Journal of Radioanalytical and Nuclear Chemistry, Articles, Vol. 162, No. 1 (1992) 139-144

PREPARATION, CHARACTERIZATION AND BIODISTRIBUTION OF SALICYLIDENE- AND PYRIDOXYLIDENE-THYROXINE COMPLEXES OF TECHNETIUM-99m

M. S. IQBAL,*+ M. H. RIZVI,** A. NAQVI**

**Pakistan Atomic Energy Commission (DNFM), P.O. Box 1114, Islamabad (Pakistan)*

***Nuclear Chemistry Division, Pakistan Institute of Nuclear Science and Technology, P.O. Nilore, Islamabad (Pakistan)*

(Received January 9, 1992)

New Schiff bases, salicylidene- and pyridoxylidene-thyroxine have been prepared and characterized. They were labeled with ^{99m}Tc. The labeling yield of the Schiff bases was over 95%. About 60% of the activity was bound to γ-globulin and albumin fractions when the labled compounds were incubated with a serum sample. The labeled compounds, after i.v. administration to rats, rapidly cleared from blood and excreted into the small intestine. They appeared to behave as hepatobiliary agents.

Introduction

Transition metal complexes of salicylidene-amino acid and analogous pyridoxal-amino acid Schiff bases are of considerable importance in biological systems.[1-4] These ligands are divalent anions with tridentate ONO donors capable of forming very stable chelates. Some of the Schiff base complexes with technetium(99m) have been shown to be useful as radiopharmaceuticals for diagnosis.[5,6] Therefore, it is relevant to prepare various technetium(99m)-labeled Schiff bases to study their potential in nuclear medicine. In the present work technetium(99m) complexes of salicylidene- and pyridoxylidene-thyroxine are prepared and studied. Thyroxine (tetraiodothyroxine, T_4) is naturally occurring in biological system. It is produced in the thyroid. Therefore, the biodistribution of technetium(99m)-labeled Schiff bases derived from thyroxine may be of special interest to nuclear medical scientists.

+Author to whom all correspondence should be addressed.

Elsevier Sequoia S. A., Lausanne
Akadémiai Kiadó, Budapest

Materials and methods

Preparation of ligands

1-Thyroxine (78.0 mg, 0.1 mmol) was dissolved in 5 ml of 1% potassium hydroxide solution (in ethanol) with gentle warming. Salicylaldehyde (12.2 mg, 0.1 mmol) was dissolved in ethanol (5 ml). Salicylidene-thyroxine (Sal-T_4) was prepared by mixing the two solutions at room temperature. A color change from light yellow to deep lemon yellow was observed. The Schiff base was crystallized out as potassium salt on reducing the volume of the solution by evaporation. It was recrystallized from methanol. Anal. Calcd for $C_{22}H_{14}I_4KNO_5$: C, 28.73; H, 1.54; N, 1.52. Found: C, 28.70; H, 1.52; N, 149.

Pyridoxylidene-thyroxine (Pyr-T_4) was prepared similarly by using pyridoxal hydrochloride (20.4 mg, 0.1 mmol) instead of salicylaldehyde in the above procedure. Anal. Calcd for $C_{23}H_{17}I_4KN_2O_6$: C, 28.63; H, 1.78; N, 2.91. Found: C, 28.67; H, 1.79; N, 2.89.

Characterization of the ligands

Microanalysis was carried out by usual techniques. Infrared spectra were recorded on a Perkin-Elmer 882 spectrophotometer using Nujol mull and KBr disc techniques. Proton NMR spectra were obtained with a 90 MHz Perkin Elmer instrument using TMS as an internal standard and CD_3OD as a solvent. Solution spectra were recorded on a Hitachi 220S UV–Vis spectrophotometer using ethanol as the solvent.

Tagging the ligands with ^{99m}Tc

The tagging was carried out at an alkaline pH because at an acidic pH the Schiff base ligands tend to hydrolyze. The procedure followed was similar to a reported method.[5] Stannous chloride (25.6 mg/ml) was dissolved in 0.004% ascorbic acid solution. To 2 ml of this solution 0.01 mmol of Sal-T_4 or Pyr-T_4 dissolved in 5 ml KOH solution (1% in ethanol) was added. The pH of the mixture was adjusted to 9 with the KOH solution. The resulting yellow solution was stirred for 5 minutes and filtered through a Millipore filter (0.22 µm, GS type). To 1.5 ml of the yellow solution a pertechnetate (1.5 ml, containing 1 mCi ^{99m}Tc) saline eluate from the Amersham technetium generator was added and the reaction vial was incubated at room temperature for one hour to obtain the complexes in solution.

Thin layer chromatography (TLC) of the ^{99m}Tc complexes:

Pre-coated silica gel plates (0.2 mm thick from E. Merck) were used with two solvent systems. In the first system the TLC plate was charged with the solution of the complex

(obtained above) and developed to about 6 cm with acetone. The plate was dried and again developed in the same direction to about 3 cm with 0.9% saline. The dried plate was cut into strips (5 mm) and each strip was counted for the radioactivity using a well-type γ-counter. In this system pertechnetate moves with acetone to the solvent front while the bound and reduced activity remains at the origin, whereas with saline the bound activity moves to the solvent front. In the second system using methanol-water-methyl ethyl ketone (45:5:50 v/v) mixture the chromatogram was developed according to a reported method[5] and counting was carried out as above. In this system pertechnetate moves with the solvent front while ^{99m}Tc–Sn colloid remains at the origin and the labeled compound produces a peak in the middle of the chromatogram ($R_f = 0.5$).

Distribution in serum protein fractions

A serum sample (1 ml) obtained from the blood of a healthy human was incubated at 37 °C with the complex solution (0.5 ml, containing 0.28 mCi activity) for one hour. Then the mixture was subjected to electrophoresis using Sepraphore III cellulose polyacetate strips (2.5 cm × 15.2 cm) and tribarbital-sodium barbital high resolution buffer (pH 8.8) at 350 V for 30 minutes to separate the serum proteins. The separated protein bands were stained with Ponceau S solution (500 mg in 100 ml of trichloroacetic acid), washed with 5% acetic acid, dried, cut and counted using the well-type gamma-counter. This experiment was performed in triplicate.

In vivo studies

Groups of six Sprague Dawley female rats (140—160 g) were injected, intravenously (through tail vein), a 0.3 ml dose of the complex solution containing about 30 μCi activity. The animals were kept in metabolic cages till the time of sacrifice to collect urine. The cumulative urine thus obtained was counted for the radioactivity. Then animals were killed with chloroform vapors and dissected 5, 10, 20, 40, 60, 90 and 120 minutes after the injection. Various organs (small intestine, kidneys and liver) were isolated in plastic cups and counted for radioactivity in the well-type gamma-counter. The blood (5–8 ml) was collected by aortic puncture with a heparinized syringe just before killing the animals.

Results and discussion

Characterization of the ligands

The ligands are readily formed at an alkaline pH according to the scheme shown in Fig. 1. At an acidic pH the Schiff bases hydrolyze and regenerate the aldehyde and thyroxine.

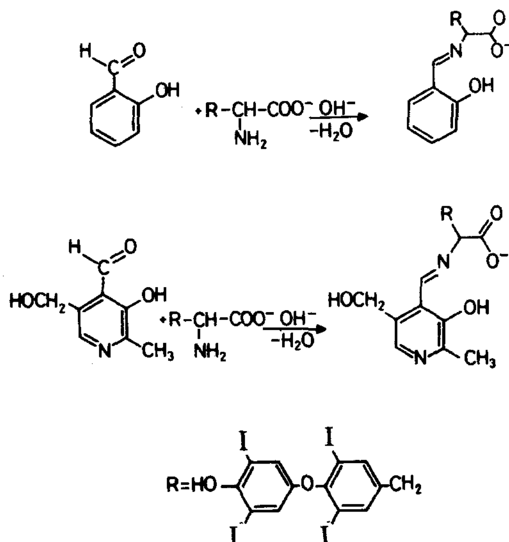

Fig. 1. Reaction scheme for preparation of the Schiff bases

The micro-analytical data for the Schiff bases agreed with the proposed composition of the ligands. The spectroscopic evidence discussed below supports Schiff base formation from the reactants. Along with other bands due to the components present in the Schiff bases the IR spectra of the title ligands contained a strong band at 1620 cm^{-1}, which is due to the $\nu C = N$ and the absence of sharp bands at 3400 and 3500 cm^{-1} due to NH_2 group in thyroxine in the spectrum of Schiff bases are indicative of the fact that the NH_2 group has been condensed with the –CHO group of salicylaldehyde and pyridoxal to produce an imine (CH = N) bond. When the components were mixed, a new absorption band appeared at 375 and 390 nm in the visible region, respectively, for Sal-T_4 and Pyr-T_4, which originates from the C = N chromophore[7] produced after the reaction. The imine formation was further confirmed by the appearance of an aldimine proton signal at 7.6 ppm and disappearance of the aldehyde proton signal at about 9.8 ppm.[8]

Identification of the labeled compounds

The labeled compounds were identified by TLC procedures. The TLC results indicated over 95% labeling yield of the ligands by the procedure used. As the ligands under investigation are divalent tridentates, the Tc-complexes may be tentatively assigned the structures shown in Fig. 2.

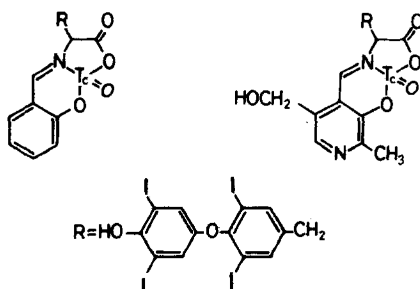

Fig. 2. Proposed structures of the ^{99m}Tc-complexes

Binding of the labeled compounds with serum proteins

For clinical use of radiopharmaceuticals, it is desirable to study their reactivity with the biological system. Therefore, in the first instance, we studied the binding behavior of the complexes under investigation with serum protein fractions in vitro. The results are presented in Table 1. It is seen that about 60% of the activity is bound to γ-globulin and albumin fractions, and that this activity is almost equally distributed among them. The binding of activity with the γ-fraction may be of some significance because of the association of γ-globulins with a sophisticated defence mechanism in the body generally known as the immune response.

Table 1
Binding of the complexes with serum protein fractions

Fraction	Activity, %	
	$Sal\text{-}T_4$	$Pyr\text{-}T_4$
Origin	19.94	12.98
γ	32.33	25.81
φ	2.11	3.75
β	3.63	7.62
α_2	1.51	6.27
α_1	11.78	13.22
Albumin	28.70	30.35

Biodistribution studies

The complexes under study produced the organ distribution graphs similar to those shown in Fig. 3 for $Pyr\text{-}T_4$. It appears that the complexes after i.v. administration, are

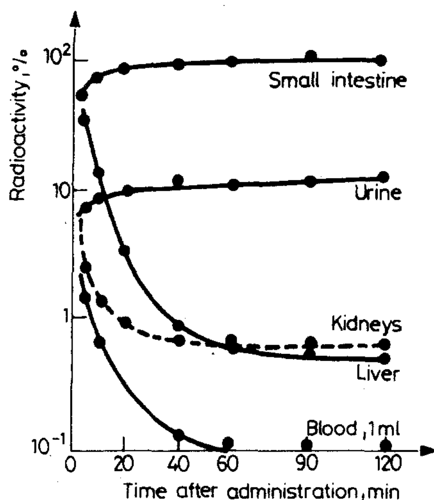

Fig. 3. Biodistribution of Pyr-T_4 in rats. Each point is a mean for six rats. The activity plotted is % of dose in the body. In the case of urine, it is % of total injected dose.The values of blood have been multiplied by 10

rapidly cleared from the blood by the liver and are then excreted into the small intestine. The urinary excretion is relatively small and reaches a plateau within a short period of time after administration. This behavior is comparable with that of the potential hepatobiliary agents,[5,6] which are also Schiff base complexes. The complexes under investigation show a rapid blood clearance as compared to the pyridoxylidene-isoleucine complex.[5] Since the reactive groups of the components (i.e., –CHO and –NH_2) are already quenched by Schiff base formation in these complexes, they are expected to be far less toxic or not at all. Moreover, the Schiff base structure provides them with a greater biocompatibility.

References

1. E. E. SNELL, D. E. METZLER (Eds), Pyridoxal Catalysis: Enzymes and Model Systems, Interscience, New York, 1968.
2. E. E. SNELL, D. E. METZLER (Eds), Chemical and Biological Aspects of Pyridoxal Catalysis, McMillan, New York, 1963.
3. V. M. DOCTOR, J. ORO, Biochem. J., 112 (1969) 691.
4. V. M. VELIKOV, Yu. N. BELOKON, V. A. KARIGINOV, N. S. MARTINKOVA, M. B. SAPOROVSKYA, Izv. Akad. Nauk. SSSR. Khim., 6 (1976) 1276.
5. M. KATO, M. HAZUE. J. Nucl. Med., 19 (1978) 397.
6. M. KATO-AZUMA, Japan J. Nucl. Med., 17 (1980) 575.
7. D. E. METZLER, J. Amer. Chem. Soc., 79 (1957) 485.
8. Yu. N. BELOKON, Izv. Akad. Nauk. SSSR, Ser. Khim., 5 (1978) 1026.