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Study on Iodine Labelling (I) Influences of Reducing Agent and Iodate-^{131}I in Sodium iodide-^{131}I solution on Labelling

Jaerok Kim

Chemistry Division, Atomic Energy Research Institute, Seoul, Korea
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Abstract

In Iodine-131 labelling of iodocompounds such as tetrachloro-P-tetraiodo R-fluorescein, sodium orthoiodohippurate and a non-iodocompound, human serum albumin (HSA), the labelling rates and yields are accurately compared with each other. The reaction systems conducted for each compounds were different conditions; sodium iodide-^{131}I containing reducing agent, sodium iodide-^{131}I free from reducing agent, and sodium iodide-^{131}I free from reducing agent but containing considerable amount of iodate-^{131}I etc.

The labelling yields were generally poor; 10% in the case of using sodium iodide-^{131}I containing reduing agent, and 50~60% in the case of using sodium iodide-^{131}I free from reducing agent but containing considerable amount of iodate-^{131}I. However, fair yields were obtained in the case of using sodium iodide-^{131}I free from reducing agent and mostly in the form of iodide-^{131}I. The reaction entities involved in these reactions are also briefly discussed.

요 약

Tetrachloro-p-tetraiodo-R-Fluorescein 및 sodium orthoiodohippurate 등 요오드화 합물과 요오드화합물이 아닌 human serum Albumin을 요오드 표지함에 있어서 표지 속도와 수율등을 비교검토하였다.

위의 각각의 화합물들을 여러가지 다른 조건하에 있는 Na^{131}I, 즉, 환원제를 함유한것, 환원제를 함유하지 않으며 거의 순수하게 $^{131}I^-$형으로 있는것, 및 환원제를 함유하지 않으며 상당량이 $^{131}IO_3^-$형으로 있는것 등을 써서 표지반응시켰다.

환원제인 $Na_2S_2O_3$를 함유한 Na^{131}I를 쓰는 경우는 위의 세가지 화합물들의 표지수율이 10% 내외로 매우 저조하였으며 환원제는 없지만 상당량이 $^{131}IO_3^-$로 존재하는 Na^{131}I를 쓰는 경우도 50~60%의 낮은 수율이었다. 환원제가 없으며 대부분 $^{131}I^-$로 존재하는 Na^{131}I를 쓰는 경우에만 70~90%의 높은 표지수율을 보였다. 이들 반응에 관여하는 중간체에 대해서도 토의하였다.

Introduction

The iodine-131 labelled tetrachloro-P-tetraiodo-R-fluorescein (Rose bengal-^{131}I), sodium ortho iodohippurate (Hippuran-^{131}I) and human serum albumin (RIHSA-^{131}I) are nowadays effectively used in hospital establishments for diagnoses and also in nuclear medicine societies for research purposes. The generally known method of ^{131}I labelling for the iodocompounds is isotopic exchange between the cold organic iodo compounds and sodium iodide-^{131} I in slightly acidic media (pH~6) using buffer (1~8), and for the organic non iodocompound such as protein is radioiodination achieved in slightly alkaline media(pH 8~9) using mild oxidizing agent such as chloramine-T[9] or iodine monochloride [10], even though there are many modifications in detail.

For labelling human serum albumin, particularly, a new method has been reported. In this method, the radioiodination of protein is carried out using iodine-131 monochloride after masking the SH-groups present in the protein molecule with inactive iodine[11].

^{99m}Tc, a milder nuclide than ^{131}I, is also used in labelling HSA recently to eliminate moderately strong irradiation in diagnotic procedures and to protect from the sell irradiation-decomposition of the labelled protein molecule. However, ^{131}I is still popular nuclide because of its easiness in labelling and its availability in small research centre. In many literatures, it is almost not treated or not emphasized or even disregarded that the isotopic exchange rate is proportional to the radioactivity per volume of a reactant. It is known on almost non-quantitative bases that the presence of reducing agent degenerate the labelling yield. The data in the literatures are not coincide with each other owing to the slight differences of reaction conditions. Also it is scarecely investigated that the reaction mechanisms including the practical entities involved in the reaction intermediates. As a part of the contribution upon it as well as for the standardization of routine syntheses of these compounds, the author has studied on the reactions using different systems of sodium iodide ^{131}I; sodium iodide containing sodium thiosulfate (^{131}I>99%), sodium iodide-^{131}I free from reducing agent ($^{131}I^-$>97%), and also sodium iodide-^{131}I free from reducing agent but considerable amount of which activity is in the form of iodate-^{131}I ($^{131}IO_3^-$>40%), respectively.

Accurate measurement of optimum time of reactions will also applied roughly to labelling with another nuclide, ^{125}I, to get rid of tedious and time-consuming procedures.

Experimental

1. Preparation of starting Material -Na^{131}I

a) Sodium iodide-^{131}I containing reducing agent, $Na_2S_2O_3$, a product of KAERI is directly used without any further purification.

b) Sodium iodide-^{131}I, free from reducing agent and mostly in $^{131}I^-$ from is prepared as following; a distillation flask of 300ml capacity was filled with 33ml of sulfuric acid, 14ml of of hydrogen peroxide, 8ml of sodium molybdate solution and 4~5ml (10-15mCi) of Na^{131}I solution containing reducing agent, and followed dilution of the mixture to 200ml in volume. The condensers were connected in series; one was in vertical for reflux and another was in downward for distillation. During reflux, the condenser for distillation was not connected to cooling water supply while the reflux conderser was connected to, and vice versa during distillation.

After 2 hours reflux, the iodine-131 was distilled into 10 ml of dilute sodium carbonate

solusion (pH 8.5). For efficient trapping of the active iodine, the condenser tip was kept dipping into the trapping solution and the distillation was aided with gentle nitrogen gas flowing from another neck of the distillation flask to the receiver so that mild distillation condition might be maintained. During the over-all process, temperature of the heating mantle was kept below 240°C.

c) Sodium iodide-^{131}I, free from reducing agent but considerable amount of its activity is in $^{131}IO_3$ form was prepared in very similar way to that of Section 1. 50ml of sulfuric acid was used instead of 33ml in the former case. The temperature of the heating mantle was within the range of 270-290°C and the pH of the trapping solution was 9.5.

d) Determination of ^{131}I and $^{131}IO_3^-$activities in the sodium iodide-^{131}I samples was carried out just before starting reactions with them by radio-paperchromatography technique using Whatman No. 1 filter paper and the developing solvent of 75% methanol. The devloped paper chromato-strips were applied to radio-chromatogram scanner to check the activities of $^{131}I^-$ and $^{131}IO_3$.

2. Isotopic Exchange between sodium iodide-^{131}I and Rose bengal

The exchange was carried out under the conditions described by Mani[3] in acetate buffered solution using each 25 mg of Rose bengal (Eastman Kodak). The weighed amount of cold Rose bengal was dissolved in 1 ml of 0.1 M sodium hydroxide and put together into the reaction tube with 3ml of sodium acetate (1M, 1 ml) and acetic acid (1M, 1 ml) buffer (pH 4.5) to get the pH of the mixture of 6, followed the addition of 5mCi of a species of Na^{131}I (1.2 ml) with 0.05 ml of 30% hydrogen peroxide. The reaction mixture was subsequently heated on water-bath for reflux. The species of Na^{131}I added to each batch of reaction were those of the products obtained from Section 1. a)~c), respectively.

The reaction systems were kept under constant conditions. At every given time interval, a portion of the reaction mixtures was taken out for radio-paperchromatographical separation using Whatman No. 1 filter paper and the solvent system of 0.25M sodium citrate. The developed chromato-strips were applied to radio-chromatogram scanner to measure the peak area for calculations of the activities of unbound ^{131}I and Rose bengal-^{131}I.

3. Isotopic Exchange between sodium iodide-^{131}I and Hippuran

The exchange was carried out under the conditions recommended by Mani[3] in acetate buffered solution using each 300mg of Hippuran (Mallinckrodt) and 5 mCi of each sodium iodide-^{131}I species (1.2ml. each) prepared in Section 1. a)~c). The exchange conditions were very similar to that carried out in Section 2. except the omission of H_2O_2-addition. At every given time interval, a portion of the reaction mixtures was taken out for radio-chomatographical separation. Whatman No. 1 filter paper and the solvent system of butanol -acetic aicd-water (4:1:1 in volume) were used. The calculations of the % activity were done in similar way to the case of Rose bengal-^{131}I.

4. Radioiodination of HSA

Radioiodinations were also carried out under the concitions recommended by Mani[3] at room temperature using phosphate buffer (pH 8). 100mg of HSA and 5mCi (1.2ml) of a species of sodium iodide-^{131}I were put into a small beaker contained 2ml of phosphate buffer (pH 7.2). 400 micrograms/ml of chloramine-T solution was added with stirring and the reaction mixture was kept at room temperature for 1

hour or so. The $Na^{131}I$ species added to each batch of the reaction were those of the products obtained from 1. a)~c), respectively. The analyses were done in similar way to that of Section 2 or 3 using 75% methanol as developing solvent.

Result and Discussion

1. Preparation of $Na^{131}I$ species

Elimination of reducing agent, $Na_2S_2O_3$, from sodium iodide-^{131}I solution by distillation is based on the following rection sequence;

$$Na^{131}I(\text{with reducing agent})+H_2SO_4 \rightarrow H^{131}I+Na_2SO_4$$

$$H^{131}I \xrightarrow[Na_2MoO_4]{H_2O_2} {}^{131}I_2 \xrightarrow[\text{dil. NaOH}]{\text{distil into}} Na^{131}I$$

(reducingagent free)·····(1)

It is important to keep mild oxidation condition and keep the pH of the trapping solution for $^{131}I_2$ to be below 8. At above of pH 8, considerable amount of iodate-^{131}I is to be formed according to the following equation;

$$^{131}I_2+2OH^- \longrightarrow {}^{131}IO^-+{}^{131}I^-+H_2O$$

$$3{}^{131}IO^- \longrightarrow 2{}^{131}I^-+{}^{131}IO^-_3 (pH>8) \cdots\cdots(2)$$

Thereupon, to prepare $Na^{131}I(^{131}I>97\%)$, the oxidation conditions should be mild and the pH of the trapping solution should be below 8, and to prepare $Na^{131}I(^{131}IO_3^->40\%)$, the oxidation conditions may be vigorous and the pH of the trapping solution should be above 8.

In either case, there was much more iodide-^{131}I in the first fractions of the distillates (Table 1) However, the distillation conditions for getting desired product were complicated owing probably to other factors. The content of $^{131}IO_3$ was varied in wide range, from batch to batch, even if the above conditions were fixed. In the paper chromatographic separation, the Rf values of $^{131}IO_3^-$ and $^{131}I^-$ were 0.5 and 0.8 respectively.

2. Exchange between sodium iodide-^{131}I and Rose bengal

In the exchange between Rose bengal and sodium iodide-^{131}I of containing reducing agent, the exchange rate was very slow.

It has been known that the Rose bengal can be labelled by exchange with either $^{131}I^-$ or $^{131}I_2$.[1] But in case of the latter, excess amount of carrier iodate should be added in acidic condition to liberate $^{131}I_2$ into the reaction mixture from added $Na\ ^{131}I$;

$$IO_3^-+H^++{}^{131}I^- \longrightarrow {}^{131}I_2 \cdots\cdots(3)$$

In present study, the exchange between Rose bengal and $Na^{131}I$ ($^{131}I<60\%$) was carried out in the presence of electron acceptor, hydrogen peroxide, instead of adding excess iodate carrier. The exchange yield was much lower than that of the exchange between Rose bengal and $Na^{131}I(^{131}I^->97\%$, and reducing agent free), (Fig. 1). It would be attributable to the incomplete reaction (3) with around 40% of $^{131}IO_3^-$ only. On the other hand, Mani[1] and others[2] have found by paper chromatography that the product obtained from the exchange in the presence of oxidizing agent is a mixture of various halogenated fluoresceins. Therefore, the use of hydorgen peroxide is not recommendable for getting pure labelled Rose bengal. In the separation of the products by paper chromatography, alkaline sodium citrate solution was preferably used in present study instead of using benzene-acetic acid-water (4:1:1 in volume) to avoid probable evaporation of iodine-131 in acidic media during separation and drying the chromato-strips.

$$4{}^{131}I^-+4H^++O_2 \longrightarrow 2{}^{131}I_2+2H_2O$$

The Rf value of $^{131}I^-$ was 0.7 and that of Rose bengal-^{131}I was 0.0, respectively. However, by using the solvent system of the above, the separation of $^{131}I^-$ and $^{131}IO_3^-$ was not accomplished. It was not so important in this study since the only significance was the

Table 1. **Preparation of Reducing agent free Sodium iodide-^{131}I**

Run No,	conc. H_2SO_4 (ml)	30% H_2O_2 (ml)	$Na^{131}I$* (ml)	$Na^{131}I$* (mCi)	15% Na_2MoO_4 (ml)	H_2O(ml)	total vol. (ml)	trapping solution (ml)	N_2 gas flow.	reflux hour	temp. of heating mantle °C	recovery (%)	$^{131}IO_4$ (%)	$^{131}IO_3$ (%)	^{131}I (%)	remarks
1	4	1	4	5	0	15	24	0.05M NaOH, 5	−	1	302	80	1	80	19	
2	33	14	1	3.8	8	144	200	$Na_2CO_3/NaHCO_3$ =1/8, v/v each 0.1 M**	+	1	310	90	1	60	39	
3	33	1	4	8	0	162	200	0.1N NaOH, 1	−	1	255	75	2	60	38	
4	5	2	3	2	0	25	60	0.05M NaOH, 3	+	1	280	25	0.5	0.9	98	
5	33	14	1	5	8	144	200	Na_2CO_3 $NaCHO_3$**	+	1	260	70	1	2	97	
6	33	14	2	5	8	143	200	$Na_2CO_3/NaCHCO_3$	+	1	262	65	2	1	97	

* sodium radio iodide with reducing agent. ** The pH of the soluction was adjusted to 8, by dilution.

activeity estimation of undound $^{131}I^-$ and bound ^{131}I to Rose bengal separately.

The reactive entity involved in these exchange reactions would probably be $^{131}I^+$, not $^{131}I^-$, considering the obtained exchange rates. Iodine, the least electronegative halogen, is actually capable of forming the cationic species I^+.

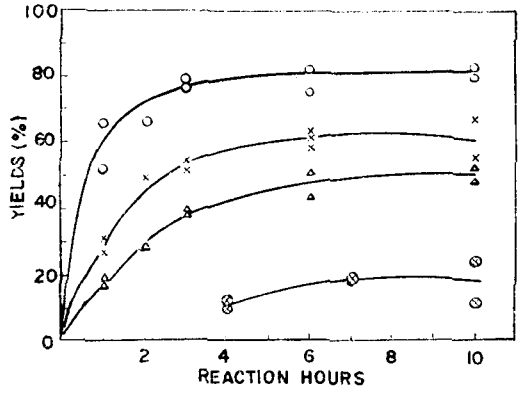

Fig. 1. The dependence of radiochemical yield on reaction time for exchange reaction of cold Rose bengal with ^{131}I in slightly *acidic solution(pH 5)*
o; sodium iodide-^{131}I, reducing agent free, $^{131}I^-$>97%
x; sodium iodide-^{131}I, reducing agent free, $^{131}IO_3^-$>40%
△; sodium iodide containing reducing agent, ^{131}I>99%
⊗; reaction is conducted without the presence of H_2O_2 using sodium iodide -^{132}I containing reducing agent

3. Exchange between sodium iodide-^{131}I and Hippuran

The general trend of this exchange reaction was similar to that of Rose bengal except the slower exchange rates. Mitta[6] has found the exchange yield after 3 hours reflux on water-bath is 80~90% (Fig. 2). The exchange was carried out using sodium iodide-^{131}I, free from reducing agent and radiochemically pure, with excess potassium iodate carrier. However, in present study, the exchange yield with sodium iodide^{131}I ($^{131}I^-$<60%) without iodate carrier was so poor that it was impossible to label.

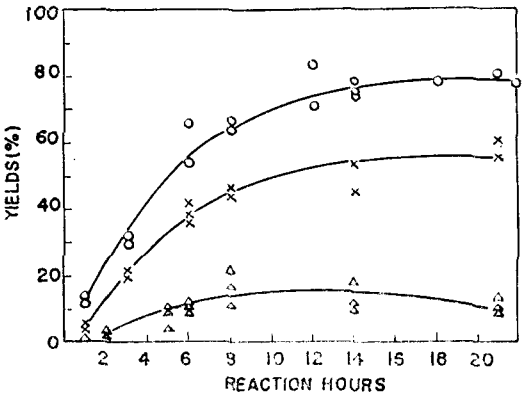

Fig. 2. The dependence of radiochemical yield on reaction time for exchange reaction of cold Hippuran with ^{131}I in slightly acidic solution (pH 6)
o; sodium iodide-^{131}I, reducing agent free, $^{131}I^-$>97%
x; sodium ioddie-^{131}I, reducing agnet free, $^{131}IO_3^-$>40%
△; sodium iodide-^{131}I containing reducing agent, $^{131}I^-$>99%

4. Radioiodination of HSA

The reaction rate was quite fast at room temperature, especially, in case of sodium iodide-^{131}I, reducing agent free and radio-

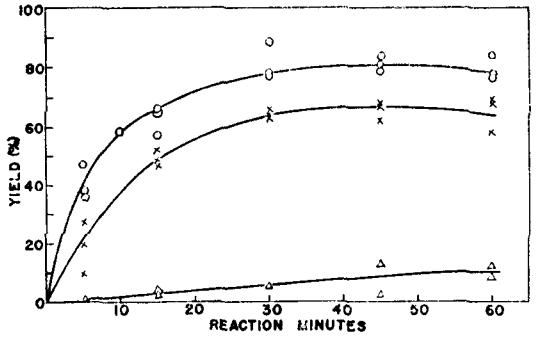

Fig. 3. The dependence of radiochemical yield on reaction time for iodination of HSA with ^{131}I in phosphate buffer(pH 8~9)
o; sodium iodide-^{131}I, reducing agent free, $^{131}I^-$>97%
x; sodium iodide-^{131}I, reducing agent free, $^{131}IO_3$>40%
△; sodium iodide-^{131}I, with reducing agent, $^{131}I^-$>99%

chemically pure ($^{131}I^{-}$>97%), is used in the presence of oxidizing agent of chloramine-T. The influences of reducing agent and iodate-^{131}I were particularly extreme in this case (Fig. 3). The obtained result strongly suggests that the reactive entity is $^{131}I^{+}$ and the reaction mechanism follows the electrophillic substitution at aromatic carbon.

Conclusion

1. The sodium iodide-^{131}I containing reducing agent and sodium iodide-^{131}I, reducing agent free but containing considerable amount of iodate-^{131}I are not useable for efficient labelling of these three compounds; ie, sodium iodide -^{131}I should be reducing agent free and radiochemically pure.

2. The exchange yields per hour in the exchange between sodium iodide-^{131}I of reducing agent free ($^{131}I^{-}$>97%) and Rose bengal, and between the same sodium iodide and Hippuran were 60%, and 12%, respectiyely. The iodination yield per hour for HSA with the same sodium iodide was 80%. The total hours for each reaction were 3, 10, and 1 to get 80%, 70% and 80%, respectively.

3. The exchange entity involved would probably $^{131}I^{+}$, and the iodination entity would also $^{131}I^{+}$. The exchage mechanism of iodination of HSA follows electrophilic substitution.

Acknowledgment

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