Radiation Protective Agents for Storage of Iodine-131 Labelled L-Thyroxine

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Solutions of iodoamino acids labelled with ^{131}I are known to undergo considerable radiation decomposition on storage.[1–7] This decomposition can be prevented to some extent by addition of a radiation protective agent, the effect of which is mainly due to reaction with free radicals and other reactive species formed by self-irradiation of the solution.

Little information is available from the literature on the effect of different protective agents on preparations of ^{131}I labelled iodoamino acids. The purpose of the present work was to study the radiation protective effect of some chemical agents on solutions of L-thyroxine (T_4) labelled with ^{131}I. Commercial preparations of ^{131}I-T_4 are usually supplied in 50 % (v/v) aqueous propylene glycol. This reagent shows good protective capacity, but cannot easily be separated from the labelled T_4, which is in some cases desirable. Emphasis was therefore laid on the study of agents which may be removed by gentle evaporation.

In general, the action of a specific additive as radiation protective agent cannot easily be predicted on a theoretical basis. Empirical data from the literature [8–10] on storage conditions for ^{3}H- and ^{14}C-labelled compounds, however, appeared to be of some use for the selection of agents to be studied. In most cases, the samples under investigation were stored in solution, and the rate of decomposition was compared to that of simultaneously prepared reference samples, stored in 50 % propylene glycol. A few samples were evaporated to dryness before storage. After 3–4 weeks' storage, the rate of decomposition was established by thin-layer chromatography. In addition, the activity concentration of each solution was measured after storage, in order to reveal possible effects due to adsorption or poor solvent action.

Results and discussion. The results of this work are summarized in Table 1. The protective capacity of each system investigated is expressed in terms of the relative decomposition Ds/Dr, where Ds is per cent decomposed T in the sample under investigation, and Dr is the corresponding figure for the 50 % propylene glycol reference sample from the same series. Correspondingly, the activity concentration of each sample after storage is expressed in per cent of the corresponding count rate for the reference sample. Some of the most promising systems were included in one or more new series. As is evident from Table 1, the reproducibility is quite satisfactory for samples stored in solution.

A considerable part of the observed decomposition may be ascribed to the action of hydroxyl radicals. In a work by Adams *et al.*[11] a number of aliphatic alcohols were found to show relatively high reaction rates with the OH-radical. Some of these alcohols have been studied in this work, and found to be effective as protective agents, and it is interesting to note that the relative protective capacities for 50 % solutions of, respectively, methanol, ethanol, propylene glycol, and glycerol, correspond closely to their comparative reactivities for the OH-radical. Benzene and some other aromatic compounds were found to exhibit still higher reaction rates with the OH-radical.[11] This fact might explain the good results obtained in the present work with solutions containing potassium hydrogen phthalate in concentrations below 1 %.

Another agent found to give quite good results, is aqueous ammonia, which appears to have significant protective effect even in low concentrations. Addition of small amounts of ammonium hydroxide to samples containing ethanol significantly improved the protective capacity at low alcohol concentrations. Similarly, the introduction of phthalate buffer to samples of ethanol or propylene glycol gave better results than for samples without buffer addition.

Attempts to use as storage medium water saturated with an inert gas, in order to avoid effects of dissolved oxygen, did not prove advantageous as compared to storage in pure water. On the other hand, storage of dry samples under various gases, as previously used for ^{14}C compounds,[8] resulted in moderate decomposition, probably due to the absence of water. The results were less reproducible, however, than those obtained for samples stored in solutions.

Table 1. Decomposition of T_4-^{131}I at various storage conditions, as compared to reference samples stored in 50 % propylene glycol. Col. A: Storage condition. Col. B: Relative decomposition Ds/Dr. Col. C: Radioactivity in solution after storage (% of reference sample).

A	B	C
25 % methanol in water (v/v)	1.3	103
50 % » » »	1.6	105
99 % » » » (approx.)	3.7	100
10 % ethanol in water (v/v)	1.8	93
12.5 % » » »	1.6	91
25 % » » »	1.4	94
30 % » » »	1.4	96
40 % » » »	1.1	98
50 % » » »	1.0, 1.1, 1.0, 1.1	104
55 % » » »	0.8	—
60 % » » »	0.9	101
70 % » » »	0.6	103
75 % » » »	1.1, 0.7	102
80 % » » »	0.5	99
86 % » » »	0.8	100
98 % » » » (approx.)	2.5	102
10 % glycerol in water (v/v)	1.7	98
25 % » » »	2.0	90
50 % » » »	1.6, 1.2	96
0.015 M ammonia in water	1.5	93
0.15 M » » »	1.4	97
1.0 M » » »	1.0	96
1.5 M » » »	1.4	103
1.8 M » » »	1.2	94
5.0 M » » »	4.0	100
Ethanol:2 M ammonium hydroxide:water =		
0.75: 0.25 :9.0	1.0, 1.0	92
1.5 : 0.5 :8.0	1.1	100
3.0 : 1.0 :6.0	0.6	95
3.75: 1.25 :5.0	1.3, 1.1, 0.6	103
6.25: 2.10 :1.65	1.1	97
3.0 : 1.0 :0	0.9	—
Phthalate buffer [a]	0.8	—
Phthalate buffer:propylene glycol:water =		
0.7 : 1.0 : 0.3	0.7, 0.7	98
Phthalate buffer:ethanol:water =		
0.7 : 1.0 : 0.3	0.7, 0.7	102
100 % water	3.2	66
Water saturated with helium	3.0	89
» » » nitrogen	3.0	47
» » » carbon dioxide	3.5	80
Evaporated to dryness, kept in air	1.0, 2.0	95
» » » » » desiccator at 20°C	0.9	91
» » » kept in helium atmosphere	0.7, 1.0	78
» » » kept in nitrogen atmosphere	0.4, 2.2	74

[a] 50 ml 0.1 M KH $C_6H_4(COO)_2$ + 28.8 ml 0.1 M NaOH, made up to 100 ml with H_2O, (pH = 5.5).

The results may be affected by at least two sources of errors. Selective adsorption of a decomposition product, which would not necessarily be revealed in the activity measurement, would leave an erroneous sample for the purity control. Possible decomposition of T_4 during the chromatographic procedure [6] could also give systematic errors. It is not believed, however, that such errors will seriously affect the more significant findings of this work.

Several systems with good protective capacity for ^{131}I-T_4 have been pointed out. In particular, ethanol seems to be a very convenient additive. The best protecting effect is obviously obtained in the concentration range 60—80 % ethanol, provided that other agents have not been added. Systems working well for ^{131}I-T_4 would most probably also be useful for preparations of other ^{131}I-labelled iodoamino acids.

Experimental. L-Thyroxine in the form of its sodium salt (Koch-Light Laboratories Ltd., pure) was labelled with ^{131}I and purified according to a procedure that has been described elsewhere.[7] The specific activity of the preparations was in the range 30—41 mCi/mg T_4, and the radiochemical purity was in most cases 97—98 %. After purification, the preparations were divided in equal parts (0.2—0.3 ml) corresponding to 0.40 mCi ^{131}I, and transferred to vials of neutral glas, which were of uniform shape and had been thoroughly washed and rinsed before use. Usually 10—12 samples were obtained from one preparation. The reagents under investigation were added in fixed proportions to a total volume of 2.0 ml, and the vials were left for storage in a refrigerator at 4—6°C. For each preparation, a vial with 50 % aqueous propylene glycol as additive was prepared, to serve as a reference sample for that particular preparation.

After 3—4 weeks' storage, the samples were investigated by means of thin-layer chromatography on silica gel with *tert*-butanol-acetone-10 M ammonium hydroxide in the ratio 50:25:18.[7] The distribution of ^{131}I on the plates was determined by means of a Packard Radiochromatogram Scanner Model 2701. In most samples, iodide and 3,5,3′-triiodo-L-thyronine were the only radioactive impurities identified. In the cases of storage in dry state the sample was gently evaporated to dryness, and after the storage dissolved in 50 % aqueous propylene glycol. The TLC analysis was always started within 3 h from the removal of a series of samples from the refrigerator.

In addition to the radiochemical purity determination, a measurement of the activity concentration in each solution after storage was undertaken. Of the solution 0.100 ml was taken for γ-activity measurements with a well-crystal scintillation counter, and the activity was compared with that of the reference sample. A low figure obtained for a particular sample would indicate difficulty due to precipitation of T_4 or possibly to adsorption effects. In the case of 50 % propylene glycol, no appreciable disappearance of activity from the solution was observed.

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Received January 28, 1970.