Chemical and Thermodynamic Characteristics of the Isotopic Exchange Reaction between Radioiodine and Iodohippuric Acid Isomers

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The parameters affecting the absolute radiochemical yield of the isotopic exchange reaction between radioiodine (^{125}I) and iodohippuric acid isomers on molten ammonium acetate as a medium exchange at 120°C without any carrier added (radioiodine, ^{125}I) was determined. The isotopic exchange reactions of radioiodine as $^{125}\text{I}^-$ for iodine-127 of *o*- and *p*-iodohippuric acid isomers occur more rapidly than *m*-iodohippuric acid isomer. These reactions proceed by nucleophilic second order substitution reaction. The kinetics and thermodynamic parameters of these isotopic exchange reactions were determined. The absolute radiochemical yield and radio pharmaceutical purity were determined by HPLC and TLC techniques.

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Introduction

Recently, radioiodinated compounds labeled with iodine radionuclides have been developed as clinical radiopharmaceuticals.¹ Such compounds are intended for the incorporation of highly toxic effects associated with the auger electrons emitted from radionuclides onto the DNA or close to the tumor cell membrane.^{1,2} Radioiodinated sodium iodohippurate is a diagnostic radiopharmaceutical currently used in nuclear medicine for the non-invasive determination of renal function, renal blood flow and urinary tract obstruction.³⁻⁷

The properties of radioiodine are reviewed in relation to its use in labeled compounds.^{3,8} Iodine is characterized by a varied spectrum of oxidation states and can form a variety of complex ions and multivalent complex species.⁹ The picture of exchange reaction mechanisms is complex, but can be rationalized in terms of the stabilization of intermediate complexes formed with different (negative, neutral or positive) iodine species.¹⁰

The radioiodination of sodium iodohippurate, or hippuric acid isomers, by the isotopic exchange reaction between the substrate and Na^*I exchange isotopes leads to the formation of other labeling products *e.g.*, glycyl-*o*-iodohippurate (*g-o*-IHA) and *o*-iodobenzoic acid (*o*-IBA).¹¹⁻¹⁹ Thus the mechanism of the isotopic exchange reaction between the iodohippurate isomers and radioiodine (as iodide, $^*\text{I}^-$) in the molten state ($160 - 170^\circ\text{C}$) with no-carrier added (nca) is of great importance.¹⁹⁻²¹

The goal of the present study was focused on the application of the no-carrier added (nca) radioiodine, $^{125}\text{I}^-$ in molten ammonium acetate (m.p. 114°C) for an isotopic exchange reaction between *o*-, *m*- or *p*-iodohippuric acid isomers and radioiodine as iodide ion, $^{125}\text{I}^-$. Ammonium acetate melt controls the formation of other labeling products rather than iodohippuric acid isomers.

Experimental

Reagents and chemicals

All chemicals used were of analytical reagent grade. Sodium iodide [^{125}I] solution was purchased from Amersham (England) of radiochemical grade carrier free and reductant free. A sodium iodide concentration of $(0.8 - 1.0) \times 10^9$ Bq/ μl was diluted with 0.1 M NaOH up to 20 μl to keep the specific activity constant $(0.8 - 1.0) \times 10^9$ Bq/mg iodohippuric acid isomer, during the experiment. Thin-layer chromatography was performed on aluminum backed TL sheets coated with silica gel 60 (Merck). Solvents of HPLC grade were delivered from Sigma. Water used throughout the experiments was obtained from a Milli-Q (MQ) water purification system (Millipore). All glassware were cleaned by soaking in an aqueous 1% Decon-100 for at least 24 h, followed by rinsing with MQ water, and then acetonitrile prior to use.

Apparatus

A Shimadzu a high-performance liquid chromatography (HPLC) equipped with a LC-dual pump, U6K injector, M 441 spectrophotometric detector, RP-C₁₈ column (250×4 mm i.d.) and M 740 recorder was used for the analysis of the radioiodinated iodohippuric acid isomer. A Scalar rate meter (SR 7), scintillation counter and a Metrohm pH-meter were used for radioactivity and pH measurements, respectively.

Synthesis of [^{125}I] iodohippuric isomers

In a quick-fit glass vessel described earlier^{21,22} 6 mg amount of the iodohippuric acid isomer, 20 μl of Na^{125}I $(0.8 - 1.0) \times 10^9$ Bq/mg IHA in 0.1 M NaOH and 0.2–0.25 ml ammonium solution (10% w/v) were mixed in a V-shaped bottom tube. The mixture was gently swirled to dissolve the contents and to insure complete homogeneity. After a gentle stream of dry nitrogen gas was applied to evaporate the solvents, the closed reaction vessel was then kept in an oil bath at $120 \pm 1^\circ\text{C}$ for 10

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min under a vacuum. The reaction mixture was cooled in an ice bath and the solidified melt was then redissolved in 50.0 μ l ethanol, and finally submitted for TLC. The reaction products were characterized and determined after developing with benzene-acetic acid-water-*n*-butanol (5:5:2:1 v/v) on silica-gel plates and drying. The radiochemical yield (*Y*) was calculated by cut and count of the integrated chromatogram peaks as follows:

$$Y, \% = \frac{\text{Radioactivity of the isotopic exchange product}}{\text{Total activity of the TLC plate}} \times 100 \quad (1)$$

The retention factors (R_f) of the IHA isomers, *g*-*o*-IHA, *o*-IBA and Na¹²⁵I were found to equal 0.5 ± 0.02 , 0.25 ± 0.02 , 0.81 ± 0.05 and 0 ± 0.05 , respectively, in good agreement with the data reported by Saeed and Gaffar.²³

HPLC analysis of the radioiodinated iodohippuric acid isomers

Two aliquots (10–20 μ l) were withdrawn from the reaction vessel solution at various time intervals. The first aliquot was pipetted into a test tube for an analysis of the total activity. The second aliquot was injected onto an RP-C₁₈ column with trace amounts of inactive *o*-, *m*- and *p*-iodohippuric acid. The mobile phase, methanol:water:acetic acid (300:700:1 v/v), was passed through C₁₈ column with a flow rate of 1.0 ml/min until the absorbance appeared to be constant at 254 nm. The temperature of the column was controlled by a temperature controller, and was kept at $25 \pm 1^\circ\text{C}$. A frontal chromatogram was recorded while the mobile phase was eluted and collected discontinuously under the optimum conditions. The radioactivity of the fractions and the aliquot were determined by a γ -counter. The absolute radiochemical yield was calculated by collecting the radioactive peaks and comparing their activities with that of a sample of the same volume that was not HPLC chromatographed. The HPLC peak of each radioactive iodohippuric isomer has the same retention time (t_R) as the inactive sample. The t_R of *o*-, *m*- and *p*-IHA isomers were found to equal 3.48 ± 0.1 , 10.6 ± 0.08 and 11.32 ± 0.05 min, respectively.

Results and Discussion

Consider that the thermodynamics of the exchange labeling is a degenerate substitution reaction, where the reaction products are chemically identical to the starting materials. Therefore, the energy of the products will be the same as the reactants and the equilibrium constant will be equal to unity. Thus, the mechanism of the isotopic exchange reaction could be proceeded as follows:⁶

$$K = \frac{[\text{R}^*\text{I}][\text{I}^-]}{[\text{RI}][{}^*\text{I}^-]} = 1 \quad (2)$$

$$\frac{[\text{R}^*\text{I}]}{[{}^*\text{I}^-]} = \frac{[\text{RI}]}{[\text{I}^-]} \quad (3)$$

$$\% \text{ Exchange} = \frac{[\text{R}^*\text{I}]}{[\text{R}^*\text{I}] + [{}^*\text{I}^-]} \times 100 = \frac{100}{1 + [\text{I}^-]/[\text{RI}]} \quad (4)$$

At equilibrium, the proportion of the incorporated radioiodine *i.e.* the labeling yield, is an inverse function of the ratio of the carrier iodine to the substrate. The yield drops dramatically

above a ratio of about 0.1 iodide to the substrate ratio. For no-carrier added radioiodine, the exchange yield based on the equilibrium considerations should be greater than 99.9% for the displacement of halogen or other leaving groups from iodohippuric acid isomers.

The parameters affecting the isotopic exchange reaction of ^{*}I[−] with IHA isomers

A preliminary investigation showed the importance of the parameters: pH, temperature, exchange medium and substrate concentration on the rate of the isotopic exchange reaction of radioiodine as ^{*}I[−] and iodohippuric acid isomers in a molten ammonium acetate. Three components (Fig. 1) were formed corresponding to *g*-*o*-IHA, IHA isomer and *o*-IBA when the isotopic exchange was performed in molten medium of the substrate, itself, at 160–170°C. These data are not in good agreement with the known principles of nucleophilic substitution.^{6,21}

The influence of sodium hydroxide concentration (0.02–0.1 M) containing most of the dispensed radioactive iodine on the radioiodination of iodohippuric acid was studied. An excess of sodium hydroxide concentration up to 0.1 M inhibited the yield of the isotopic exchange reaction to less than 75%. A good radiochemical yield was obtained at 0.03 M sodium hydroxide concentration. The isotopic exchange reactions proceed very rapidly close to the neutralization equivalent point.⁶ Thus, in subsequent work, neutralization of the radioactive sodium iodide solution was carried out before performing the experimental procedures and the isotopic radiodination exchange reaction of IHA isomers was also performed at 0.03 M NaOH.

The influence of the iodohippuric acid isomer content on the radiochemical yield in a molten medium of ammonium acetate at 120°C was investigated. The radiochemical yield gradually increased up to 98.2, 97.2 and 98.5% for *o*-, *m*- and *p*-¹²⁵IHA isomers, respectively at 6 mg of each IHA isomer. Thus, a 6 mg portion of the iodohippuric acid isomer was used in the subsequent work.

The influence of ammonium acetate (0–50 mg) as a molten medium on the isotopic exchange reaction of the IHA isomers and radioiodine (^{*}I[−]) at 120°C for 10 min reaction time was investigated. The radiochemical yield of *o*-, *m*- and *p*-¹²⁵IHA isomers increased linearly from ~82, 79.4 and 86 to 98.2, 97.3 and 98.3 upon increasing ammonium acetate from 5 to 25 mg, respectively. The observed increase in the radiochemical yield is most likely attributed to the *in situ* thermal decomposition of ammonium acetate rendering the final pH of the reaction mixture on the acidic side required to perform the isotopic exchange reaction.⁶

The effect of the temperature (25–120°C) and reaction time (0–30 min) on the radiochemical yield of the radioiodinated IHA isomer was studied with 6 mg IHA isomer, 25 mg ammonium acetate and 20 μ l Na¹²⁵I in dry state in a thermostat glycol path. A radiochemical yield of 98.2, 97.2 and 98.5% was obtained for *o*-, *m*- and *p*-¹²⁵IHA, respectively, at 120°C within 10 min. Thus, in subsequent experiments, the temperature was selected to be 120°C.

Kinetics and thermodynamic studies

The isotopic-exchange radioiodination reaction of the IHA isomer and the radioactive iodine as ^{*}I[−] can be expressed as

This reaction can be considered as a simple homogeneous

Fig. 1 Radiochromatograms of the nucleogenic isotopic exchange radioiodination reaction of *o*-IHA with (-----) and without (—) ammonium acetate.

radioisotope exchange reaction, and its kinetics follows the exponential exchange law:^{6,20}

$$-\ln(1 - F) = \frac{[A] + [B]}{[A] \cdot [B]} R t \quad (5)$$

where F is the fraction of the exchange, $[A]$ for the concentration of IHA in g mol^{-1} , $[B]$ the concentration of Na^{125}I (nca), R for isotopic exchange rate and t for time in min. The fraction of the exchange was calculated employing

$$F = \frac{X}{X_{\infty}} \quad (6)$$

where X and X_{∞} are the radiochemical yields (% labeling) at time t and at the equilibrium time, $t = \infty$, respectively. Upon plotting $\log(1 - F)$ versus time, a straight line with a negative slope passing through the origin was obtained at the reaction temperatures employed for *o*-, *m*- and *p*-IHA isomers (Fig. 2). These data suggest that the kinetics of the reaction depend on the concentration of both IHA isomer and the radioiodine as iodide ion. These results strongly suggest a simple second-order isotopic exchange reaction of radioactive iodine as I^- with each of the iodhippuric acid isomer.^{19,20}

The values of the specific rate constant (k) of the proposed isotopic exchange were calculated from the slope (p) of the corresponding straight line of the plot of $\log(1 - F)$ versus time (Fig. 2) employing the following equations.¹⁹

$$-2.303 \log(1 - F) = P t \quad (7)$$

$$k = \frac{2.303 P}{a} \quad (8)$$

where $a = [^{125}\text{I}] + \text{IHA}$. The amount of the radioactivity of the no carrier added [^{125}I] iodide tracer (20 μl , 20×10^3 Bq) used in each run was negligible compared to the total chemical quantity

Fig. 2 Variation of $\log(1 - F)$ versus time at different temperatures in 25 mg molten ammonium acetate, 6 mg *o*-IHA isomers and radioactive iodine.

of each IHA isomer. Thus, the amount of $[^{125}\text{I}] + \text{IHA} \approx \text{IHA} = 0.39334 \text{ g mol}^{-1}$. The values of the specific rate constant for *o*-, *m*- and *p*-IHA isomers were found equal to 0.8034, 0.698 and $0.933 \text{ mol}^{-1} \text{ min}^{-1}$ at 120°C , respectively. The average k values at all temperatures for the radioiodination of iodhippuric acid isomers follows the sequence $p\text{-IHA} > o\text{-IHA} > m\text{-IHA}$.

The activation energy (E) of the isotopic exchange reaction of radioactive iodide ions with each isomer of IHA was obtained, employing the Arrhenius equation

$$\ln k = \frac{-E}{RT} + \text{constant} \quad (11)$$

where R is the universal gas constant ($1.987 \text{ cal mol}^{-1}$) and T is the absolute temperature. Upon plotting $\log k$ versus $1/T$ straight line with a slope of $-E/2.303R$ is obtained. The results obtained for *o*-, *m*- and *p*-IHA isomers are summarized in Fig. 3. The E values (Table 1) were found to equal 10.01, 10.11 and $9.92 \text{ kcal mol}^{-1}$ for *o*-, *m*- and *p*-IHA, respectively at 120°C in molten ammonium acetate. These data are in good agreement with the sequence of the specific rate constant of the radioiodination reaction of $p > o > m$ -IHA isomer. These results can further be based on the electronegativity (*i.e.* electron withdrawing character) of the iodine atom in *p*- and *o*-positions compared to the *m*-position of the IHA isomer.

The dependence of the radioiodination reaction of the IHA isomers on the temperature have also been evaluated from a thermodynamic point of view. The numerical values of ΔG , ΔH and ΔS were obtained from the slopes and intercepts in Fig. 3. The results are summarized in Table 1. The values were estimated with a correlation factor of 0.9919. The numerical value of E evaluated from equation 10 is $9.92 \pm 0.21 - 10.11 \pm 0.12$, which reflects the non-spontaneous nature of the proposed isotopic exchange reaction. The positive values of ΔG and ΔH and the fact that the reaction proceeds more favorably at high temperatures confirm the endothermic and non-spontaneous nature of the reaction, respectively. It is worth noting that the

Table 1 Kinetic and thermodynamic parameters of the isotopic exchange reactions of *o*-, *m*-, and *p*-IHA with radioactive iodine as $^{125}\text{I}^{\text{a}}$

Activation parameters	<i>o</i> -IHA	<i>m</i> -IHA	<i>p</i> -IHA
E , kcal mol $^{-1}$	10.01 ± 0.1	10.11 ± 0.12	9.92 ± 0.021
K_{120} , L kcal mol $^{-1}$ min $^{-1}$	0.8034 ± 0.06	0.698 ± 0.05	0.933 ± 0.07
ΔH , kcal mol $^{-1}$	9.23 ± 0.09	9.33 ± 0.08	9.14 ± 0.06
ΔG , kcal mol $^{-1}$	23.397 ± 1.2	23.505 ± 1.2	23.271 ± 1.4
ΔS , kcal mol $^{-1}$ deg $^{-1}$	$(-3.605 \pm 0.2) \times 10^{-2}$	$(-3.607 \pm 0.16) \times 10^{-2}$	$(-3.5 \pm 0.18) \times 10^{-2}$

a. Average $\pm$ standard deviation.

Fig. 3 Rate constant ($\log k$) versus $1/T$ of the radioiodination of IHA isomers with radioactive iodine in 25 mg molten ammonium acetate.

pK_a of the *o*-, *m*- and *p*-IHA, the possible intermolecular H-bonding formation in the *o*-IHA isomer and the intermolecular H-bonding formation in the *m*- and *p*-IHA isomers¹⁷ are participating factors in the thermodynamic values (Table 1) and could account for the sequence of the radioiodination of IHA isomers.

Conclusion

This article has demonstrated the use of ammonium acetate as a molten medium for the nucleophilic isotopic exchange reaction of radioiodine (as iodide) with iodohippuric acid isomers to enhance the radiochemical yield of each isomer without the formation of bi-products *e.g.*, *g*-*o*-IHA and *o*-IBA. The method is simple and reliable for routine use with an excellent radiochemical yield and purity of the radioactive IHA isomers. Adjusting the pH of radioiodinated NaI before performing the isotopic exchange reaction restored the quantitative substitution reaction with a high radiochemical purity > 99%. The positive values of ΔH and ΔG indicate exothermic and non-spontaneous nature of the isotopic exchange reaction, respectively.

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