Synthesis and Kinetic Study on the Chromium(III) Complex [Cr(ASA)(en)\(_2\)]Cl\(\cdot\)2H\(_2\)O

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In order to explore the transfer mechanism of chromium(III) in mammals, a novel complex [Cr(ASA)(en)\(_2\)]Cl\(\cdot\)2H\(_2\)O, bis(ethylenediamine-κ\(_2\)N,N')(4-aminosalicylic acid-κ\(_2\)O,O') chromium(III) monochloride dihydrate was synthesized (4-aminosalicylic acid = H\(_2\)ASA, ethylenediamine = en). The crystal structure belongs to orthorhombic system with the space group \(P_{2_1}2_12_1\) by means of X-ray diffraction. The characteristic for transfer of Cr\(^{3+}\) from the compound to the low-molecular-mass chelator EDTA and the iron-binding protein apoovotransferrin (apoOTf) was followed by UV-visible (UV-Vis) and fluorescence spectra in 0.01 mol•L\(^{-1}\) Hepes at pH 7.4. The second order rate constants were calculated. Those spectra in conjunction were used to obtain more accurate information about the interaction of chromium complex with apoOTf. The experimental results indicate that Cr\(^{3+}\) can be transferred from the complex to apoOTf with the retention of the 4-aminosalicylic acid acting as a synergistic anion.

Keywords Chromium(III), 4-aminosalicylic acid, apoovotransferrin, kinetics

Introduction

Chromium is thought to activate enzymes, maintain protein stability and enhance carbohydrate metabolism. Clinical trials have demonstrated that supplementation with chromium compounds can lower blood glucose levels in diabetic patients. Chromium(III) picolinate, Cr(pic)\(_3\), has been studied one of these synthetic products and most thoroughly become a very popular nutritional supplement today. However, recent reports have indicated that the complex can efficiently cleave DNA in the presence of biologically reducing agents and induce strand breakages in chromosomes of intact cells. Hence, a search has been under way to identify the biologically active form of chromium and seek other safe synthetic chromium complex.

Up to date, the mechanism of chromic ion absorption remains uncertain. Little is known of the fate of Cr\(^{3+}\) uptaken orally. Essentially no data exists on the forms of chromium(III) in food as a result of its very low concentration. The fate of chromium(III) after it enters the bloodstream is better elucidated. The iron-transport protein transferrin (OTf) is likely to serve as the major chromium transport agent in blood. The transferrin-bound Cr(III) will then be transformed to low-molecular-weight chromium-binding substance (LMWCr) in blood. Vincent has studied the interaction of Cr(pic)_3 with apoOTf by UV-Vis spectrum. The result showed that Cr\(^{3+}\) could not be transferred from Cr(pic)_3 to apoOTf unless the metal is reduced to the Cr\(^{2+}\) level.

Here in we report the synthesis of a novel chromium(III) complex with 4-aminosalicylic acid (H\(_2\)ASA) and ethylenediamine (en) and the transfer kinetics studies of chromium from the complex to apoOTf by a combination of UV-visible (UV-Vis) and fluorescence spectra. As a control experiment, EDTA was employed firstly as a simple competition agent. The ligand 4-aminosalicylic acid was chosen originally because of its low toxicity and the similar structure with that of aspirin.

Experimental

General procedure

All manipulations were performed under aerobic conditions, and all chemicals were analytical grade reagents and used without further purification. Deionized water was used throughout. All glassware, including absorption and fluorescence cuvettes (1×1 cm), was routinely soaked in 1 mol•L\(^{-1}\) HNO\(_3\) and then rinsed with deionized water. ApoOTf was obtained from Sigma (Lot 115H7080) and purified according to the literature. The final concentration was determined from the absorbance at 280 nm using an extinction coefficient of 91200 mol\(^{-1}\)•L\(^{-1}\)•cm\(^{-1}\).

UV-visible (UV-Vis) spectra were measured with an HP8453 UV-Vis spectrophotometer. Fluorescence spectra were measured with a Hitachi 850 fluorescence spectrophotometer. The temperature of the solutions was maintained at 37 °C by a jacketed cell holder connected to an external circulation water bath (Shimadzu TB-85 or...
Huber). Chemical analyses for carbon, hydrogen, and nitrogen were performed by microanalysis using a Perkin-Elmer 2400B elemental analyzer. The X-ray data were collected on a Smart Apex CCDX diffractometer.

**Synthesis of the title complex**

The complex \([\text{Cr(ASA)(en)}_2]\text{ClO}_4\) was made by Masayuki in 1973. A new synthesis method of the chromium complex using a reductive method is reported here. \(\text{CrCl}_3\times6\text{H}_2\text{O}\) (0.67 g, 2.5 mmol), 4-aminosalicylic acid (0.38 g, 2.5 mmol) and granular (Mesh size 20) zinc (0.10 g, 1.5 mmol) were added into methanol (30 mL) and then refluxed. To the mixture (0.10 g, 1.5 mmol) were added into methanol (30 mL) and the resulting mixture was allowed to re flux for 45 min until a pink precipitate formed. After 2 d at room temperature, the compound was dissolved in methanol and beautiful X-ray quality crystals were obtained. The cell constants and orientation matrix were refined on \(\text{SHELX}97\) and subsequent Fourier difference map and constrained to ride on their parent atomic positions. Displacement ellipsoids are drawn at the 30% probability level for non-H atoms.

**Result and discussion**

**Crystal structure and spectral analysis**

The structure of \([\text{Cr(ASA)(en)}_2]\text{Cl} \times 2\text{H}_2\text{O}\) was determined by X-ray crystallography. The perspective structure and the atomic numbering scheme for the chromium complex are shown in Figure 1. A summary of the crystal data, experiment details and refinement results is given in Table 1. Selected bond lengths and angles are given in Table 2. The main structure of the title compound is made up of one \([\text{Cr(ASA)(en)}_2]\text{Cl} \times 2\text{H}_2\text{O}\) complex cation, one \(\text{Cl}^-\) and two solvating water solvate molecules. The chromium(III) is located at the centre of a compressed octahedron formed by four nitrogen atoms (Cr—N, 0.2060—0.2100 nm) from two en molecules, one phenolic hydroxyl oxygen (Cr—O, 0.1917 nm) and one carboxylate oxygen (Cr—O, 0.1936 nm) from one dianionic ASA ligand.

**Reaction of \([\text{Cr(ASA)(en)}_2]^{3+}\) with EDTA or apoOTf**

To determine the transfer of \(\text{Cr}^{3+}\) from \([\text{Cr(ASA)(en)}_2]^{3+}\) to EDTA, 1.2 mmol•L\(^{-1}\) EDTA was added to 0.12 mmol•L\(^{-1}\) [Cr(ASA)(en)]\(^{3+}\) in 0.01 mol•L\(^{-1}\) Heps at pH 7.4. The reaction mixture was stored at 37 °C. Both the UV-Vis spectra and fluorescence spectra were monitored as a function of time until the spectra became constant with time. The sample was excited at 280 nm and the emission was monitored from 350 to 500 nm.

To monitor the transfer of \(\text{Cr}^{3+}\) from \([\text{Cr(ASA)(en)}_2]^{3+}\) to apoOTf, solution of 19 µmol•L\(^{-1}\) [Cr(ASA)(en)]\(^{3+}\) with 19 µmol•L\(^{-1}\) apoOTf in 0.01 mol•L\(^{-1}\) Heps at pH 7.4 was stored at 37 °C for one week (period I). Then excessive EDTA was added and the reaction mixture was stored at 37 °C again (period II). Both the difference UV-Vis spectra (blanked as the same concentration of apoOTf) and the fluorescence spectra were monitored as a function of time throughout the course.
Table 1 Crystal data and structure refinement for [Cr(ASA)(en)$_2$]Cl•2H$_2$O

<table>
<thead>
<tr>
<th>Crystal data</th>
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<tbody>
<tr>
<td>Cr$_3$H$_6$N$_5$O$_5$Cl</td>
</tr>
<tr>
<td>$M_r = 394.81$</td>
</tr>
<tr>
<td>Orthorhombic, $P2_12_12_1$</td>
</tr>
<tr>
<td>$a = 0.8369$ (13)</td>
</tr>
<tr>
<td>$b = 0.9650$ (15)</td>
</tr>
<tr>
<td>$c = 2.402$ (4) Å</td>
</tr>
<tr>
<td>$β = 90.00°$</td>
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<tr>
<td>$V = 1.9396(5)$ nm$^3$</td>
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<table>
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<th>Data collection</th>
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<tr>
<td>Bruker SMART CCD area-detector</td>
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<tr>
<td>$ω$ and $φ$ scans</td>
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<tr>
<td>Absorption correction: multi-scan</td>
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<tr>
<td>(SADABS; Sheldrick, 2000)</td>
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<td>$T_{min} = 0.7522, T_{max} = 0.8637$</td>
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<td>7975 measured reflections</td>
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<th>Refinement</th>
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<tr>
<td>Refinement on $F^2$</td>
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<td>$R[F^2 &gt; 2σ(F^2)] = 0.0643$</td>
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<tr>
<td>$wR(F^2) = 0.1816$</td>
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<tr>
<td>$S = 1.212$</td>
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<td>3409 reflections</td>
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<td>209 parameters</td>
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<td>H-atom parameters constrained</td>
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Table 2 Selected bond lengths (nm) and angles (°)

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<tr>
<th>Bond</th>
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<th>Bond</th>
<th>Length (nm)</th>
<th>Bond</th>
<th>Length (nm)</th>
</tr>
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<tr>
<td>Cr(1)—O(1)</td>
<td>0.1936(4)</td>
<td>Cr(1)—O(2)</td>
<td>0.1917(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr(1)—N(1)</td>
<td>0.2060(5)</td>
<td>Cr(1)—N(2)</td>
<td>0.2095(5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr(1)—N(3)</td>
<td>0.2100(5)</td>
<td>Cr(1)—N(4)</td>
<td>0.2081(5)</td>
<td></td>
<td></td>
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<tr>
<td>O(1)-Cr(1)—O(2)</td>
<td>91.25(17)</td>
<td>O(1)-Cr(1)—N(1)</td>
<td>90.07(19)</td>
<td></td>
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<tr>
<td>O(2)-Cr(1)—N(1)</td>
<td>94.02(18)</td>
<td>O(1)-Cr(1)—N(4)</td>
<td>95.0(2)</td>
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<td></td>
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<tr>
<td>O(2)-Cr(1)—N(4)</td>
<td>92.1(2)</td>
<td>N(1)-Cr(1)—N(4)</td>
<td>172.0(2)</td>
<td></td>
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<tr>
<td>O(1)-Cr(1)—N(3)</td>
<td>176.1(2)</td>
<td>O(2)-Cr(1)—N(3)</td>
<td>89.76(18)</td>
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<td>N(1)-Cr(1)—N(3)</td>
<td>93.64(19)</td>
<td>N(4)-Cr(1)—N(3)</td>
<td>81.2(2)</td>
<td></td>
<td></td>
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<tr>
<td>O(1)-Cr(1)—N(2)</td>
<td>87.27(18)</td>
<td>O(2)-Cr(1)—N(2)</td>
<td>175.74(19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N(1)-Cr(1)—N(2)</td>
<td>81.99(19)</td>
<td>N(4)-Cr(1)—N(2)</td>
<td>92.0(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N(3)-Cr(1)—N(2)</td>
<td>92.0(2)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

The UV-Vis spectrum of [Cr(ASA)(en)$_2$]Cl is shown in Figure 2. The characteristic absorption peak of the free H$_2$ASA occurred at 298 nm (curve a), while in the complex this peak exhibited a considerable red shift to 315 nm (curve b). The electronic spectrum in the visible region of the complex (curve c, attributed to d-d transitions of the Cr$^{3+}$) was altered in intensity and shifted in position of the adsorption bands relative to the corresponding aquo Cr$^{3+}$ ions. Figure 3 displays the fluorescence spectrum of the free H$_2$ASA in 0.01 mol•L$^{-1}$ Hepes at pH 7.4 with a maximum emission peak near 410 nm (curve b). With the coordination of H$_2$ASA to Cr$^{3+}$ the fluorescence intensity at 410 nm was quenched and the title complex hardly exhibited any fluorescence intensity.
in the same condition, as shown in Figure 3 (curve a).

**Figure 2** Absorption spectra of \([\text{Cr}(\text{ASA})(\text{en})_2]^+\) and \(\text{H}_2\text{ASA}\).
(a) \([\text{H}_2\text{ASA}]=6.7 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}\), (b) \([\text{Cr}(\text{ASA})(\text{en})_2]^+]=5.5 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}\), (c) \([\text{Cr}(\text{ASA})(\text{en})_2]^+]=2.0 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}\).

**Figure 3** Fluorescence spectra of \([\text{Cr}(\text{ASA})(\text{en})_2]^+\) and \(\text{H}_2\text{ASA}\).
(a) \([\text{Cr}(\text{ASA})(\text{en})_2]^+]=1.0 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}\), (b) \([\text{H}_2\text{ASA}]=1.0 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}\).

Transfer of \(\text{Cr}^{3+}\) from \([\text{Cr}(\text{ASA})(\text{en})_2]\text{Cl}\) to EDTA
Chromium(III) complexes are substitutionally inert, which are usually required many days for the transfer studies of \(\text{Cr}^{3+}\). In order to research the interaction of the complex with transferrin, EDTA was employed as a simple competitive ligand firstly \((K_{\text{Cr-EDTA}}=10^{23})\). As can be seen in Figure 4, the absorption peak at 315 nm for coordinated \(\text{H}_2\text{ASA}\) decreased gradually and the peak at 298 nm for free \(\text{H}_2\text{ASA}\) increased (Figure 4). A well-defined isosbestic point at 310 nm was maintained throughout the course of the reaction. Meanwhile, the fluorescence intensity at 410 nm increased gradually (Figure 5). The second-order rate constant \(k\) was obtained using standard fitting procedures. The \(k\) value obtained from the absorption spectra is \(k'=2.6 \pm 0.1\times 10^{-3} \text{ mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}\), and that obtained from fluorescence spectra is \(k''=3.6 \pm 0.5 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}\). The difference between them is not statistically significant. The control experiment shows that the complex is stable in Hepes buffer at 37 °C alone. It can be concluded that \(\text{Cr(III)}\) was combined by EDTA and the ASA or en ligands were competitively replaced.

**Figure 4** Absorption spectra at different time for the mixture of \(0.142 \text{ mmol} \cdot \text{L}^{-1} \text{Cr}(\text{ASA})(\text{en})_2\text{Cl}\) with \(1.42 \text{ mmol} \cdot \text{L}^{-1} \text{EDTA}\) at 37 °C for time/h (1) 0; (2) 25; (3) 50; (4) 75; (5) 100; (6) 150; (7) 205 h. Inset: a plot of \(\ln [(a-x)/(b-x)]/(a-b)\) vs. time, \(a\) = 0.142 mmol\text{L}^{-1}, \(b\) = 1.42 mmol\text{L}^{-1}, \(x=a(A_0-A_t)/(A_0-A_\infty), r=0.995\).

**Figure 5** Fluorescence changes with time (a→d) for the mixture of \(0.12 \text{ mmol} \cdot \text{L}^{-1} \text{[Cr}(\text{ASA})(\text{en})_2]\text{Cl}\) with \(1.42 \text{ mmol} \cdot \text{L}^{-1} \text{EDTA}\) at 37 °C in 0.01 mol\text{L}^{-1} Hepes at pH 7.4 for time (h): (a) 0; (b) 3; (c) 30; (d) 50 h. Inset: a plot of \(\ln [(a-x)/(b-x)]/(a-b)\) vs. time, \(a=85 \mu\text{mol} \cdot \text{L}^{-1}, b=1.42 \text{ mmol} \cdot \text{L}^{-1}, x=a(F_0-F_t)/(F_0-F_\infty), r=0.995\).

For convenience, all the characteristic fluorescence peaks or UV-Vis absorption peaks appeared in this paper are summarized in Table 3.

**Table 3** Characteristic fluorescence peak or UV-Vis absorption peak

<table>
<thead>
<tr>
<th>H$_2$ASA</th>
<th>ASA-Cr</th>
<th>ApoOTf</th>
<th>Cr-OTf</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV peak/nm</td>
<td>298</td>
<td>315</td>
<td>280</td>
</tr>
<tr>
<td>Molar absorptivity/(\varepsilon_{298}) (mol$^{-1}$L$^{-1}$cm$^{-1}$)</td>
<td>8610</td>
<td>6600</td>
<td>91,200</td>
</tr>
<tr>
<td>Fluorescence peak</td>
<td>410</td>
<td>410</td>
<td>336</td>
</tr>
</tbody>
</table>

Previously we synthesized the similar complex \([\text{Cr}(\text{SSA})(\text{en})_2]^+\) \((\text{H}_3\text{SSA} = 5\text{-sulfosalicylic acid})\). The comparisons of \(\text{Cr—O}\) bond lengths and kinetics constants \(k\) between \([\text{Cr}(\text{SSA})(\text{en})_2]^+\) and \([\text{Cr}(\text{ASA})(\text{en})_2]^+\) are shown in Table 4. It can be seen the kinetics constants

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the [Cr(ASA)(en)]2+ are slightly bigger than those of Cr(SSA)(en). The differences arise from the different stability of the two complexes, which can be reflected by the average band lengths in Table 4.

Table 4 Comparisons of Cr—O bond lengths and rate constants k between [Cr(ASA)(en)]2+ and Cr(SSA)(en)

<table>
<thead>
<tr>
<th>k</th>
<th>[Cr(ASA)(en)]2+</th>
<th>Cr(SSA)(en)2</th>
</tr>
</thead>
<tbody>
<tr>
<td>+EDTA</td>
<td>(3.6±0.5)×10^-10</td>
<td>(3.3±0.3)×10^-10</td>
</tr>
<tr>
<td>+apoOTf</td>
<td>(3.0±0.2)×10^-10</td>
<td>(2.6±0.2)×10^-10</td>
</tr>
<tr>
<td>Cr—O bond lengths/nm</td>
<td>0.1936(4), 0.1899(4), 0.1929(4)</td>
<td>0.2084, 0.2076</td>
</tr>
</tbody>
</table>

Transfer of Cr^3+ from [Cr(ASA)(en)]2Cl to apoOTf

The iron-transport protein transferrin, the second most abundant protein in blood serum, has been proposed to serve as the major chromium transport agent. Binding constants of chromium to transferrin was reported to be the major chromium transport agent. The iron-transport protein transferrin, the second most abundant protein in blood serum, has been proposed to serve as the major chromium transport agent.

In period I, the fluorescence of apoOTf at 336 nm (characteristic of tryptophan residue) was quenched as the reaction progressed (a → c), as shown in Figure 6. Of interest is that a peak at 410 nm for the free H2ASA does not appear as expected, although the free H2ASA exhibits a strong fluorescence peak at 410 nm under the same conditions (shown in Figure 7). The k value was calculated by monitoring the change of fluorescence intensity at 410 nm with time, k = (3.0±0.2)×10^-10 mol•L^-1•s^-1. Figure 8 shows the changes of difference UV spectra of the mixture, which was blanked as the same concentration of apoOTf solution. It reveals a significant enhancement in the absorbance in 291—240 nm gradually. Surprisingly, changes of the peak at 315 nm for the coordinated H2ASA are barely visible. The poor quality data of difference UV-Vis spectra in Figure 8 is due to the high concentration of the protein. The k value was calculated by monitoring the change of absorption at 291 nm, k = (2.6±0.3)×10^-10 mol•L^-1•s^-1. Both the changes of absorption spectra and fluorescence spectra show that Cr^3+ has been transferred from the title complex to apoOTf.

![Figure 6](image1)

**Figure 6** Fluorescence spectra at different time for the reaction of 19 μmol•L^-1 [Cr(ASA)(en)]2+ with 19 μmol•L^-1 apoOTf at 37 °C. Inset: a plot of 1/(a-x) vs. time, a = 19 μmol•L^-1, x = a(F0 – F)/F0, r = 0.992.

In period I, the fluorescence of apoOTf at 336 nm (characteristic of tryptophan residue) was quenched as the reaction progressed (a → c), as shown in Figure 6. Of interest is that a peak at 410 nm for the free H2ASA does not appear as expected, although the free H2ASA exhibits a strong fluorescence peak at 410 nm under the same conditions (shown in Figure 7). The k value was calculated by monitoring the change of fluorescence intensity at 410 nm with time, k = (3.0±0.2)×10^-10 mol•L^-1•s^-1. Figure 8 shows the changes of difference UV spectra of the mixture, which was blanked as the same concentration of apoOTf solution. It reveals a significant enhancement in the absorbance in 291—240 nm gradually. Surprisingly, changes of the peak at 315 nm for the coordinated H2ASA are barely visible. The poor quality data of difference UV-Vis spectra in Figure 8 is due to the high concentration of the protein. The k value was calculated by monitoring the change of absorption at 291 nm, k = (2.6±0.3)×10^-10 mol•L^-1•s^-1. Both the changes of absorption spectra and fluorescence spectra show that Cr^3+ has been transferred from the title complex to apoOTf.
the fate of en and H$_2$ASA ligands. According to the coordination demands or binding abilities of the transferrin site, an en molecule does not fit for the demands of a synergistically bound anion.$^{27}$

Then, in order to confer the fate of ASA in period I, excessive EDTA was added to the solution in period II. The sample is referred for simplicity as [ASA-Cr-OTf]$^{+}$EDTA. With the addition of 250—2500 equiv. of EDTA, the absorption bands at 315 nm and in ca. 240—291 nm all markedly decreased, while a new peak at 298 nm for free H$_2$ASA appeared (Figure 9). Meanwhile, the quenched fluorescence intensity of apoOTf at 336 nm was enhanced with the concomitant appearance of fluorescence for free H$_2$ASA at 410 nm (Figure 10). It clearly implies that the coordinated Cr-apoOTf and Cr-ASA decreased, while the free apoOTf and H$_2$ASA are increased in the presence of excess EDTA. Therefore, it can be concluded that a very stable complex [ASA-Cr-apoOTf] is formed in period I, and then destroyed by excessive EDTA in period II.

A number of literatures$^{28,29}$ have indicated that dozens of organic anions could substitute carbonate as the synergistically bound anions in an iron-transferrin complex. A carboxylate group and a proximal polar group (hydroxy, keto, amino, or carboxylate group) within 0.63 to 0.70 nm of the carboxylate are required for synergistic binding. Dubach and his coworkers$^{29}$ used EPR spectra to examine the mode of synergistic anion binding. It was proposed that these anions behaved as bidentate ligands, with coordination to the iron through both the carboxylate and proximal polar groups. An SA-Fe$^{3+}$-transferrin complex shows a characteristic charge transfer band in the visible spectrum with $\lambda_{\text{max}}$=450 nm, implying a direct metal-anion bonding. Salicylic acid (H$_2$SA) and its substituted derivate 4-aminosalicylic acid (H$_2$ASA) have the similar carboxylic group and phenolic hydroxyl (The $pK_a$ values of salicylic acid are 2.97 and 13.40 for the carboxylic and phenol groups, respectively. The dissociation constants of H$_2$ASA are not known and expected to be of the same order$^{31}$). Does the dianionic H$_2$ASA ligand have also the same function as the synergistically bound anion? What we concern about is the role of amino group of the H$_2$ASA molecule in the hydrophobic pockets of apoOTf; the results obtained in this paper indicated that the dianionic ASA served as the role of a synergistically bound anion, and the additional amino group did not prevent the ASA from inserting into the hydrophobic pockets of apoOTf. Based on the above discussion, it can be inferred that a dianionic ASA serves as the role of a synergistically bound anion when Cr$^{3+}$ is transferred to the protein from the title complex and an ASA-Cr$^{3+}$-transferrin ternary complex is formed. Cr$^{3+}$ is six coordinated; two bonds are from Cr-ASA and the others four from Cr-OTf, respectively. Perhaps the summing-up can be illustrated in Scheme 1.

**Scheme 1**

Crystallographic data for the compound has been deposited with the Cambridge Crystallographic Data Centre by a CCDC No. 289528. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: 0044 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

**References**
