THE TRANSFER OF RADIOACTIVITY OF HSA-CONTAINING SAMPLES OF ^{125}I INSULIN PREPARATIONS DURING THEIR STORAGE

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Summary

In ^{125}I insulin preparations, preserved in the form of lyophilized solutions with human serum albumin, the transfer of radioactivity from the insulin molecules to the higher molecular weight fractions was observed. After one month storage this transfer corresponded to 7 % of the total radioactivity and it increased proportionally to the length of the storage of iodinated preparations under simultaneous decrease of their biological activity. The results obtained with stored ^{125}I insulin preparations and these preparations irradiated with external gamma-source were compared and discussed.

Key Words: ^{125}I insulin, HSA-containing samples, storage, gamma-radiolysis

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INTRODUCTION

From the review article by Yamamoto[1] it follows that radiation aggregation belongs among the main radiation reactions of higher peptides and proteins, irradiated either in solid state or in aqueous solutions. The formation of radiation aggregates was also demonstrated during the irradiation of aqueous solutions of insulin with an external gamma-radiation source[2]. Radiation aggregates of insulin are also formed in consequence of an internal irradiation of ^{125}I insulin stored in aqueous solution of 1.5 μg concentration and 100 μCi activity[3]. The stability of insulin could be increased by storing iodinated insulin preparations in a 1 % bovine serum-albumin solution. In spite of this (as is evident from the mentioned paper), after 6 weeks of storing, about 10 % of the radioactivity passes into the fraction of high-molecular substances even in this case.

We were interested to find how and whether this transfer of radioactivity proceeds when the storage time of iodinated preparations of ^{125}I insulin is prolonged, and to learn which type of aggregation is involved in this case. Therefore we devoted attention to preparations of ^{125}I insulin, used in medical diagnostics, which are usually supplied in the form of freeze-dried solutions in phosphate buffer, with an addition of human serum albumin (HSA) and merthiolate.

We observed the changes taking place during their storage over a period exceeding the one month guarantee set by the producer and we compared them with the changes proceeding after irradiation of these samples with an external gamma-radiation source.

EXPERIMENTAL

Samples of ^{125}I insulin of Isocommerz were used, which are supplied in the form of lyophilized solutions in 0.04 M phosphate buffer, additioned with human serum albumin (0.25 %) and merthiolate. The content of insulin in one ampoule corresponded approximately either to 0.1 μg of substance of 10 μCi activity, or to 1 μg of substance of about 100 - 150 μCi activity. The samples were stored at + 4 °C.

For testing, both various preparations with expired guarantee term, and freshly supplied preparations were used, from which aliquots were taken at gradual time intervals. Individual samples were dissolved in about 0.5 ml of dist.water, acidified with acetic acid and 1.5 to 2 mg of inactive insulin standard (Novo Co.) were added to it. The samples prepared in this manner were applied on a Sephadex G 75 column, (76 cm high and 1.6 cm diameter) and eluted with 2.5 M acetic acid. The fractions eluted from the column were measured spectrophotometrically at 276 nm (Spekord UV-VIS) and radiometrically (gamma

system Nuclear Chicago).

For preliminary determination of molecular weight, thin layer chromatography on Sephadex G 150 (superfine) and standard samples of gamma-globulin, serum albumin, cytochrome C and ribonuclease(of Koch-Light) were used. In this case 1M acetic acid was used for development. The isolated fraction of high-molecular substances was also checked by means of paper electrophoresis on paper Whatman No.3 in 20 % formic acid (5 V/1 cm, 5 h).

For comparison aliquot samples of lyophilized preparations of ^{125}I insulin were irradiated with an external source of gamma-radiation. For this purpose ^{60}Co of 16.6 rad/sec intensity was used. The doses ranged from 30 to 180 krad. After irradiation the samplen were worked up on a Sephadex G 75 column in the same manner as described above.

RESULTS AND DISCUSSION

Fig. 1 represents the elution profile of the preparation of ^{125}I insulin supplied with 1 μg starting mass and 100 μCi activity, which was stored in the form of a lyophilized solution at + 4 °C for 1 month. The first peak (I) eluted from the column within the 40-58 ml volume range corresponds to HSA present in the preparation supplied. The second peak (II), eluted from 90 to 130 ml corresponds to insulin. The last peak (III), eluted from 140 to 153 ml corresponds to the liberated iodide. Similarly all ^{125}I

insulin preparations which had been stored for various time intervals were worked up and evaluated. The results are shown in Table 1 a.

It is evident that during the storage of iodinated insulin preparations a decrease in the radioactivity in the insulin fraction takes place while the radioactivity in the HSA fraction increases proportionally to the length of storage (Fig.2a). This shift in radioactivity was also followed in preparations which were supplied with a decreased content of insulin ^{125}I (0.1 μg of 10 μCi activity each). As is evident from Fig. 2b the shift in radioactivity takes place still more rapidly in this case.

In contrast to this, when aliquot samples from the supplied iodinated insulin preparations were irradiated externally in the form of lyophilized solutions, no distinct changes in the distribution of radioactivity took place (Table 1b). The presence of HSA and merthiolate, present in the preparations supplied, protect the insulin molecule practically completely from the effect of the ionizing radiation even at relatively high doses of external gamma--radiation when the preparations are irradiated in the form of lyophilized solutions. Only in the case when the samples were dissolved in water before irradiation and irradiated in aqueous solutions the protecting effect of the added substances decreases to some extent. A more pronounced drop in radioactivity in the insulin fraction takes place

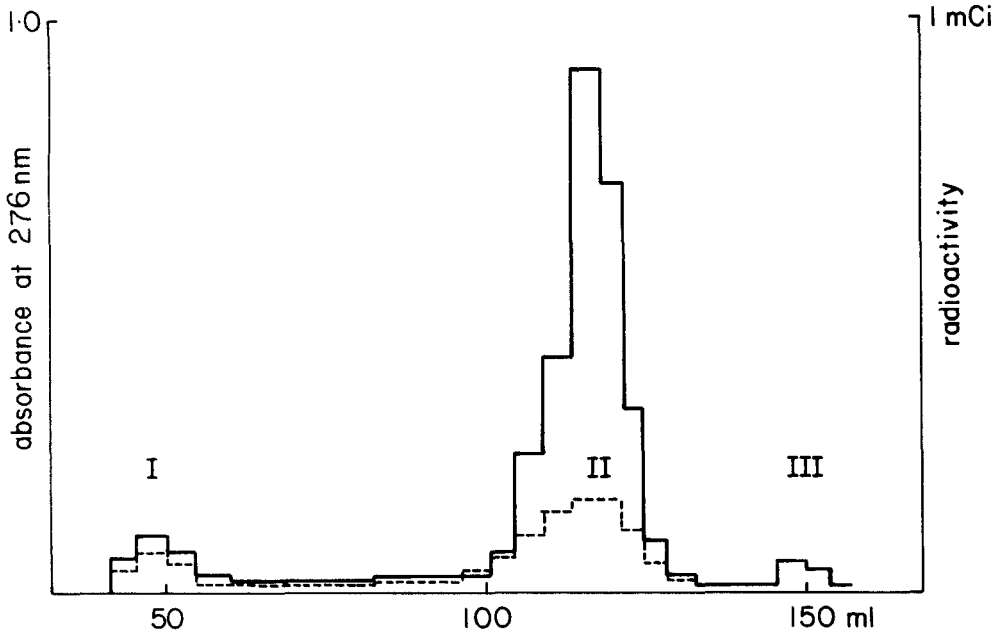

Fig. 1 Gel filtration of ^{125}I insulin preparation stored 30 days at 4 °C in the form of lyophilized solution with human serum albumin) on the Sephadex G 75 column. Eluted fractions were evaluated spectrophotometrically (---) and radiometrically (——).

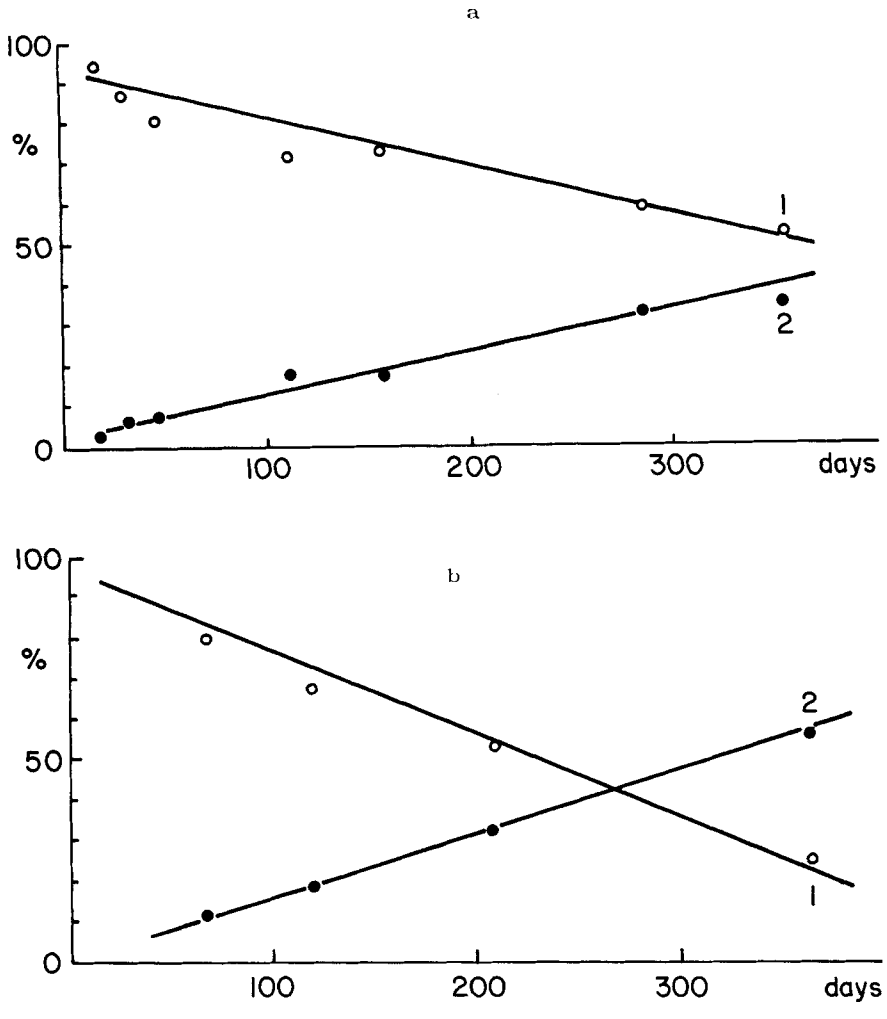

Fig.2 Decrease of radioactivity in the fraction of insulin (1) and increase of radioactivity in the higher molecular weight fraction (2) in dependence on the length of the storage.
a-samples supplied with starting mass 1 μg and radioactivity 100 μCi, b-samples with starting mass 0.1 μg and activity 10 μCi.

Table 1

a.)				
Storage of ^{125}I insulin preparations at 4 °C of specific activity: (radioactivity/mass)	Length of storage (number of days)	Distribution of radioactivity (% of the total sum of the radioactivity applied) in fractions:		
100 μCi/μg		I	II	III
sample A	30	6.8	87.0	3.5
sample B	48	8.0	80.5	6.0
sample B	112	18.0	70.0	7.0
sample A	160	17.0	73.0	8.5
sample A	285	33.0	60.0	6.8
sample B	350	34.5	51.0	7.5
10 μCi/0.1 μg				
sample C	68	11.5	79.5	4.7
sample D	120	19.0	68.0	7.0
sample E	210	33.0	51.5	8.0
sample F	365	57.0	26.5	7.0
b.)				
Gamma-irradiation of ^{125}I insulin preparation of specific activity: 100 μCi/μg In the form of lyophilized solution (a preparation stored for 1.5 months was used)	Dose of external gamma-irradiation (krad)			
	0	6.5	81.5	6.0
	30	7.8	80.5	6.2
	60	8.0	80.0	6.0
	180	11.0	78.0	5.5
In the form of an aqueous solution: (an aliquot of the ^{125}I preparation was dissolved in water before irradiation)	180	9.5	59.5	19.0

in this case in consequence of a radiation liberation of iodine.

The total doses of internal beta-radiation absorbed by iodinated ^{125}I insulin preparations during storage are substantially lower: in samples with 100 μCi radioactivity they do not exceed 10 krad even after prolonged storage. In spite of this a distinct shift in the distribution of radioactivity from the insulin fraction to the fraction of high-molecular substances takes place. In the course of storage of iodinated preparations adsorption of insulin molecules onto the HSA molecules evidently takes place, probably under formation of an insulin-HSA complex.

By paper electrophoresis in 20 % formic acid it was found that the position of the radioactive complex (after its isolation from stored iodinated insulin preparation) coincides with the position of a standard of HSA, which indicates a relatively high stability of the bond in this complex.

The formation of complexes of insulin with a number of serum proteins formed in vitro on incubation of insulin with the serum at 35-37 °C, is described in a number of papers, surveyed by Kraml[4]. As is evident from our results the formation of insulin-HSA complex should be reckoned with even during the storage of lyophilized solutions of iodinated insulin in the presence of HSA at + 4 °C. The energy gained by iodinated insulin molecules during the decomposition

of atoms of ^{125}I evidently enables them to interact with the closest HSA molecules and to form new irreversible bonds. This is in agreement with the results obtained during the development of the isolated high-molecular fraction I on thin layers of Sephadex G 150 (superfine) (Fig.3). This fraction gives an elongated radioactive spot located partly in the position of the standard HSA and partly shifted toward substances with a higher molecular mass.

The formation of the insulin-HSA complexes is directly proportional to the length of storage of iodinated preparations of ^{125}I insulin, under simultaneous decrease of free insulin and the biological activity of the stored samples[5].

In this paper we did not make detailed study of the mechanism according to which the insulin-HSA complex is formed. However, we considered it useful to point out this phenomenon, taking place during the storage of the ^{125}I preparations in the presence of serum albumin, which can negatively affect the course of their further biological application.

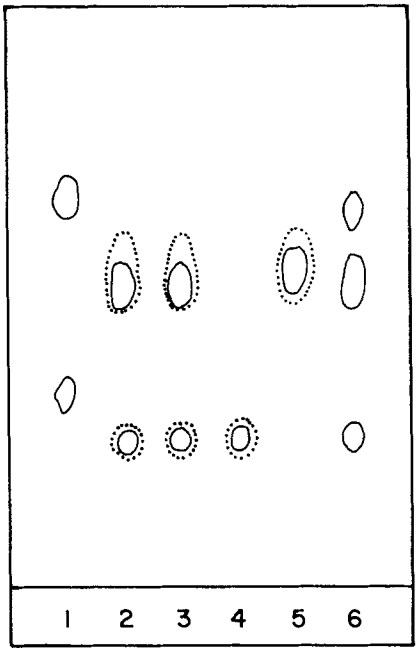

Fig.3 Paper replica on the thin gel layer chromatogram (Sephadex G 150 superfine) detected radiometrically (---) and with Bromocresol Green (——):

1-standards of cytochrome C/m.w. 13 500/ and gamma-globulin /m.w. 160 000/; 2 and 3-samples of ^{125}I insulin preparations A and B with the spec.act. 100 μCi/μg, stored 160 and 112 days, with added standards of insulin and HSA; 4 and 5-lyophilized fractions II and I separated previously on the column of Sephadex G 75; 6-standards of insulin/m.w. 5 800/BSA/m.w. 67 000/ and its dimer /m.w. 134 000/.

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