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A Spectrofluorimetric Study of Finasteride and Bovine Serum Albumin Interaction and Its Application for A Simple Quantitative Determination of Finasteride in Tablet Dosage Form

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Abstract

Based on the finasteride (FIN) ability to quench the intrinsic fluorescence of bovine serum albumin (BSA), a new spectrofluorimetric method for the determination of FIN in tablet dosage form was developed. BSA was monitored at 337 after excitation at 280 nm, the quenching effect of FIN on BSA was monitored at its maximum (332–344 nm). Upon investigating the interaction, negative values of $\Delta S^\theta$, $\Delta H^\theta$ and $\Delta G^\theta$ were computed and showed that a spontaneous binding is taking place with the main contribution from van der Waals power but without eliminating the role of hydrogen bonding from the interaction. The UV absorption spectra of FIN-BSA system confirmed the interaction with absorption intensity of BSA increasing gradually with the increased FIN concentrations, referring to the extended peptide strands of BSA molecules upon addition of FIN. Three dimensional and synchronous fluorescence spectra were used to detect conformational changes of BSA in the FIN-BSA complex. The well known Stern–Volmer and Lineweaver–Burk equations were used to determine the quenching type in the FIN-BSA system. Additionally, the linear Lineweaver–Burk plot was successfully used as a calibration curve for the analytical application of the method in the range of 0.5-15 $\mu$gmL$^{-1}$. To quantify FIN, the fluorescence intensity was measured at 334 nm after excitation at 280 nm. The developed method of analysis is accurate, precise and has shown good sensitivity with an LOD and LOQ values of 0.16 and 0.49 $\mu$gmL$^{-1}$, respectively. Recovery of FIN with the proposed method was 97.70±4.65 % for tablet dosage form.

Keywords:

Finasteride, Fluorescence quenching, Human plasma, Spectrofluorimetric analysis

1. Introduction
Finasteride (FIN), N-(1,1-dimethylethyl)-3-oxo-(5α,17β)-4-azaandrost-1-ene-17-carboxamide (Figure 1), is a type II 5 α reductase inhibitor, an intracellular enzyme that converts the androgen testosterone into 5α-dihydrotestosterone. FIN was developed by Merck & Co., Inc. (New Jersey, USA) and marketed under the names Proscar® (FIN 5 mg) and Propecia® (FIN 1 mg) tablets. Proscar® was approved by the FDA in 1992 for the treatment of bothersome symptoms in men with benign prostatic hyperplasia (BPH) and to reduce the risk of urinary retention or the need for surgery related to BPH 1. Few years later, Propecia® was also FDA approved in 1997 for the use in males only and is indicated for the treatment of male pattern hair loss (androgenetic alopecia) 2. On the other hand, serum albumins are multi-functional storage proteins and transport carriers for many ligands, they are also regarded the predominant circulatory proteins in various organisms 3. Their interaction with the different drugs can significantly influence the distribution volume and elimination rate of the drugs. Therefore, investigations of the drug-albumin binding shall help interpreting their metabolism and transport pathways. An important member of the albumins family, is the bovine serum albumin (BSA) which also possess various physiological functions including binding, transport, and delivery of several molecules 4-7. BSA is also responsible for certain binding aggregation, and conformational dynamics in solution 8. BSA is structurally homologue to the human serum albumin (HSA) and its structure was well studied 9-13. Therefore, investigating the interaction of drugs and serum albumins is an important factor to determine the pharmacokinetic and pharmacodynamic properties of drugs. Several previous studies have reported the BSA-drugs binding via spectroscopic techniques 14-16. Additionally, various reports have been published on the determination of finasteride either alone or in combinations in tablet dosage form and biological fluids using high-performance liquid chromatography (HPLC) 17-22, HPTLC 23, micellar electrokinetic chromatography 24, polarography 25, ELISA assay 26, voltametry 27, UV-spectrophotometry 28, liquid chromatography–tandem mass spectrometry 29-33. In the current study, quenching of the intrinsic fluorescence of BSA was investigated by selectively exciting BSA. Moreover, because FIN doesn’t possess native fluorescence, hence its interaction with BSA was used to develop a method for determination of FIN in tablets and human plasma. Thorough literature survey revealed that no single study with detailed description to investigate FIN and serum albumin binding was reported and no reports were found for FIN determination using fluorescence spectroscopy. Therefore, the present work
is designed to study the interaction of BSA and FIN utilizing fluorescence spectroscopy and to provide a new method for the determination of FIN in tablets.

Figure 1: Chemical structure of FIN

2. Experimental

2.1. Materials

Finsteride (FIN) primary standard was a kind donation of Dr. Haya Al-Johar from the Saudi Food and Drug Authority (Riyadh, KSA). Bovine serum albumin (BSA) purchased from Techno Pharmchem (Bahadurgarh, Haryana, India). HPLC grade methanol purchased from BDH laboratory supplies (Poole, UK). Potassium dihydrogen orthophosphate anhydrous purchased from Central drug house Ltd. (New Delhi, India). Sodium phosphate dibasic, anhydrous purchased from Bio Basic Inc. (NY USA). Extra pure sodium chloride purchased from Loba Chemie Pvt Ltd. (Mumbai, India) Potassium chloride purchased from WINLAB Ltd. (Leicestershire, UK). Ultrapure water of 18 μΩ obtained from a Millipore Milli-Q® UF-Plus purification system (Millipore, Bedford, MA, USA) was used throughout the study..

2.2. Instruments

A Jasco FP-8200 spectrofluorimeter (JASCO International Co. Ltd. Tokyo, Japan) was used for all the fluorescence measurement, with excitation and emission slits at 5 nm, λ_ex = 280 nm and 1-cm quartz cell. In the study, BSA was excited at 280 nm and monitored at 337 nm and the quenching effect of FIN on BSA was monitored at its maximum (332–344 nm), Quantification of FIN was carried out at 334 nm. All absorption spectral recordings and absorbance measurements were performed on a Nanodrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). All measurements were performed in 1X phosphate buffered
saline (PBS buffer) pH 7.4, the pH was measured on an Adwa AD1030 pH-meter (ADWA Instruments Inc., Romania).

2.3. Sample preparation

Standard solutions preparation was conducted at room temperature, and samples were stored at −20°C. FIN standard solution was prepared in methanol to produce a final concentration of 1.0 mg mL⁻¹. The working standard solution was prepared by diluting 1.0 mL of stock solution to 10 ml with methanol to give a 100 µg mL⁻¹ concentration. The stock solution was further diluted with PBS buffer pH 7.4 to produce a working standard solution of 20 µg mL⁻¹.

A standard bovine serum albumin (BSA) solution of 1.0 mg mL⁻¹ was prepared by dissolving 0.1 g pure BSA in PBS buffer pH 7.4 and making up the volume to 100 mL in a volumetric flask and kept in the cool, dark. The stock solution was further diluted with PBS buffer pH 7.4 to produce a working standard solution of 200 µg mL⁻¹.

2.4. Tablet samples preparation

Twenty tablets of FIN (Proscar® 5.0 mg) weighed and powdered. A quantity equivalent to one tablet (5.0 mg) of FIN was transferred to a 50 mL volumetric flask and 40 mL of HPLC grade methanol was added. The solution was sonicated for 30 min, and the final volume was diluted to the mark with methanol. Solution was then filtered through a Whatmann filter paper, the filtrate was subsequently filtered through a Millex-GP, 0.45 µm syringe filter (Millipore, Billerica, MA, USA) to remove all insoluble excipients.

2.5. Procedures

2.5.1. FIN–protein interactions

Upon optimizing the interaction conditions, BSA concentration was kept constant at 200 µg mL⁻¹ and FIN concentration was in the range of 0.5 to 15 µg mL⁻¹. Fluorescence spectra of BSA in absence and presence of FIN were recorded at three temperatures (288, 298 and 309 K) in the range of 290–500 nm upon excitation at 280 nm. This procedure was also used for the determination of FIN in tablet solutions but only at a temperature of 298 K.

2.5.2. UV measurements
The UV measurements of BSA in the presence and absence of FIN were made in the range of 220–350 nm. BSA concentration was fixed at 1.0 mgmL⁻¹ while the drug concentration was 100, 300 and 500 µgmL⁻¹

3. Results and discussion

3.1. Fluorescence quenching spectra

Fluorescence quenching refers to a process which reduces the fluorescence intensity of a given fluorophore through various molecular interactions, viz. molecular rearrangements, excited-state reactions, ground state complex formation, energy transfer, and collisional quenching. The fluorescence spectra of BSA in absence and presence of different FIN concentrations were measured in the concentration range of 290–500 nm upon excitation at 280 nm. There was no native fluorescence emission for FIN at the range measured and it caused a linear quenching of the BSA fluorescence intensity (Figure 2) with a slight red shift of the emission maximum which may indicate increase polarity of the FIN-BSA system. The results also refer to formation of a non-fluorescent complex upon the interaction of FIN and BSA.

Figure 2: Fluorescence spectra of BSA (200 µg.mL⁻¹) in the absence (a) and in presence of FIN at different concentrations. FIN concentrations were 0.5 µg.mL⁻¹ (b) 1.0 µg.mL⁻¹ (c) 3.0 µg.mL⁻¹ (d) 5.0 µg.mL⁻¹ (e) 7.0 µg.mL⁻¹ (f) 10 µg.mL⁻¹ (g) 12 µg.mL⁻¹ (h) 15 µg.mL⁻¹ (i)

3.2. Fluorescence quenching mechanism

Mechanisms of fluorescence quenching are usually divided into two types, namely, dynamic or static quenching. Dynamic and static types of quenching are caused by diffusion and...
ground-state complex formation, respectively. Both types have different temperature dependence viz. in dynamic type quenching constants are supposed to rise at higher temperatures, the opposite is always true for static quenching where increased temperature is likely to result in reduced stability of complexes, and thus lower values of the quenching constants. In the present work, fluorescence quenching data were interpreted using the Stern–Volmer equation (Eq. 1)\(^{38}\).

\[
\frac{F_0}{F} = 1 + K_{SV} C_Q = 1 + K_q \tau_0 C_Q
\]  

(1)

Where \(F_0\) and \(F\) as the steady-state fluorescence intensities of BSA in the absence and presence of FIN, respectively, \(C_Q\) as the concentration of FIN and, \(K_{SV}\) is the Stern–Volmer constant. \(K_q\) is the quenching rate constant; \(\tau_0\) is the average lifetime of the protein without the quencher.

Similarly, Lineweaver–Burk equation (Eq. 2)\(^{39}\), was also used to determine the quenching type of FIN–BSA system.

\[
(F_0 - F)^{-1} = F_0^{-1} + K_{LB}^{-1} F_0^{-1} C_Q^{-1}
\]  

(2)

Previous reports have demonstrated that, within certain concentration range, if the quenching type is single static or dynamic quenching then the curve of \(F_0/F\) versus \(C_Q\) (Stern–Volmer curve) would be linear\(^{14}\). Similarly, a linear curve of \((F_0 - F)^{-1}\) versus \(C_Q^{-1}\) (Lineweaver–Burk curve) is an indication of a static quenching\(^{40}\). However, an upward curvature of the Stern–Volmer plot is indicative of a combined quenching (both static and dynamic)\(^{41}\). Stern–Volmer and Lineweaver–Burk curves of FIN–BSA system at three different temperatures are shown in Figure 3. It can be noted that the Stern–Volmer curves were linear at lower FIN concentrations, while they were upward bent at higher ones. This recommends that the quenching type was probably a combined quenching, (both static and dynamic) at higher FIN concentrations.
Figure 3: The Stern–Volmer (a) and Lineweaver–Burk (b) curves at different temperatures

Additionally, Figure 3b of the Lineweaver–Burk curves show that under the studied FIN concentration range, the curves of \((F_0 - F)^{-1}\) versus \(C_Q^{-1}\) were linear, which reveals the presence of clear characteristics of a static quenching. Furthermore, the \(K_{SV}\) and \(K_{LB}\) values summarized in tables 1 and 2 are decreasing with the gradual increase in temperature which in turn is consistent with static quenching.

Table 1: Stern-Volmer and Lineweaver–Burk correlation parameters for BSA–FIN interaction

<table>
<thead>
<tr>
<th>Temperature (T) (K)</th>
<th>Stern-Volmer parameters</th>
<th>Lineweaver–Burk parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(K_{SV} \times 10^4) (Lmol(^{-1}))</td>
<td>(K_q \times 10^{12}) (Lmol(^{-1})s(^{-1}))</td>
</tr>
<tr>
<td>288</td>
<td>6.90±0.71</td>
<td>6.90</td>
</tr>
<tr>
<td>298</td>
<td>6.64±0.75</td>
<td>6.64</td>
</tr>
<tr>
<td>309</td>
<td>6.44±0.77</td>
<td>6.44</td>
</tr>
</tbody>
</table>

*Average of three determinations

The formation of a non-fluorescent complex was further confirmed from the values of quenching rate constants, \(K_q\), which were evaluated using equation 3

\[ K_q = K_{SV}/\tau_0 \] (3)

The value of \(\tau_0\) of the biopolymer is \(10^{-8}\) s\(^{-1}\) \(^{42}\) thus \(K_q\) values were computed to be in the order of \(10^{12}\) LM\(^{-1}\)s\(^{-1}\) (Table 1). This \(K_q\) value is higher than the previously reported values for the maximum scatter collision quenching constant, \(K_q\) of various quenchers with the biopolymer which was \(2 \times 10^{10}\) LM\(^{-1}\)s\(^{-1}\) \(^{42}\). This in turn refers to the fact that the quenching here is due to a complex formation and not initiated by dynamic collision.

3.3. Binding mode and binding sites

Presuming that small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules can be described by the following equation (Eq. 4) \(^{41,43}\):

\[ \log \left( \frac{F_0 - F}{F} \right) = \log K + n \log C_Q \] (4)
In this equation (Eq. 4) $K$ is the binding constant and $n$ is the number of binding sites on a BSA molecule. Hence, a plot of $\log(F_0 - F)/F$ versus $\log C_Q$ could be used to calculate $K$ and $n$ values.

Table 2 summarizes the different $K$ and $n$ values at different temperatures and demonstrates a decrease in the binding constant with the increase in temperature, yielding a less stable FIN–BSA complex. Moreover, $n$ values were found to be nearly $\sim$1, which indicates that only one association site exists between FIN and BSA.

Table 2: Thermodynamic parameters of BSA–FIN binding along with binding constant $K$ and the number of binding sites $n$

<table>
<thead>
<tr>
<th>Temperature (T) ($K$)</th>
<th>$\Delta G^0$ (kJmol$^{-1}$)</th>
<th>$\Delta H^0$ (kJmol$^{-1}$)</th>
<th>$\Delta S^0$ (Jmol$^{-1}$K$^{-1}$)</th>
<th>$K \times 10^4$ (Lmol$^{-1}$)</th>
<th>$n$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>288</td>
<td>-25.82</td>
<td></td>
<td></td>
<td>5.02</td>
<td>0.99</td>
<td>0.9768</td>
</tr>
<tr>
<td>298</td>
<td>-25.63</td>
<td>-31.28</td>
<td>-18.95</td>
<td>2.88</td>
<td>0.94</td>
<td>0.9758</td>
</tr>
<tr>
<td>309</td>
<td>-25.43</td>
<td></td>
<td></td>
<td>2.07</td>
<td>0.89</td>
<td>0.9767</td>
</tr>
</tbody>
</table>

3.4. Thermodynamics parameters and nature of the binding forces

Thermodynamic parameters, enthalpy ($\Delta H^0$) and entropy ($\Delta S^0$) of FIN–BSA interaction are significant to confirm binding mode. Due to the temperature dependence of the binding constant, the thermodynamic process is considered responsible for the complex formation. In general, the binding forces between a small molecule and a biological macromolecule could be hydrophobic, hydrogen bonding, van der Waals and/or electrostatic forces. Previous reports on the thermodynamic parameters of the various types of interactions $^{44}$ concluded that, hydrophobic binding enhances $\Delta H^0$ and $\Delta S^0$ of a system, while the opposite is always true for van der Waals force, and electrostatic force usually makes $\Delta H^0 \sim 0$ and $\Delta S^0 > 0$. In the current study, thermodynamic parameters of the FIN-BSA system were computed using the Van't Hoff and Gibbs–Helmholtz $^{21, 22}$ equations 5 and 6:

\[
\log K = \frac{-\Delta H^0}{2.303RT} + \frac{\Delta S^0}{2.303R} \tag{5}
\]

\[
\Delta G^0 = \Delta H^0 - T \cdot \Delta S^0 \tag{6}
\]
In equation 5, $K$ and $R$ are the binding and gas constants, respectively. $\Delta H^0$, $\Delta G^0$ and $\Delta S^0$ are, respectively, enthalpy change, free energy change and entropy change, respectively. Accordingly, a plot of $\log K$ vs. $T^{-1}$ (Figure 4) can be used to calculate $\Delta H^0$, $\Delta G^0$ and $\Delta S^0$ values.

Figure 4: Van’t Hoff plot for the binding between FIN and BSA, variation of $\ln K$ as a function of $1/T$

Previous reports have shown that a positive change of the entropy is usually regarded as an evidence for hydrophobic binding $^{28, 44}$. While, negative enthalpy refers to either electrostatic forces or hydrogen bonding, however, as mentioned previously, in electrostatic binding, enthalpy is anticipated to be $\sim 0$ $^{28, 44}$. Table 2 lists the computed thermodynamic parameters for FIN-based on these rules the main binding power between FIN and BSA can be regarded as van der Waals power and the reaction was mainly entropy driven. Based on the large binding constant, we think, beyond van der Waals power, however, hydrogen bonding role cannot be excluded in the FIN and BSA interaction.

3.5. UV–vis absorption spectra

The UV–vis absorption spectral data shown in Figure 5 for FIN-BSA system has proven the formation of a complex between FIN and BSA. Moreover, intensity of the UV absorption of BSA increased gradually upon the addition of different concentrations of FIN.
Figure 5: Absorbance spectra of BSA (a) FIN 0.3 mgmL⁻¹ (b) and FIN-BSA system at FIN concentrations of 100 µgmL⁻¹, (c) 300 µgmL⁻¹ (d) and 500 µgmL⁻¹ (e)

3.6. Effect of FIN on BSA Conformation.

3.6.1. Synchronous fluorescence

Synchronous fluorescence spectra conserve the sensitivity associated with fluorescence and allow spectral bandwidth reduction, spectral simplification and avoidance of different perturbing effects.

Figure 6: Effect of FIN on the synchronous fluorescence spectra of BSA with ∆λ=15 nm (a) and ∆λ=60 nm (b). BSA concentration= 200 µg.mL⁻¹, FIN concentrations (a-i) = (0, 0.5, 1.0, 3.0, 5.0, 10, 12 and 15 µg.mL⁻¹)

It refers to the fluorescence of tyrosine residues (Tyr) and tryptophan residues (Trp) of BSA when the wavelength interval ∆λ is 15 nm and 60 nm, respectively 45 and 46. Figure 6 shows that the emission intensity of both Tyr. and Trp. was reduced with no obvious shift in both peaks. A stronger fluorescence quenching effect of Trp. compared to Tyr. residues after the addition of different FIN concentrations. Subsequently, this can indicate that the binding site of FIN may be closer to tryptophan than to tyrosine residues.

3.6.2. Three dimensional fluorescence
Three-dimensional (3D) fluorescence spectra were determined, and some characteristic 3D parameters are reported in table 3. Two fluorescence peaks can be seen in the 3D fluorescence spectra (Figures 7 and 8). Peak 1, (224→333) mainly refers to the fluorescence characteristic of \( n \rightarrow \pi^* \) transition of the polypeptide backbone of protein structure, C=O \(^{47}\). The second peak at (280→337) represents the spectral characteristic of Trp and Tyr residues \(^{16}\). It is obvious that both fluorescence peaks of BSA have been quenched by FIN, but to different extents.

**Figure 7:** Three dimensional spectra of BSA (200 \( \mu \)g.mL\(^{-1} \)) in the absence (a) and presence (b) of FIN (10 \( \mu \)g.mL\(^{-1} \))

**Figure 8:** Contour fluorescence intensity spectra of BSA (a) and FIN-BSA system (b)

**Table 3:** Three-dimensional fluorescence characteristic parameters of FIN-BSA binding

<table>
<thead>
<tr>
<th></th>
<th>BSA</th>
<th>FIN-BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak position</td>
<td>Peak 1</td>
<td>Peak 1</td>
</tr>
<tr>
<td>((\lambda_{ex}/\lambda_{em}, \text{nm/nm}))</td>
<td>224/333</td>
<td>224/334.5</td>
</tr>
<tr>
<td>Relative intensity ((I_F))</td>
<td>6777.12</td>
<td>3036.18</td>
</tr>
</tbody>
</table>


### 3.7. Analytical application

As proven by the current study that BSA fluorescence quenching degree has a good relationship with the concentration of FIN in the range of 0.5–15.0 µg mL⁻¹. Hence, a new fluorescence method was developed and validated to determine FIN in pharmaceutical tablet formulation.

#### 3.7.1. Linearity, LOD and LOQ

As was previously demonstrated in section 3.2, Lineweaver–Burk double-reciprocal curve provided better linearity than Stern–Volmer curve. Therefore, Lineweaver–Burk double-reciprocal curve was used as a calibration curve for the analysis of FIN. Under the experimental conditions described in section 2.4.1, three independent calibration curves were constructed determining the reciprocal peak height of the quenched BSA versus the reciprocal nominal concentrations of FIN (mol L⁻¹). Regression analysis for the results was carried out using the least square method and the correlation coefficient, \( r^2 = 0.9983 \) (±0.003) over the concentration range used. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the signal-to-noise ratio. The intercept was then equal to \( S D_0 \) (the estimated SD at a concentration of zero). LOD and LOQ were then defined as 3\( S D_0 \), 10\( S D_0 \), respectively. The computed values were 0.49 and 0.16 µg mL⁻¹ for LOD and LOQ, respectively.

#### 3.7.2. Analysis of Fin in human plasma and tablets

The proposed method was applied for the determination of FIN in tablet dosage form. As summarized in Table 4, for Proscar® tablets the results obtained by the proposed method were in good agreement with the target value (5 mg/tablet).

**Table 4: Determination of FIN in tablet dosage form (Proscar® 5mg)**

<table>
<thead>
<tr>
<th>Number of Tablet determinations</th>
<th>Found concentration (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.69</td>
</tr>
<tr>
<td>2</td>
<td>4.98</td>
</tr>
<tr>
<td>3</td>
<td>5.02</td>
</tr>
<tr>
<td>4</td>
<td>5.13</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Mean (mg/tablet)</strong></td>
<td>4.88</td>
</tr>
<tr>
<td><strong>Standard deviation (SD)</strong></td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Accuracy (%)</strong></td>
<td>97.70</td>
</tr>
<tr>
<td><strong>RSD (%)</strong></td>
<td>4.65</td>
</tr>
</tbody>
</table>

### 4. Conclusions

The present work provided a new method to determine FIN in tablet dosage form utilizing the found interactions of BSA with FIN for the first time using fluorescence-quenching technique. The interaction of FIN with BSA was studied as the binding affects the drug bioavailability as well as its elimination rate. Upon binding of BSA to FIN, its fluorescence intensity was quenched through ground state complex formation. Binding constant was calculated for the FIN-BSA complex to be in the order of $10^4$ Lmol$^{-1}$ with one binding site on BSA for FIN. The negative values of the thermodynamic parameters ($\Delta G^\theta$, $\Delta H^\theta$ and $\Delta S^\theta$) indicate mainly involvement of van der Waals power but this does not exclude hydrogen bonding which might also play a role in the FIN and BSA binding. Ultimately, the FIN linear quenching effect on BSA fluorescence intensity was used for the quantitative determination of FIN in tablet dosage form. The analytical method was applied successfully for FIN determination in the range of 0.5-15 µg.mL$^{-1}$.

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### References


