Pre-formed iron-thiosemicarbazone chelates as ribonucleotide reductase inhibitors

Anupa Murugkar & Subhash Padhye*
Department of Chemistry, University of Pune,
Pune, 411 007, India
and
Lena Vorobjeva
Biological Faculty, Department of Microbiology, Moscow State
University, Moscow 119899, Leninskie Gori, USSR

Received 28 June 2002; revised 4 April 2003

Pre-formed iron(III) complexes of naphthoquinone thiosemicarbazone ligands, viz. 2-hydroxy-1,4-naphthoquinone-1-thiosemicarbazone (HNQTSC) and 2-thiosemicarbazido-1,4-naphthoquinone (TSCNQ) have been synthesized, characterized by various physical techniques and their potential for inhibition of ribonucleotide reductase (RDR) enzyme, crucial for DNA synthesis, has also been evaluated. It is observed that the pre-formed iron(III) complexes possess higher RDR inhibitory activity than the respective free ligands.

Ribonucleotide reductase (RDR) plays a central role in the formation and maintenance of the optimal levels of deoxyribonucleoside triphosphates which are required for DNA replication and DNA repair processes. The enzyme is found in mammalian cells, DNA viruses of the herpes group and some prokaryotes. Due to its importance in DNA synthesis, RDR is a potential target for the development of anticancer and antiviral agents.

Heterocyclic thiosemicarbazones have been shown to be effective inhibitors of RDR by Sartorelli et al., due to their chelating ability which can result in scavenging out the iron centers necessary for the enzyme activity. The other known inhibitors of RDR include hydroxyurea, guanazole and pyrazole-imidazole derivatives. Supplementation with exogenous iron was found to reverse the inhibition by hydroxyurea and guanazole but only partially reversed the inhibition of DNA synthesis by the thiosemicarbazones. This has led researchers to suspect that the active form of the inhibitor is in fact an iron complex formed in situ and hence metal complexes of several thiosemicarbazone derivatives have been investigated as putative inhibitors of the enzyme. This led us to evaluate the RDR inhibitory activities of two ferric complexes of naphthoquinone thiosemicarbazones in the bacterial cultures of Propionibacterium jenseni. The ferric compounds were earlier shown to possess excellent antitumor properties. The quinone motif present in the present compounds is a common structural unit present in many anthracycline anticancer drugs and hence the present studies have a relevance to the anticancer drug design.

Experimental

The ligands, 2-hydroxy-1,4-naphthoquinone-1-thiosemicarbazone (HNQTSC) and 2-thiosemicarbazido-1,4-naphthoquinone (TSCNQ) were prepared according to literature methods with a slight modification. Fe(III) complexes of these ligands were synthesized by the reaction of equimolar quantities of the corresponding thiosemicarbazone ligands and ferric chloride hexahydrate at pH 6-7 in methanol solvent under nitrogen atmosphere at 50°C for 3 h. The reaction mixture was stored in a refrigerator overnight and the microcrystalline solid separated out was filtered, washed with cold methanol and ether and then dried in vacuum at room temperature. The compounds were analyzed as follows:

\[
[\text{Fe(HNQTSC)Cl}_2] \cdot 2\text{H}_2\text{O}, \text{ Cal (\%)} C = 32.30, H = 2.95, S = 7.84, \text{Cl} = 17.33, \text{Fe} = 13.65; \text{Found (\%)} C = 31.86, H = 2.93, S = 7.35, \text{Cl} = 16.56, \text{Fe} = 13.31.
\]

\[
[\text{Fe(TSCNQ)Cl}_2] \text{Cl}, \text{ Cal (\%)} C = 45.26, H = 2.76, S = 10.96, \text{Cl} = 6.08, \text{Fe} = 9.56; \text{Found : C = 44.75, H = 2.70, S = 10.65, Cl = 5.89, Fe = 9.82.}
\]

IR spectra of the compounds were recorded in nujol mulls on a Perkin-Elmer 283-B Infra-red spectrophotometer in the range 4000-600 cm\(^{-1}\), while far IR spectra were recorded on a Polytec FIR-30 spectrophotometer. Electronic spectra were recorded in DMF solvent on a Hitachi-220A spectrophotometer using 1 cm\(^2\) rectangular quartz cells. The variable temperature magnetic susceptibility measurements were carried out from 300 to 16 K on a Faraday type balance with continuous Helium gas-Flow Closed Cycle Refrigerator (Air Products). X-band powder and DMF solution EPR at room temperature and liquid nitrogen temperature were recorded on Varian 109-ESR spec-

E-mail: sbpadhye@chem.unipune.ernet.in; Fax : 91-20-5691728
trometer with 100 KHz field modulation amplitude. The field was calibrated using DPPH marker. Cyclic voltammetric measurements were made in DMSO solvent using tetraethyl ammonium perchlorate (TEAP) as supporting electrolyte with the help of BioAnalytical System (BAS) cyclic voltammetric model CV-27 with XY recorder under a dry and pure nitrogen atmosphere. The three-electrode system employed consisted of platinum working electrode, platinum wire as auxiliary electrode and Ag/AgCl as reference electrode.

The ligands and their ferric compounds were evaluated for the RDR inhibitory activities on Propionibacterium jensenii using hydroxyurea as a standard inhibitor by the method described by Theander.

The propionic acid bacteria were grown for three days at 30°C in anaerobic conditions in a minimal glucose medium supplemented with 2% glucose, 0.3% (NH₄)₂SO₄, 0.25% KH₂PO₄, 0.0005% MnSO₄.7H₂O, 0.002% NaCl, 0.001% FeCl₃.6H₂O, 0.005% ZnSO₄, 0.001% CoCl₂.6H₂O, 1000 mKg/l calcium panthonate, 200 mKg/l Vitamin B1 and 1.00 mKg/l biotin in distilled water. The pH of the culture was between 6.8 to 7.0. The biomass was separated out by centrifugation at 15000 g for 20 min and was washed twice with 0.05 M sodium phosphate buffer (pH 7.4). It was resuspended in the same buffer in 1 : 2 (w/v) ratio. The cell suspension was sonicated (40s × 3) at 4°C and then centrifuged at 200000 g for an hour at 4°C. The cell extracts were then dialyzed against 0.05 M tris-buffer of pH 7.3 to 7.5 for 15-20 h at 10°C.

RDR inhibitory assay

The incubation mixture was prepared by mixing cell extract (4 mg /ml RDR) with different concentrations of compounds ranging from 0.26 µM to 260 µM in presence of dithiothreitol (30 mM), ATP (2mM) and Tris-buffer (0.1 M, pH 7.0). After preparation of the incubation mixture, two sets of 3 probes each were taken. In one set, the inactivated RDR enzyme (heated at 100°C for 5 min) was added (control), while the active enzyme was added in another set (test). After incubation at 37°C for 1 h, the concentration of deoxyribose was determined by diphenylamine method. Difference between the activities of samples (control minus test) gives the accumulation of dADP as a result of RDR reaction.

Results and discussion

The interaction of HNQTSC with anhydrous FeCl₃ in 1:1 ratio in aqueous methanol gave a monoligand complex having the general formula [Fe(HNQTSC)Cl]₂H₂O (I). The conductivity measurements in DMF solvent indicate the non-electrolyte nature of this compound, whereas the reaction of FeCl₃ with TSCNQ yielded the compound [Fe(TSCNQ)₃]Cl (I) which is a 1:1 electrolyte (78 Ω cm mole) in DMF solvent.

In theIR spectra, the absorption due to the C2-hydroxyl group and asymmetric and symmetric stretching modes of the terminal -NH₂ group of the free ligands are observed in the range of 3180 to 3300 cm⁻¹. The absorption due to H-bonded C2-hydroxyl group is found to disappear on metal complexation indicating its replacement by metal ion. This is further supported by the disappearance of C-O stretch observed at 1210 cm⁻¹ in the free ligand on metal complexation. Similarly, the band at 1600 cm⁻¹ ascribed to the C=N stretching absorption in the ligand is shifted to lower energy side (Δ = 20 cm⁻¹) on metal complexation indicating its involvement in coordination. Finally, the thiocarbonyl stretching vibration observed at 820 cm⁻¹ is found to be lowered on complexation confirming the involvement of thione sulphur in coordination. [Fe(HNQTSC)Cl]₂H₂O exhibit an absorption at 445 cm⁻¹ indicative of the thione coordination, whereas [Fe(TSCNQ)₃]Cl show similar absorption at 415 cm⁻¹ corresponding to thiol form. The metal-oxygen vibrations for these compounds appear at 520 and 515 cm⁻¹, while metal-nitrogen vibrations at 360 and 340 cm⁻¹ respectively.

The variable temperature magnetic susceptibility measurements on [Fe(HNQTSC)Cl]₂H₂O between 16-293 K reveal that they can be best fitted for a five-coordinate species having S = 3/2 ground state. Its EPR spectrum at 77 K gives a sharp signal at 2.18 and
a poorly resolved band at \( g = 4.23 \) confirming the spin-quartet \((S = 3/2)\) ground state. On the other hand, the magnetic moment of \([\text{Fe(TSCNQ)}_2]\text{Cl}\) at 295 K is observed to be 4.42 B.M., which is lower than the one expected for a sextet ground state. It exhibits only a slight drop in the lower temperature range (3.44 B.M. at 15 K). The most plausible explanation for such a behaviour can be given on the basis of an intermediate spin \((S=3/2)\) state. The EPR spectrum of this compound at 298 K exhibits a signal around \( g = 2 \) ascribed to the \(-1/2 \rightarrow +1/2\) transition, while another signal with a reduced resolution is observed centered around \( g=4 \). At 77 K, the former band is however, found to split due to rhombic symmetry. The absence of the signal at \( g=6 \) suggests \( S \) = \( \frac{1}{2} \) as the ground state.

The absorption spectra of the two complexes is dominated by the charge transfer bands (CT), while the low intensity spin-forbidden transitions are obscured. The cyclic voltammogram of the iron complex of HNQTSC shows two irreversible reduction peaks, +0.20 V and \(-1.50\) V, of which the former corresponds to the reduction of Fe(III) to Fe(II) species, while the later represents the reduction of the azomethine group \(^{14}\) respectively. The absence of the oxidation counterpart for the peak at +0.20 V probably indicates that the Fe(II) species formed containing this ligand system is unstable. The ligand TSCNQ exhibits cathodic peaks at \(-0.56\) and \(-1.45\) V, of which the first peak corresponds to the one electron reduction of quinone ring while the second one corresponds to the conversion of semiquinone to catechol. This is in agreement with the reduction potentials found for 1,4-naphthoquinone derivatives in DMF and catechol compounds studied by several workers \(^{15}\). The ferric complex of this ligand shows a reversible peak centered at +0.25 V which can be assigned to a Fe(II)/Fe(III) redox couple. The peak corresponding to the azomethine reduction appears at \(-1.33\) V in this compound.

The % RDR inhibitory activities of all compounds were determined and compared with hydroxyurea as a positive control. It is observed that the ligand HNQTSC and its ferric complex have higher RDR inhibitory activity than hydroxyurea (HU) which is a standard inhibitor of RDR while the ligand TSCNQ and corresponding complex exhibit lower inhibitions. Since the parent naphthoquinone ligands differ only in the presence of a hydroxyl group at C-2 position it can be concluded that it is essential for the RDR inhibitory activity. Similar observation has recently been made by Smith & Douglas in case of lapachol \(^{16}\).

The present work has thus revealed that the preformed iron complexes of naphthoquinone thiosemicarbazones are potent inhibitors of RDR enzyme exhibiting higher activities than their parent ligands which provides support to the suspicion of some researchers that the active forms of the thiosemicarbazone ligands are actually their iron compounds formed \textit{in situ}. It needs to be investigated further which stereochemistries are more suitable for the task and which metal redox potentials favour such inhibitions as it would offer very useful criterion for designing active anticancer drug molecules.

**Acknowledgements**

Dr Rajeev Chikate and Dr. A.R.Belapure are thanked for providing samples of metal complexes for comparison. APM would like to acknowledge CSIR, New Delhi for financial support.

**References**


