Purification and characterization of sulfite oxidase from goat liver

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Sulfite oxidase (EC 1.8.3.1) catalyzes the physiologically vital oxidation of sulfite to sulfate, the terminal reaction in the degradation of sulfur-containing amino acids. Genetic deficiency related to human sulfite oxidase is associated with the severe clinical abnormalities with no effective therapies known, making the enzyme of immense biomedical importance. In the present study, sulfite oxidase was purified from the goat tissues, a hitherto unexplored source, in particular from the liver, and its physico and biochemical properties were characterized. The liver was chosen as it showed the highest activity, compared to kidney and muscle. The enzyme was purified to homogeneity by salting out, gel filtration and ion-exchange chromatography. It was a dimer (113 kDa) having two identical subunits (56 kDa) and did not contain free sulfhydryl groups. Its spectral analysis showed the presence of heme and molybdenum. circular dichroism (CD) spectra in near and far-UV regions showed the presence of significant amounts of secondary structures (45% α helix, 9% β structure and 26% β turn and remaining random coil) in the native molecule. The kinetic and hydrodynamic properties of the enzyme were also determined. Results also showed that ferricyanide was 8-times more effective electron acceptor than its physiological acceptor cytochrome c. The limited N-terminal analysis of the enzyme revealed the sequence up to six amino acids Trp-Glu-Pro-Ser-Gly-Ala. Together, these results suggested the liver was a major source of sulfite oxidase in goat and most of its physico-chemical, except secondary structure and amino acid sequence from N-terminal and biological properties were fairly similar to the sulfite oxidase isolated from other mammalian species/organisms.

Keywords: Goat liver, Sulfite oxidase, Molybdoenzyme, Molybdenum, Heme, Purification, Physico-chemical properties, CD spectra, Absorption spectra, N-terminal analysis

The enzyme sulfite oxidase (EC 1.8.3.1) catalyses the oxidation of sulfite to sulfate with the reduction of two equivalents of ferricytochrome c1-3, according to the following equation:

\[
\text{SO}_3^2- + \text{H}_2\text{O} + 2\text{Fe(III) Cyt c} \rightarrow \text{SO}_4^{2-} + 2\text{Fe(II) Cyt c} + 2\text{H}^+
\]

This is the terminal reaction in the oxidative degradation of sulfur-containing amino acids such as cysteine or methionine and is physiologically essential. The enzyme also plays an important role in detoxifying exogenously supplied sulfite and sulfur dioxide4,5. Genetic deficiency related to human sulfite oxidase is associated with the severe clinical abnormalities, namely mental retardation, seizures, characteristic dysmorphic features and dislocated lenses and in severe cases, attenuated growth of the brain6 and early death with no effective therapies known4,7, making the enzyme of immense biomedical importance.

The enzyme contains a pterin-molybdenum cofactor8,9 at the catalytic site and a b-type heme in a separate domain, which is similar in sequence to cytochrome b510. It was first isolated from bovine liver11, 12 and subsequently also purified from sources such as rat13, human and chicken14-17 liver. It has also been isolated from prokaryotes such as Thiobacillus novellus18-20, T. thioparus21 and Deinococcus radiodurans22. Its homologue is also reported in E. coli23.

In the present study, purification of sulfite oxidase is reported from goat liver, a hitherto unexplored source and its physico and biochemical properties have been characterized.

Materials and Methods

Fresh tissues/organs from goat were obtained from local slaughterhouse. DEAE-cellulose (DE-52) from Whatman International Ltd., England, Sephacryl
S-200 and blue dextran 2000 from Pharmacia Inc., Sweden, PVDF membrane from BioRad Laboratories, Hercules, CA were purchased. Dialysis tubes and all the electrophoresis materials and chemicals used in sequence analyses were purchased from Sigma Chemical Co.; St. Louis, MO. Sodium dithionite was obtained from E. Merck, Germany. All other chemicals/reagents used were of analytical grade.

**Sulfite oxidase assay**

Sulfite oxidase activity was determined by measuring the reduction of ferricyanide \((\varepsilon = 1020 \text{ M}^{-1} \text{ cm}^{-1} \text{ at } 420 \text{ nm})\) or reduction of cytochrome \(c\) \((\varepsilon = 19 \, 630 \text{ M}^{-1} \text{ cm}^{-1} \text{ at } 550 \text{ nm})\). One enzyme unit per mL was defined as the amount of enzyme that catalyzed the transformation of one nmole of potassium ferricyanide into potassium ferrocyanide or ferricytochrome \(c\) to ferrocytochrome \(c\) per min at 25°C.

**Purification of sulfite oxidase**

Sulfite oxidase was isolated and purified from goat liver according to methods described elsewhere\(^{11,15}\). The fractions containing the enzyme as judged by UV-visible spectrum and SDS-PAGE analysis were pooled (A\(_{413}\) nm/A\(_{280}\) nm ratios of 0.70 or greater), concentrated either by ultrafiltration membrane (Amicon, USA) with molecular weight cut-off limit of 10 kDa or lyophilized by using Martin Christ Alpha 1-2 lyophilizer (Germany) and used in the subsequent studies. Dialysis tubings were essentially prepared according to the previously described procedure\(^{25}\). Refrigerated centrifuge (Remi C-24, India) was used for all routine centrifugation. The detailed purification procedures of the enzyme are described as below:

**Acetone powder, ammonium sulfate precipitation, heat treatment and chromatography**

The acetone powder was prepared as described previously\(^{11}\) using liver (150 g) as starting material. Suspensions of acetone powder in extraction buffer and 2.5 mM sodium molybdate stirring resulted in the extraction of the enzyme into solution. This preparation was referred to as “crude extract” of the enzyme. To the crude extract, 20% ammonium sulfate was added while heating the content. Supernatant was taken and ammonium sulfate concentration was increased to 50% and precipitate taken after centrifugation. The precipitate was re-suspended in minimum volume of 25 mM Tris-Cl buffer, pH 8.0 and dialyzed against same buffer at 4°C to remove the ammonium sulfate. Further purification of the enzyme was achieved by ion-exchange chromatography on DEAE-cellulose (DE-52) column, equilibrated with extraction buffer (25 mM Tris-Cl, pH 8.0 buffer containing 0.1 mM EDTA and 0.02% sodium azide). The column was washed with the extraction buffer till the absorbance of the effluent at 280 nm reached to almost zero. The bound protein was eluted from column by applying a linear gradient of NaCl (0 to 0.5 M) in the extraction buffer.

The column was monitored by measuring the absorbance at 280 nm and 413 nm. The enzyme activity was measured in all fractions that showed A\(_{413}\)/A\(_{280}\) ratios of 0.70 or greater after dialyzing them separately to remove the sodium chloride (as Cl\(^-\) inhibits internal electron transport, Ahmad \textit{et al}, unpublished result) before the enzyme assay. The active fractions were then pooled, concentrated and subjected to gel chromatography on Sephacryl S-200 column. The column was monitored by measuring the absorbance at 413 nm. The active fractions of the enzyme were pooled, concentrated and subsequently used throughout the study.

**Determination of purity, molecular weight and subunit structure of the enzyme**

Purity of the enzyme was assessed after the chromatography on Sephacryl S-200 column, and was checked by SDS-PAGE\(^{26}\) in the presence and absence of β-mercaptoethanol. The electrophoretogram was obtained after staining the gel with Coomassie brilliant blue R-250 stain and also by silver staining. Native-PAGE\(^{27}\) was also carried out to check the charge homogeneity of preparation and the gel was stained with amido black.

Under native conditions, the molecular mass of the enzyme was determined by gel chromatography on Sephacryl S-200 column in extraction buffer. The column was calibrated with standard proteins of known molecular weight, namely cytochrome \(c\), α-chymotrypsinogen, ovalbumin and bovine serum albumin. The data obtained were treated according to Andrews\(^{28}\) and a plot of V/V\(_o\) and log M (M = molecular wt) was prepared to determine the molecular weight. Under denaturing condition, the molecular mass of sulfite oxidase was determined by SDS-PAGE in 10% gels in presence of β-mercaptoethanol. The molecular weight standards were also electrophoresed simultaneously.

**Spectroscopic methods**

UV-visible spectra in 250–600 nm range were recorded using either Perkin-Elmer Lambda Bio 20
UV/Vis double-beam spectrophotometer or Spekol 1200 (Analytikal Jena, Germany) using quartz cuvettes of 1 cm path length. The temperature was maintained at 25°C by circulating water using PTP-Peltier systems and circular dichroism (CD) spectrum (in 200-320 nm range) of the enzyme was measured on JASCO (J-715) Spectropolarimeter, equipped with Peltier type temperature controller PTC-383 WY. All spectral studies were carried out in 25 mM Tris-Cl buffer, pH 8.0.

### Determination of kinetic parameters

The kinetic parameters ($K_m$ and $V_{max}$) for the enzyme were determined in 0.1 M Tris-Cl buffer, pH 8.5 at 25°C. The data were analyzed according to Line-Weaver and Burk. The molybdenum (Mo) level in the enzyme was calculated from the calibration curve. The Mo was calculated from the calibration curve. The total sulphhydryl groups in the enzyme was determined using Ellman's reagent [5, 5'-dithiobis-(2-nitrobenzoic acid)] (DTNB) as a standard and with standards.

Different tissues of goat, namely kidney, liver, and muscles were examined for the levels of protein content and activity of sulfite oxidase. As shown in Table 1, liver showed the highest activity amongst the tissues, thus was chosen for further purification of enzyme. The results are represented in Table 2. Heat treatment of the crude extract and 20% ammonium sulfate salt saturation resulted in the removal of a substantial amount of extraneous proteins. Most of the enzyme was precipitated when ammonium sulfate saturation was increased to 50%. The bound protein from DEAE-cellulose column was eluted as one major and 2-3 minor peaks (Fig. 1). The enzyme preparation was eluted from Sephacryl-200 column as a single major peak and hump (Fig. 2).

### Results

#### Isolation and purification of sulfite oxidase

Different tissues of goat, namely kidney, liver, and muscles were examined for the levels of protein content and activity of sulfite oxidase. As shown in Table 1, liver showed the highest activity amongst the tissues, thus was chosen for further purification of enzyme. The results are represented in Table 2. Heat treatment of the crude extract and 20% ammonium sulfate salt saturation resulted in the removal of a substantial amount of extraneous proteins. Most of the enzyme was precipitated when ammonium sulfate saturation was increased to 50%. The bound protein from DEAE-cellulose column was eluted as one major and 2-3 minor peaks (Fig. 1). The enzyme preparation was eluted from Sephacryl-200 column as a single major peak and hump (Fig. 2).

### Purity, Molecular weight and subunit structure of the enzyme

In the SDS-PAGE system, the enzyme moved as a single band, indicating the size homogeneity of the preparation (Fig. 3). In the absence of β-mercaptoethanol

<table>
<thead>
<tr>
<th>Organ/Tissue</th>
<th>Protein (mg/mL)</th>
<th>Activity (EU/mL)</th>
<th>Specific activity (EU/mg of Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>7.50</td>
<td>20.72</td>
<td>2.76</td>
</tr>
<tr>
<td>Liver</td>
<td>9.51</td>
<td>51.76</td>
<td>5.44</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.78</td>
<td>2.50</td>
<td>0.90</td>
</tr>
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</table>
**Fig. 1**—Elution profile of sulfite oxidase on DEAE-Cellulose (DE-52) column (1.6 × 22.0 cm) [Sulfite oxidase activity (EU/mL) and A_{413} were plotted as a function of fraction number. Units of the enzyme activity were given in terms of the reduction of potassium ferricyanide. The sample size and total protein were 3.5 mL and 113.75 mg, respectively. The flow rate was maintained at 30 mL/h and fraction size was 2.0 mL. Fractions from 53 to 64 were pooled and concentrated for subsequent chromatography using Sephacryl S-200 column.]

**Table 2**—Purification for sulfite oxidase

<table>
<thead>
<tr>
<th>Steps</th>
<th>Volume (mL)</th>
<th>Protein conc. (mg/mL)</th>
<th>Total protein (mg)</th>
<th>Activity (Unit/mL)</th>
<th>Specific activity (Unit/mg protein)</th>
<th>Total activity</th>
<th>Yield (%)</th>
<th>Fold purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>600</td>
<td>7.10</td>
<td>4,260</td>
<td>30.96</td>
<td>4.36</td>
<td>18,756.00</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>20% Salt saturation and heat treatment</td>
<td>520</td>
<td>3.90</td>
<td>2,028</td>
<td>34.50</td>
<td>8.85</td>
<td>17,940.00</td>
<td>96.60</td>
<td>2.03</td>
</tr>
<tr>
<td>50% Salt saturation</td>
<td>35</td>
<td>32.50</td>
<td>1,300</td>
<td>374.50</td>
<td>11.52</td>
<td>12,107.50</td>
<td>70.60</td>
<td>2.64</td>
</tr>
<tr>
<td>Ion-chromatography (DEAE-cellulose, DE-52)</td>
<td>20</td>
<td>0.55</td>
<td>11</td>
<td>121.5</td>
<td>220.91</td>
<td>2,430.00</td>
<td>13.10</td>
<td>50.57</td>
</tr>
<tr>
<td>Gel chromatography (Sephacryl S-200)</td>
<td>8</td>
<td>0.42</td>
<td>3.36</td>
<td>169.5</td>
<td>403.57</td>
<td>1,356.00</td>
<td>7.30</td>
<td>92.56</td>
</tr>
</tbody>
</table>

**Fig. 2**—Elution profile of sulfite oxidase on Sephacryl S-200 column (2.6 × 57.0 cm) [The sample size and total protein applied were 2.5 mL and 10 mg, respectively. The flow rate was maintained at 30 mL/h and fraction size was 2.0 mL. The enzyme activity was measured by determining the reduction of potassium ferricyanide.]

**Fig. 3**—A Coomassie brilliant blue R-250 stained SDS-PAGE (10%) of the final pool from five different purification steps employed to purify sulfite oxidase (Lane 1, crude; lane 2, 20% ammonium sulfate precipitation; lane 3, 50% ammonium sulfate precipitation; lane 4, pooled fractions after ion-exchange chromatography; lane 5, gel chromatography; and lane 6, enzyme resolved without β-mercaptoethanol (gel chromatography). The sulfite oxidase subunit band is indicated with an arrow.

**Fig. 4**—A Coomassie brilliant blue R-250 stained SDS-PAGE (10%) of purified sulfite oxidase [The amount of sulfite oxidase was 75 µg. The goat liver sulfite oxidase subunit indicated with arrow corresponded to a molecular mass of 56 kDa. The molecular weight marker proteins used in lane 1 included: BSA (66 kDa); egg albumin (43 kDa); glyceraldehyde-3-phosphate dehydrogenase (36 kDa); bovine carbonic anhydrase (29 kDa); bovine pancreas trypsinogen (24 kDa); and soybean trypsin inhibitor (14.2 kDa).]
also, the enzyme moved as a single band (Fig. 3, lane 6), indicating the same mobility pattern as that of the enzyme treated with SDS and β-mercaptoethanol. In the native-PAGE, the purified enzyme also moved as single band, indicating the charge homogeneity of the preparation (Fig. not shown). Under native condition, the molecular weight of the enzyme was determined to be 113 kDa. Under denaturing condition, the enzyme moved as a single band (Fig. 4) and the molecular mass of the subunit was calculated to be 56 kDa.

Spectral characteristics

Absorption and CD spectra

The absorption spectrum of sulfite oxidase was determined in 25 mM Tris-Cl buffer, pH 8.0 at 25°C in the range of 250-600 nm. The spectrum showed one maximum in UV region at 278 nm and another one at 413 nm in visible region (Fig. not shown). In the near-UV region (Fig. 5a), the CD spectrum of the enzyme showed a positive band at 293 nm, wiggles in the wavelength range of 288 to 255 nm at a deep trough at 243 nm. The CD spectrum of in the far-UV region exhibited troughs at 222 and 215 nm (Fig. 5b).

Kinetic parameters

The kinetic parameters (K_m and V_max values) were calculated to be 6.98 × 10^{-4} M and 0.5824 nmole/mL/min (or 5.824 × 10^{-7} M/min), respectively.

Other characteristics

Stokes' radius and other related hydrodynamic parameters of the enzyme data treated according to Laurent and Killander. A plot of (-log K_av)\(^{1/2}\) and Stokes' radius was prepared and the line was drawn by least squares analysis of the data. The value of Stokes' radius was found to be 5.01 nm and the frictional ratio was calculated to be 1.57. The Mo content was determined to be 25 ppb. There was no increase in absorption at 412 nm after mixing the Ellman’s reagent with sulfite oxidase, as the absorbance of sample mixture and the blank was the same at this wavelength, indicating no free sulfhydryl group in the enzyme. The pH optimum was found to be 8.5 (Fig. or data not shown).

Effect of storage time on enzyme activity showed that the enzyme could safely be stored at -20°C for more than 2 months without any loss in the activity. As such freeze-thawing had no significant effect on the activity; however, about 8% of the activity was lost after 10 days, about 70% after 25 days on storage at -5°C and about 60% activity after 6 days storage at 4°C.

N-Terminal analysis of the enzyme showed the first six-residues from N-terminus were tryptophan, glutamate, proline, serine, glycine and alanine (WEPSGA), respectively. Absorption spectra of reduced and oxidized enzyme are shown in Fig. 6. The reduced enzyme showed three peaks at 550, 526 and ~420 nm in the visible region, designated as: \(α\), \(β\) and \(γ\) starting with peak at longest wavelength. Upon reduction with sodium dithionite, the \(γ\) or soret band increased in intensity and shifted to a slightly higher wavelength. The comparison of enzymatic activity
with electron acceptors revealed that ferricyanide was 8-times more effective than ferricytochrome c. This might indicate that the ability of sulfite oxidase to transfer electron to different acceptor was sensitive to the ionic composition of assay medium.

**Discussion**

Amongst all tissues tested for the sulfite oxidase, liver showed maximum enzyme content/activity (Table 1), as expected, since liver is the main tissue where detoxification reactions occur. These results were in agreement with the earlier reports\(^\text{36-38}\), wherein the highest sulfite oxidase activity was found in the liver amongst the mammalian tissues. As liver showed maximum enzyme content/activity, the enzyme was purified to homogeneity from this source only. During preparation of acetone powder, it was observed that if less than 12 mL of acetone per g of liver was used, removal of lipids was not complete and the enzyme could not be extracted into buffer completely.

Heat treatment of the 20% ammonium sulfate saturated extract resulted in the loss of about 52% of protein, but only about 4% loss of total activity giving 2-fold purification at this step. Heating up to 50°C did not result in any precipitation of extraneous proteins. A substantial amount of extraneous proteins was removed by ion-exchange chromatography. In elution profile of active pooled fractions obtained from ion-exchange on Sephacryl S-200 column, a small shoulder was observed before the main active peak (Fig. 2). The fractions under this shoulder showed the enzyme activity, which might indicate the aggregated form of the enzyme. Earlier\(^\text{17}\), aggregated form of the enzyme was also observed in the polyacrylamide gel under native condition.

The yield of sulfite oxidase was found to be ~7%. Earlier, variation in the yield (from 4.5 to 36%) of sulfite oxidase was observed from various sources\(^\text{12,15,17}\). The purity of the enzyme was analyzed by SDS-PAGE and native-PAGE. The faint protein bands in SDS-PAGE near the top of the gels in Fig. 3 might indicate the aggregated form of the enzyme.

The molecular mass (113 kDa) of goat liver sulfite oxidase was comparable to that reported for bovine (110 kDa)\(^\text{11}\) and chicken\(^\text{36}\) liver; however, in another study\(^\text{12}\), molecular mass of 83 kDa was reported for bovine liver, while molecular mass of 122 kDa and 120 kDa was reported for sulfite oxidase for human\(^\text{14}\) and rat\(^\text{39}\) liver, respectively. Subunit molecular mass of goat liver sulfite oxidase (56 kDa) was in agreement with the values reported for bovine\(^\text{16}\) and chicken\(^\text{15,17}\) and rat\(^\text{39}\) liver. However, subunit molecular mass of 43 kDa for the enzyme from *Deinococcus radiodurans*\(^\text{22}\) and 61 kDa from human liver was reported\(^\text{14}\). By comparing the values of molecular mass in the native state and denatured state under reducing condition, it could be concluded that the native enzyme comprised two subunits of identical molecular mass. As the molecular mass of the subunits of goat liver sulfite oxidase obtained from SDS-PAGE performed in the absence (Fig. 3, lane 6) and presence of β-mercaptoethanol was similar, indicating that the subunits were held together only by non-covalent interactions and inter-chain disulfite bonds were not involved. Similar observations were also reported for the enzyme from bovine\(^\text{11}\) and chicken liver\(^\text{15}\).

The absorption spectra (250-600 nm range) showed two maxima (at 278 and 413 nm) and two minima (at 316 and 251 nm) which was comparable with that reported for the enzyme from chicken\(^\text{5,12}\), bovine\(^\text{11,12}\) and rat\(^\text{39}\) liver. Evidences suggest that during catalysis electrons are shuttled from sulfite to the Mo center to the heme (Fe) center and then to cytochrome c (whole process is known as intramolecular electron transfer or IET). Each subunit of the hepatic sulfite oxidase contained a Mo center and a cytochrome b\(_5\) type heme. Atomic absorption spectroscopy of goat liver sulfite oxidase revealed the presence of Mo and reduction with sodium dithionite showed the characteristic spectrum of b\(_5\) heme, indicating the presence of heme prosthetic group in the goat liver sulfite oxidase, which acts as electron acceptor during IET (Fig. 6). The visible spectra of oxidized and reduced states of sulfite oxidase were strikingly similar to the enzymes that displayed cytochrome b\(_5\) type spectra, such as L-lactate cytochrome c oxidoreductase (EC 1.1.2.3) from yeast and which contains FMN as well protoheme prosthetic groups and the TPNH-nitrate reductase (EC 1.6.6.2) from *Neurospora*, which depends upon Mo, protoheme and FAD as prosthetic groups\(^\text{40}\). These observations established that goat liver sulfite oxidase is a heme protein. The absorption of Mo co-factor was completely masked by much stronger absorption of the heme chromophore. Isolated Mo co-factor domain of sulfite oxidase from rat and chicken liver exhibited an absorption maximum at 350 nm\(^\text{13}\).

The fine structures in the CD spectra in the near and far-UV regions (Figs 5a and b) suggested the
presence of significant amounts of secondary structures in the native molecule. The positive band in the near-UV region indicated that the presence of aromatic amino acids residues in asymmetric environment. The deep trough around 210 nm and a shoulder at 222 nm in the far-UV region suggested the presence of α helix. Analysis of CD spectra in the far-UV region indicated the presence of 45% α helix, 9% β structure and 26% of β turn and remaining random coil in the native molecule. These results were at variance to the secondary structures (9% α helix and 31% β structure) found in rat liver sulfite oxidase.

The $K_m$ (6.8 $\times$ 10$^{-4}$ M) was comparable to bovine liver sulfite oxidase (1.4 $\times$ 10$^{-4}$ M)$^{11}$, but was approximately two-fold lower than that reported sulfite oxidase from $T$. Thiobacillus novellus$^{18}$, rat liver$^{39}$, chicken liver$^{44}$ and recombinant human liver$^{42}$. Higher value of goat liver sulfite oxidase indicated that the enzyme might have less affinity for sulfite as substrate than sulfite oxidases isolated from other animal sources and bacteria. This could be explained on the basis of amino acid sequence analysis of sulfite oxidase of bovine and chicken liver (only available crystal structure of the enzyme to date), as goat is phylogenetically very close to bovine in animal kingdom. An important basic residue Lys 281 (Lys 200 in chicken)$^{42}$, which is not within the hydrogen bonding distance to substrate, but contributes positive charge to substrate binding pocket, is replaced by another basic residue arginine in bovine and its guanidium group provides extra positive charge which might destabilize the substrate binding and catalysis.

The value of stokes radius (5.01 nm) for the goat liver sulfite oxidase was in agreement with that reported (5.06 nm) for rat liver$^{39}$. However, a value of 3.67 nm was reported for bovine liver having the molecular mass of 83 kDa$^{39}$. The value of fractional ratio was also similar to that reported from rat liver$^{39}$, indicating the structure asymmetry of the enzyme. The goat liver sulfite oxidase did not react with Ellman's reagent, suggesting that the absence of sulfhydryl group in the enzyme.

The $p$H optimum (8.5) for goat liver sulfite oxidase was similar to that reported from the bovine liver$^{11,45}$. Goat liver sulfite oxidase showed a typical $p$H-rate profile with inflection points at $p$H 7.5 and 9.5, suggesting that its activity depends on the two active site residues having $pK_a$ values of 7.0 and 9.0. The substrate-binding pocket of chicken liver sulfite oxidase is formed by three arginines, tryptophan and tyrosine; the $pK_a$ values of these amino acid residues were in the range of 9.0 to 9.3$^{42}$. Probably, these amino acids residues also formed the substrate-binding pocket of goat liver sulfite oxidase. Tryptophan was found to be N-terminus residue of goat liver sulfite oxidase; however, alanine$^{10,42}$ in chicken and methionine in human$^{44}$ and rat$^{45}$ were the N-terminal residues. Results also showed that ferricyanide was 8-times more effective than its physiological electron acceptor cytochrome c, though bovine liver enzyme was 10-times more effective electron acceptor.$^{41}$

In conclusion, sulfite oxidase was purified and characterized from the goat liver and most of its physico-chemical and biological properties were found to be similar to the sulfite oxidase isolated from other mammalian species/organs, as well as non-mammalian sources.

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References