttle between Mo(V) and OH$^\cdot$ or H$_2$O (10). Fig. 1B gives a detailed schematic view of part of a possible CEPT mechanism in which Tyr-322 acts as an intermediary proton shuttle. A water molecule bound to the molybdenum active site pocket is poised to donate a proton to the equatorial Mo(V) = O group during reduction to Mo(V); for the reverse process an OH$^\cdot$ anion in the pocket accepts a proton from Mo(V) = OH. Competing hydrogen-bonding interactions of the Mo(V) = O moiety with Tyr-322 and with the anion occupying the active site may also be responsible for the well known equilibrium between two EPR-distinct forms of SO.
observed for the sulfite-reduced enzyme (15). This tyrosine residue is conserved in all SOs from animals (6), plants (19), and bacteria (20). The mutation of this conserved tyrosine should provide direct evidence for its role in IET, and this is the goal of the present study.

The most extensively studied examples of SO are from rat, human, and chicken livers, and all three enzymes show a very high degree of sequence similarity with 68% sequence identity between human and chicken SO (21). The close similarity of the pulsed EPR spectra for native chicken and human SO indicates that the structures of their molybdenum centers are essentially identical (13, 14). Native SO has been purified from bovine, chicken, rat, and human livers. Rat (22, 24) and human (25–27) SO have been successfully cloned and expressed in active forms. Due to the intricacies of assembling and inserting

**FIG. 2.** Postulated oxidation state changes occurring at the molybdenum and iron centers of SO during the catalytic oxidation of sulfite and the concomitant reduction of cyt e. The one-electron reduction indicated by a dashed arrow connecting MoVFeIII and MoVFeII can be initiated with a laser pulse in a solution containing dRF and a sacrificial electron donor (semicarbazide in this study).

**SCHEME 1.** Proposed CEPT mechanism for IET in SO (adapted from Ref. 10).
the molybdenum center into the overexpressed apoenzyme, it is extraordinarily difficult to develop suitable expression systems for recombinant SO having high levels of enzyme activity. Expression of these proteins in *Escherichia coli* makes it possible to use site-directed mutagenesis to incisively probe the roles of specific residues in catalysis (25, 26, 28). This is particularly true for human SO where, in addition to structural studies, site-directed mutagenesis can be expected to critically evaluate the involvement of specific amino acid residues in pathological human SO deficiency. In the present experiments we explore the role of the conserved Tyr-343 in human SO (the equivalent of Tyr-322 in chicken SO) by comparative steady-state kinetic and flash-induced IET studies of the wild-type and the Y343F mutant.

**EXPERIMENTAL PROCEDURES**

Site-directed Mutagenesis—The Y343F mutation was introduced into pT7G918 (24) using the Transformer Site-directed Mutagenesis kit (Clontech) with the mutagenic primer Y343F (GGCCGGATTTCAAAG-GCTTCTC). Mutations were verified by sequence analysis performed at the Duke University DNA Analysis facility.

Expression and Purification of Wild-type and Y343F Human SO—Both recombinant wild-type and Y343F human SO were expressed and purified as previously described (24, 25) with the following modifications. After the phenyl-Sepharose column, fractions exhibiting an A414/A523 ratio greater than 0.89 were pooled and further purified using the gel filtration column Zorbax GF-250 (Agilent Technologies). Fractions exhibiting an A414/A523 ratio of 0.96 or greater were then pooled and used in the experiments described in this study. The molybdenum content of purified SO proteins was determined using a Perkins-Elmer Zeeman/3030 atomic absorption spectrometer as previously described (25, 29). Enzyme concentrations were determined by using molar extinction coefficients of 99,900 and 115,000 M cm⁻¹ at 414 nm for the oxidized chicken and human SO, respectively.

Laser Flash Photolysis—Laser flash photolysis experiments were performed anaerobically on 0.50-ml solutions containing ~90 μM 5-deazariboflavin (dRF) and 0.5 mM freshly prepared semicarbazide as a sacrificial reductant. The methodology used has been described previously (10, 30). The published method (30) was used for studying the effect of solution viscosity on IET in human SO. The laser apparatus and associated visible absorbance detection system have been extensively described (31) as has the basic photochemical process by which 5-deazariboflavin semiquinone (dRFH) is generated by reaction between tripyrimidine tetrathione dRF and the sacrificial reductant and used to reduce redox-active proteins (32–34). Further details concerning the photochemical process, which are of particular relevance to the SO system, are presented below. Non-linear least-squares fitting of experimental data at 513 nm was generally performed using an implementation of the Levenberg-Marquart algorithm (10), provided as part of the Microcal Origin (version 7.0; Northampton, MA) software package for data processing and display. Transient absorbance changes at 555 and 523 nm were obtained by a Gaussian fit to the data. Steady-state Kinetics—Steady-state enzyme kinetic studies were performed using an appropriate pH with NaOH. The total concentrations of the wild-type and Y343F human SO were 100 mM Bis-Tris (pH 6.0) exhibiting an A280 ratio greater than 0.89 were pooled and further purified using the gel filtration column Zorbax GF-250 (Agilent Technologies). Fractions exhibiting an A414/A523 ratio of 0.96 or greater were then pooled and used in the experiments described in this study. The molybdenum content of purified SO proteins was determined using a Perkins-Elmer Zeeman/3030 atomic absorption spectrometer as previously described (25, 29). Enzyme concentrations were determined by using molar extinction coefficients of 99,900 and 115,000 M cm⁻¹ at 414 nm for the oxidized chicken and human SO, respectively.

**RESULTS**

**Photochemical Reduction of Human SO by Deazariboflavin Semiquinone**—The photochemical reduction of the Fe³⁺ heme moiety of human SO was monitored by laser flash photolysis-induced transient absorbance changes. The flash-induced difference spectrum is shown in Fig. 3. The peak wavelengths (555 and 523 nm) and isobestic points (513, 533, 545, and 565 nm) observed in the flash-induced difference spectra are identical to those observed in the steady-state reduced minus oxidized difference spectrum for heme reduction (inset of Fig. 3). These spectra confirm that the transient absorbance changes observed at 555 nm are directly related to reduction and re-oxidation of the b-type heme prosthetic group (11). No detectable spectral contribution from the molybdenum cofactor was observed. It is important to note that human SO (wild-type and Y343F mutant) has photochemical reduction properties that are similar to the native chicken SO.

Fig. 4, A and B show typical transient kinetic traces of absorbance changes at 555 nm upon laser flash photoexcitation of a solution containing oxidized wild-type human SO (Fig. 4A) or native chicken SO (Fig. 4B), dRF, and semicarbazide. The kinetic behavior can be fully described in terms of the minimal set of reactions shown in Equations 2–5. dRFH is generated by the laser pulse in the presence of the sacrificial electron donor semicarbazide (AH₂) (Equation 3). The initial positive deflection of absorbance from zero in Fig. 4 is due to net reduction of the SO heme center to the Fe²⁺ form (Equation 4), which has an absorbance maximum at 555 nm (Fig. 3). The slow decrease in absorbance that follows the initial rapid increase is due to net reduction of the SO heme center to the Fe²⁺ form (Equation 4), which has an absorbance maximum at 555 nm (Fig. 3). The slow decrease in absorbance that follows the initial rapid increase is due to net IET from Fe³⁺ to Mo⁵⁺, which establishes an equilibrium between the Mo⁵⁺Fe⁴⁺ and Mo⁵⁺Fe⁶⁺ forms of SO (Equation 5). The kinetics of this latter process is independent of the concentrations of human and chicken SO, indicating that it is due to a first-order IET process, which is the reaction that is of particular interest to us in this study.

$$\text{hv} \rightarrow \text{dRFH} \rightarrow \text{dRF} \rightarrow \text{Fe}^{2+}$$

$$\text{dRF} + \text{AH} \rightarrow \text{dRFH} + \text{AH}$$

**Fig. 3. Flash-induced difference spectrum of wild-type human SO obtained 20 ms after the laser flash. The dotted lines with peaks at 555 and 523 nm were obtained by a Gaussian fit to the data. Anaerobic solutions contained 10 μM SO, 0.5 mM semicarbazide hydrochloride and ~90 μM dRF. Within the resolution of the experiment, the flash-induced difference spectrum is identical to that obtained by steady-state reduction (inset). Difference spectra obtained at longer times were similar in appearance indicating that no further reduction occurred.**
For a case such as that shown in Fig. 4, in which the photochemically induced reduction of SO occurs much faster than subsequent IET, excellent values for the overall IET rate constant $k_{rt}$ (= $k_f + k_r$) and parameters $a$ and $b$ can be obtained by fitting the heme re-oxidation phase with the simple exponential function given in Equation 6 (the geometrical meanings of the parameters $a$ and $b$ are shown in Fig. 4A).

$$A_{555} = a + b \exp (-k_{rt}t) \quad \text{(Eq. 6)}$$

Based on Equation 5, the parameters $a$ and $b$ in Equation 6 should have the meanings expressed in Equations 7 and 8 (where $A_0$ is simply the absorbance extrapolated to $t = 0$, assuming that the photochemically induced reduction of SO is instantaneous).

$$a = A_0 \frac{k_r}{k_{rt}} = A_0 \frac{k_r}{k_f + k_r} \quad \text{(Eq. 7)}$$

$$b = A_0 \frac{k_f}{k_{rt}} = A_0 \frac{k_f}{k_f + k_r} \quad \text{(Eq. 8)}$$

Thus the individual IET rate constants $k_f$ and $k_r$ can be calculated from $k_{rt}$ and $K_{eq}$ (= $b/a$, Equation 9). The IET rate constants and $K_{eq}$ values for the wild-type human SO (Fig. 4A) and the native chicken SO (Fig. 4B) under the same conditions are shown in Table I.

The IET rate constant in wild-type human SO exhibits a linear dependence on the negative 0.6th power of the viscosity (Fig. 5), which is similar to that for chicken SO (~0.7th power, Ref. 30). Control experiments using either sucrose or polyethylene glycol 400 as viscosogen indicate that it is viscosity itself that is responsible for the dependence of IET rates on the solution composition. The viscosity effect suggests that the IET in human SO may also involve significant conformational change, which has been suggested to involve domain rearrangement in the chicken enzyme (30, 37).

The observed rate constant for the initial heme reduction is protein concentration-dependent as expected for a bimolecular process (Equation 4). Electron transfer from $dRFH$ to the heme of SO can best be observed spectrophotometrically as a decrease in absorbance at 513 nm, which is close to the absorbance maximum for $dRFH$ and is an isosbestic point for oxidized and reduced heme cofactor. The calculated second order rate constants ($k_1$) for the reduction of wild-type human SO and

![Figure 4](image-url)

**Fig. 4.** Transient kinetic trace obtained at 555 nm upon photoexcitation of a solution containing (A) 10.8 $\mu$M wild-type human SO or (B) 14.9 $\mu$M chicken SO, ~90 $\mu$M $dRF$, and 0.5 mM semicarbazide in 10 mM Tris buffer (pH 7.4). pH was adjusted with HCl. The solid line indicates a single-exponential fit to the IET phase. $K_{eq} = b/a$.

![Figure 5](image-url)

**Fig. 5.** Fit of viscosity dependence of $k_{rt}$ for wild-type human SO in sucrose solution using modified Kramer’s theory (30).

### Table I

|                      | $k_1 \times 10^{-8}$ | $k_{at}$ | $K_{eq}$ | $k_f$ | $k_r$ |
|----------------------|----------------------|----------|----------|-------|-------|
|                      | m$^{-1}$s$^{-1}$      | s$^{-1}$ | s$^{-1}$ | s$^{-1}$ | s$^{-1}$ |
| Wild-type human SO*  | 4.0 ± 0.1             | 491 ± 11 | 0.73 ± 0.08 | 207 ± 5 | 284 ± 6 |
| Chicken SO           | 4.2 ± 0.1             | 1318 ± 28 | 1.63 ± 0.04 | 817 ± 18 | 501 ± 10 |

*Expressed as K108R. This recombinant protein has no effect on the sulfite:cytochrome c and sulfite:ferricyanide activities, which are identical to those of native human SO (25).
Role of Tyrosine 343 in Human Sulfite Oxidase

Fig. 6. The pH dependence of $k_{\text{cat}}$ values of wild-type and Y343F human SO. Experiments were performed in 0.1 M buffers (see “Experimental Procedures”) between pH 7.0 and 9.5 at 25 °C, with varying concentrations of sulfite and 15 μM cytochrome c.

![Image of Fig. 6 showing pH dependence of $k_{\text{cat}}$ values](image)

Table II

| pH   | $k_{\text{cat}}$ (s$^{-1}$) | $K_{\text{m}}$ sulfite (μM) | $k_{\text{cat}}/K_{\text{m}}$ sulfite (M$^{-1}$s$^{-1}$) |
|------|----------------------------|-------------------------------|--------------------------------------------------------|
| Wild-type |
| 8.02 | 35.2 ± 0.4                 | 2.4 ± 0.1                     | 1.5 × 10$^7$                                           |
| 8.25 | 31.8 ± 1.9                 | 3.8 ± 0.3                     | 8.4 × 10$^6$                                           |
| 8.48 | 28.8 ± 1.1                 | 2.4 ± 0.1                     | 1.2 × 10$^7$                                           |
| Y343F mutant |
| 8.02 | 6.8 ± 0.3                  | 10.2 ± 2.1                    | 6.6 × 10$^5$                                           |
| 8.25 | 9.1 ± 1.0                  | 24.8 ± 7.6                    | 3.7 × 10$^5$                                           |
| 8.48 | 8.5 ± 0.8                  | 50.2 ± 9.7                    | 1.7 × 10$^5$                                           |
| Ratio (Wild-type/Y343F) |
| 8.02 | 5.3                        | 0.24                          | 22                                                     |
| 8.25 | 3.4                        | 0.15                          | 22                                                     |
| 8.48 | 3.4                        | 0.048                         | 71                                                     |

![Image of Table II](image)

The Dependence of IET Rates on pH Values—The steady-state oxidation of sulfite to sulfate as catalyzed by human SO using cytochrome c as the electron acceptor yields plots of initial velocity versus substrate concentration that display typical saturation kinetics. The $k_{\text{cat}}$ values of both wild-type and Y343F were found to vary significantly over the pH range 7.0–9.5 (Fig. 6), and both exhibit bell-shaped pH dependences. Optimum pH values for activities of wild-type and Y343F are ~8.1 and ~8.3, respectively, which are not significantly different. The values of $k_{\text{cat}}$, $K_{\text{m}}$ sulfite and $k_{\text{cat}}/K_{\text{m}}$ sulfite around the optimum pH value are shown in Table II.

![Image of Fig. 7 showing pH dependence of rate constants](image)

Fig. 7. Dependence of the rate constants $k_f$ and $k_r$ of wild-type human SO on hydroxide ion concentration. A, 0.5 mM semicarbazide hydrochloride, 6 mM HCl; B, 20 mM Na$_2$SO$_4$, 0.5 mM semicarbazide hydrochloride, 6 mM HCl. For all experiments, the Tris base concentration was chosen so as to achieve the appropriate pH value while keeping [Cl$^-$] between 5 and 6 mM.

![Image of Table II showing pH dependence of rate constants](image)

 constants of the Y343F mutant were determined between pH 5.8 and 6.2. The SO activities at these low pH values were measured to make certain that the enzyme was still active. It was observed that the mutant remains functional even at pH 5.6 ($k_{\text{cat}}$ 1.25 s$^{-1}$). In addition, the magnitudes of the signal change at 555 nm in flash photolysis experiments were comparable to those observed with the wild-type protein. Fig. 8 shows the effects of pH on $K_{\text{eq}}$ for IET in the wild-type and Y343F human SO. Note the shift in the pH range for the mutant compared with the wild-type enzyme. Fig. 9 compares the kinetic traces of the absorbance changes at 555 nm upon laser flash photoexcitation of solutions of Y343F or wild-type human SO at pH 6.0. Note the significant difference in the time scale for these two proteins. Fig. 10 shows the pH dependence of IET rates in the Y343F mutant at low anion concentrations ([Ac$^-$] ~12 mM, [Cl$^-$] ~0.5 mM) between pH 5.8 and 6.2. Due to the slow IET, acetic acid was used to adjust the pH value of the buffer, and experiments on Y343F at high anion concentrations were not conducted at various pH values. The pH profiles of the IET rate constants for the mutant show significant differences from those for wild-type protein (compare Fig. 10 with Fig. 7A). Note that $k_r$ for the mutant increases slightly with increasing [OH$^-$], but $k_f$ is actually unchanged.

**DISCUSSION**

IET Rates of Wild-type Human and Chicken SO—The observed IET rate constant for the wild-type human SO at pH 7.4...
is 491 s\(^{-1}\), which is 37% of that obtained for chicken SO (1318 s\(^{-1}\)) under the same conditions (Fig. 4, Table I). The \(K_{eq}\) values for IET in wild-type human SO and chicken SO are 0.73 and 1.63, respectively, which must contribute to the difference in IET rates of these two proteins. Note that the \(k_{cat}\) value of wild-type human SO (35 s\(^{-1}\)) is also about 50% of that for chicken SO (73 s\(^{-1}\)) under standard assay conditions. Taken together, the steady-state kinetic and flash photolysis results are consistent with a recent comprehensive kinetic study of chicken SO (36), which suggests that at pH 7.4 the IET step contributes to the overall kinetic barrier to catalysis. The similarity in the heme reduction rates (\(k_1\)) of human and chicken SO is consistent with the very high degree (68%) of amino acid sequence homology in human and chicken SO and suggests that the exposure of the heme cofactors in the two proteins is similar.

The pH Dependence of IET Rates in Wild-type Human SO—In wild-type human SO, at low anion concentrations, both \(k_f\) and \(k_r\) decrease significantly with increasing \([\text{OH}^-]/[\text{H}^+]+[\text{Ac}^-]\) (Fig. 7A), which is quite different from the pattern observed previously for chicken SO (10) for which \(k_f\) decreased but \(k_r\) remained constant. The reason for this difference is not clear yet. A possible explanation is that the interdomain docking for human SO during the IET reaction may be disfavored upon increasing pH values, making both forward and reverse electron transfer slower.

In the presence of high anion concentrations for wild-type human SO only \(k_f\) decreases with increasing \([\text{OH}^-]\) (Fig. 7B). However, under comparable conditions, \(k_r\) for chicken SO increases with increasing \([\text{OH}^-]\), while \(k_f\) is effectively unchanged (10), which is remarkably different from wild-type human SO. These differences can be explained by the previously proposed mechanism (10) involving CEPT processes (Scheme 1).

Electron transfer processes in biological systems are often coupled to protonation/deprotonation events, and these effects may influence the kinetics of electron transfer (38). In the case of molybdoenzymes Stiefel has proposed that one-electron transfers are coupled to proton transfers (39). Although this hypothesis is widely accepted and several model systems designed to investigate such processes have been reported (40, 41), only a few studies on CEPT in molybdaden enzymes have been carried out (10, 42).

Scheme 1 can be algebraically expressed by Equations 10 and 11 (derived by a similar procedure to that shown in the supplemental materials for Ref. 10) as follows.

\[
\frac{k_{\text{app}}}{c} = \frac{d}{[\text{OH}^-]} \quad \text{Eq. (10)}
\]
The magnitude of the changes of $k_d$ are from 0.36 to 0.24 (Table III). The observed significant shift in $K_{eq}$ for the Y343F mutant (Fig. 8) indicates that the mutation in Tyr-343 disfavors the forward IET process (Equation 5), which may be due to the removal of hydrogen-bonding between the equatorial Mo$^{5+}$--OH group and the hydroxyl group of Tyr-343 (Fig. 1B). Hydrogen bonding to Mo$^{5+}$--OH can stabilize the +5 oxidation-state, thereby making the reduction of Mo$^{7+}$ to Mo$^{5+}$ more favorable. The absence of this hydrogen bonding in the Y343F mutant is expected to decrease $K_{eq}$ substantially (see below). Modulation of reduction potentials by hydrogen bonding is a general phenomenon in biological systems (43, 44). The effect of hydrogen bonding on the reduction potential in molybdenum complexes has been extensively studied (45–48). The NH--S hydrogen bond makes a significant contribution to the positive shift of the reduction potential of Mo$^{5+}$/Mo$^{4+}$ in monooxomolybdenum complexes with o-(acylamino)benzenethiolate ligands (48).

At pH 6.0, the $k_{cat}$ value of Y343F is 46 s$^{-1}$ (compared with 411 s$^{-1}$ for the wild-type under the same conditions, see Fig. 9). For the mutant, $K_{eq}$ does decrease from 0.36 to 0.24 (Table III). However, such a small shift in $K_{eq}$ (i.e. a thermodynamic factor) cannot account for such a large change in the IET rate constants (10-fold). The most plausible explanation is that the hydrophobic phenylalanine in the Y343F mutant may hinder direct access of water or H$^+$ to the equatorial Mo=O group (Fig. 1B), thus retarding efficient CEPT (i.e. a kinetic factor). This is clearly shown by the shift in the dependence of $K_{eq}$ for IET to lower pH values in the Y343F mutant (Fig. 8).

For the Y343F mutant at low anion concentrations, $k_{cat}$ increases with increasing [OH$^-$], while $k_d$ is effectively unchanged (Fig. 10), which is quite different from the wild-type behavior (Fig. 7A). These data can also be explained by the mechanism discussed above for the wild-type enzyme (Scheme 1; Equations 10 and 11). For the sake of brevity, only the analysis for $k_{cat}$ will be presented. For Y343F at the sufficiently high dissociation constant $K_{eq}$ and low association constant $K_{X1}$ (making $d$ [OH$^-$]), the apparent value for $k_d$ could become pH-independent over the entire pH range investigated, which suggests that the binding of [X$^-$] to the molybdenum active site may not be as effective in the absence of the OH group of Tyr-343. It is reasonable to expect hydrogen-bonding of the hydroxyl of Tyr-343 to anions because of its close proximity to the anion-binding site (Fig. 1A). Thus it is possible that Tyr-343 plays a role in anchoring the anion close to the molybdenum center.

Role of Tyr-343 in the Inhibitory Effect of Sulfate—At pH 6.0, $k_{cat}$ values of the Y343F mutant without and with 50 mM sulfate are 46 and 2.4 s$^{-1}$, respectively, while for the wild-type under the same conditions the corresponding $k_{cat}$ values are 434 and 7.7 s$^{-1}$, respectively. The inhibition by SO$_4^{2-}$ anions in the wild-type SO is still apparent in the Y343F mutant, which demonstrates that the OH group of Tyr-343 is not required for sulfate inhibition. Furthermore, the data presented in this paper clearly demonstrate that the OH group of Tyr-343 in human SO plays an important role in IET whether or not anions are bound to the enzyme active site, in contrast to an earlier suggestion that this is only true when sulfate is bound (10).
In conclusion, this study has provided direct evidence for the important role of the conserved active site tyrosine (Tyr-343 in human SO) in transferring protons during the CEPT process and the binding of substrate to the active site. In addition, these results provide experimental rationale for the close proximity of the equivalent tyrosine residue (Tyr-322) to the coordinated ligands of the molybdenum center observed in the crystal structure of chicken SO (4).

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