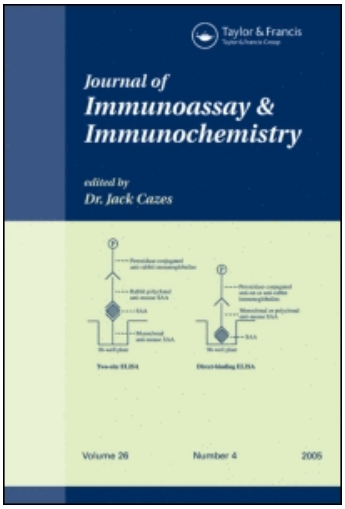

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Gerhard Baumann[a]; Klaus Amburn[a]
[a] Center for Endocrinology, Metabolism and Nutrition Northwestern University Medical School Chicago, Illinois

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THE AUTODECOMPOSITION OF RADIOLABELED HUMAN GROWTH HORMONE

Gerhard Baumann and Klaus Amburn

Center for Endocrinology, Metabolism and Nutrition
Northwestern University Medical School
Chicago, Illinois

ABSTRACT

Human growth hormone (hGH) was radiolabeled with ^{125}I, using a gentle lactoperoxidase technique. The stability and decomposition products of this tracer were studied by frequent periodic analysis by Sephadex G-100 chromatography on a long column. Monomeric ^{125}I-hGH showed an exponential decline, with a half-life of 61 days. The main radioactive degradation product was iodide, which appeared with a fractional appearance rate of 0.01136 per day. Secondary degradation products were a series of radioactive oligomers of hGH, which appeared with an overall fractional rate of 0.00525 per day. The kinetic data obtained should provide guidelines for the shelf-life and repurification schedule of radioiodinated polypeptides.

(KEY WORDS: Human growth hormone, Radioimmunoassays, Radiation damage, Autodecomposition).

INTRODUCTION

The shelf-life of radiolabeled compounds is limited due to progressive decomposition. In most instances, the chemical nature of the resulting products is unknown, and the decomposition process

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is simply summarized as "radiation damage". Radioiodinated peptides and proteins are widely used as tracers in radioimmunoassays and radioreceptor assays. The quality of such tracers declines over time, as assessed by a variety of techniques, such as binding to antibody or receptor preparations, trichloroacetic acid precipitation, and adsorption methods. However, neither the precise reasons for this declining molecular integrity nor the factors determining its rate are completely understood. We recently had opportunity to serially examine radioiodinated human growth hormone (hGH) in the course of our studies on a novel hGH-binding component in human plasma (1). The resulting repeated analysis of the hGH tracer used yielded precise kinetic data on its decay, which form the basis of this report.

MATERIALS AND METHODS

Materials

hGH (lot no. 306-13-3) was kindly supplied by Dr. U.J. Lewis, La Jolla, CA. High specific activity ^{125}INa (cat. no. IMS.30) was purchased from Amersham, Arlington Heights, IL. Sephadex G-100 was obtained from Pharmacia, Piscataway, NJ, fatty acid-free bovine serum albumin (BSA) from Sigma, St. Louis, MO, and lactoperoxidase (lot no. 130086) from Calbiochem, La Jolla, CA. All chemicals were reagent grade.

Radioiodination

hGH was iodinated with ^{125}I to specific activities ranging from 52-81 μCi/μg by a lactoperoxidase method (2,3). This method results in primarily monoiodinated hGH of high molecular integrity (3,4). The radioiodination mixture was fractionated on a 1 x 50 cm Sephadex G-100 column at 4°C. The column was developed with phosphate-buffered saline (PBS; 0.14 M NaCl, 0.01 M Na-phosphate, pH 7.4, 0.02% NaN_3) containing 1 mg/ml BSA. Fractions corresponding to monomeric ^{125}I-hGH were stored either at -20°C or +4°C to compare the effect of temperature and freeze-thawing cycles on tracer stability.

Gel filtration

The purity of ^{125}I-hGH was periodically examined by Sephadex G-100 chromatography on a 1.5 x 100 cm column at 4°C, as described (1). Approximately 100,000 cpm ^{125}I-hGH were chromatographed in PBS, containing 1 mg/ml BSA. Fractions of 1.2 ml were collected. The flow rate was 0.1 ml/min. The column was calibrated with Blue Dextran, ^{125}I Na, and globular proteins of known molecular weight (phosphorylase B, BSA, ovalbumin, chymotrypsinogen, hGH, ribonuclease). Molecular weights were derived from a calibration curve of K_{av} of standard proteins vs. the log of their molecular weights (5). Recovery of radioactivity in gel filtration ranged from 92 to 97%.

Measurements

Radioactivity was measured in a well counter equipped with a 3 inch NaI crystal with an efficiency of 71%. Spectral analysis was performed in a Nuclear Data 62 multichannel analyzer (courtesy of Dr. Stewart Spies, Department of Nuclear Medicine, Northwestern Memorial Hospital).

Data analysis

The various radioactive components observed in gel filtration were quantitated by peak integration and expressed as a percentage of the total radioactivity recovered. The disappearance of ^{125}I-hGH and the emergence of new radioactive compounds were then plotted as a function of time.

RESULTS

Representative Sephadex G-100 profiles of fresh ^{125}I-hGH and a corresponding aged ^{125}I-hGH preparation are shown in Fig. 1. The initially homogenous ^{125}I-hGH peak decreased as a function of time, with several other radioactive species accumulating. They corresponded to iodide and a series of hGH aggregates. The most abundant decomposition product in several preparations tested was iodide.

The decomposition of ^{125}I-hGH followed an exponential function of the form $y = ae^{-kt}$, where y denotes the amount of

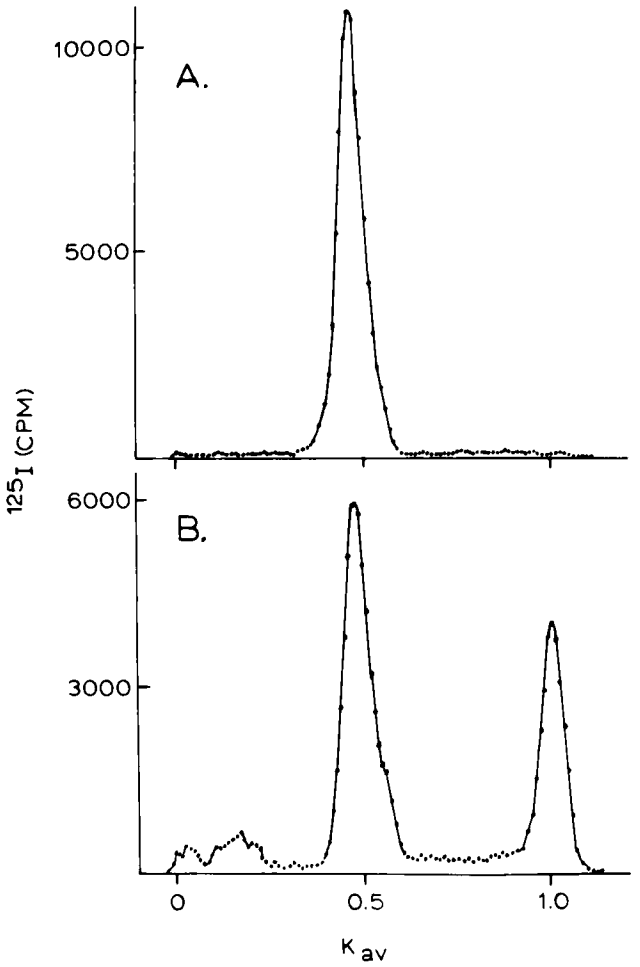

FIGURE 1 - Representative elution profiles of ^{125}I-hGH in Sephadex G-100 chormatography. A. Profile obtained with fresh tracer immediately (3 h) after labeling and purification. B. Profile of the same tracer 31 days later. K_{av} denotes partition coefficient.

intact monomeric ^{125}I-hGH at a particular point in time, a the amount of intact ^{125}I-hGH present initially, and k the fractional decay constant (Fig. 2). Conversely, the appearance of iodide or aggregates followed exponential functions of the form $y = a-ae^{-kt}$ (Fig. 3). The constants k for the decomposition of hGH and the appearance of iodide and aggregates, respectively, as well as the corresponding half-times, are listed in Table 1. These kinetics were indistinguishable in three different ^{125}I-hGH preparations tested. Storage temperature (either -20° or +4°) and freeze/thawing cycles did not appreciably affect the rate or type of tracer decomposition.

The rate constant for ^{125}I-hGH deiodination was, to our surprise, very similar to the physical decay constant of ^{125}I (0.01136 vs. 0.01153 day^{-1}), suggesting a causal link between the two processes. To further investigate the possible reasons for such linkage, the radiochemical nature of the decomposition products was assessed by determination of their energy spectra, physical decay constants and precipitability with $AgNO_3$. In particular, the radioactivity eluting at the total column volume (i.e. the salt peak) was of interest in this regard. The physical properties of this material corresponded to ^{125}I, both in terms of half-life and energy spectrum. Its chemical nature corresponded to I^-, as indicated by its quantitative precipitation with $AgNO_3$ in the presence of excess phosphate and stable iodide.

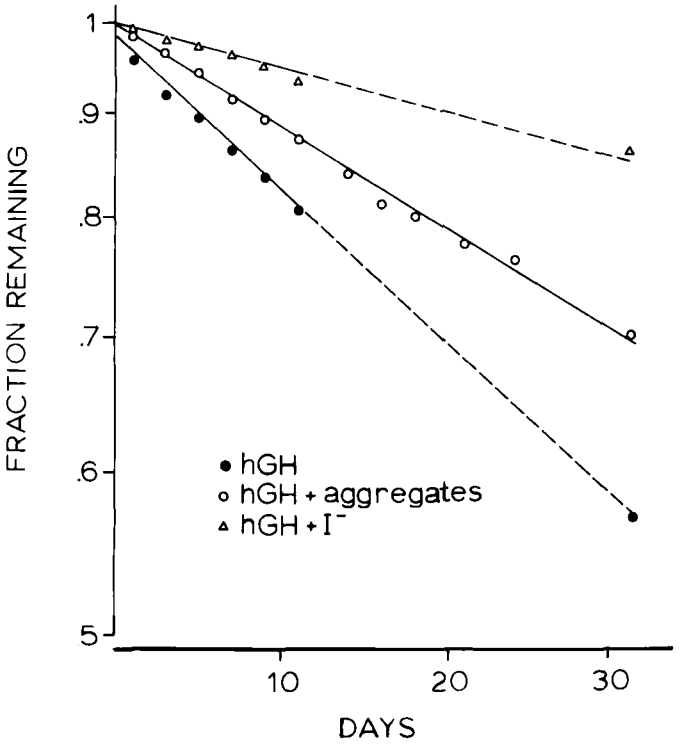

FIGURE 2 - The autodecomposition of ^{125}I-hGH is expressed as a function of time in terms of the fraction of original ^{125}I-hGH remaining (●). Each of the two major decomposition products and hGH in combination is also shown. Data shown represent averages of three separate radiolabeled ^{125}I-hGH preparations. Note the logarithmic ordinate.

DISCUSSION

The present data provide information on both the qualitative nature of ^{125}I-hGH decomposition and on its quantitative aspects. The main degradation product is iodide, with a number of aggregation states of ^{125}I-hGH also being generated. These findings are in agreement with previous observations (6). Frequent examination of

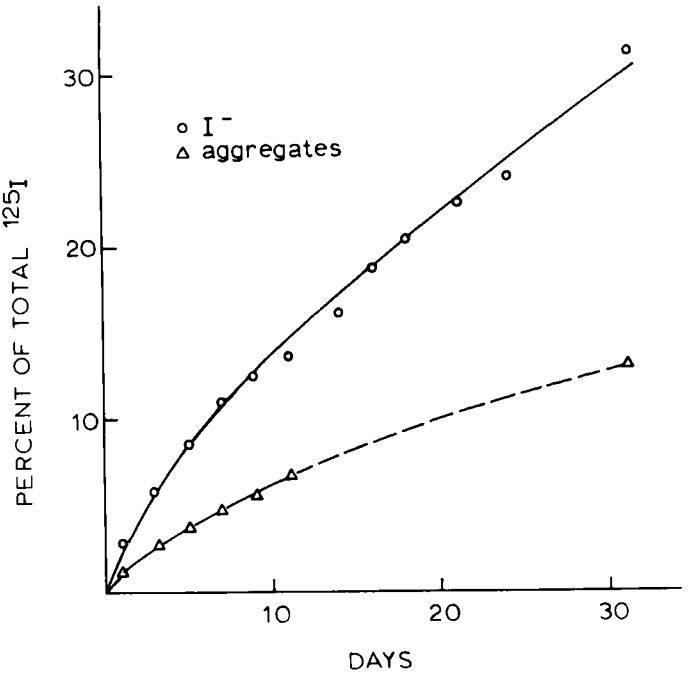

FIGURE 3 - Accumulation of the major radioactive decomposition products of ^{125}I-hGH as a function of time. Data represent averages of three radiolabeled preparations.

TABLE 1

Fractional Rate Constants for the Autodecomposition of ^{125}I-hGH and the Appearance of Decomposition Products.

	k (day^{-1})	$t_{\frac{1}{2}}$ (days)
^{125}I-hGH decomposition	0.01824	38
generation of free $^{125}I^-$	0.01136	61
^{125}I-hGH aggregation	0.00525	132

the tracer allowed us to derive the kinetic constants of decomposition. It appears that tracer decomposition is a continuous process following exponential laws.

A surprising finding was the apparent coincidence of one aspect of hGH decomposition, namely deiodination, and the physical decay of ^{125}I. The connection between these processes may seem logical at first, since radioactive decay of an iodine atom will alter its chemical nature such that it may be lost from iodotyrosine. In fact, this probably occurs, since ^{125}I decays by electron capture to ^{125}Te (7), which lacks the proper electron configuration for electron pairing with the tyrosyl ring. However, ^{125}Te is a stable element; it would not be counted as radioactivity in the salt peak. We considered the possibility that some impurity in the ^{125}I preparation used for radiolabeling may be responsible for the observation of a radioactive daughter in the salt peak, but we found no evidence for a radioisotope other than ^{125}I in that peak. Therefore, we suggest that radioactive decay of an iodine atom affects - on the average - one neighboring ^{125}I-hGH molecule in such a way as to cause the loss of an iodine atom. Alternatively, the deiodination of hGH and the decay of ^{125}I may fortuitously proceed at similar rates.

Although we have examined the behavior of only one radioiodinated polypeptide, i.e. hGH, this example may serve as a fairly representative paradigm. hGH was one of the first proteins

labeled to high specific activity (8). hGH is an inherently stable protein, and it contains no unusual structural features. Thus, within the limits of these considerations, it appears that deiodination and aggregation are primarily responsible for tracer polypeptide decomposition. It should be emphasized that other, more subtle alterations in molecular structure may also occur and manifest themselves by impaired antibody binding of aged tracer despite repurification efforts. However, few data addressing this possibility are available.

In summary, the present data provide initial information about the major decomposition products of radiolabeled hGH and the kinetics of their appearance. This information should be useful to immunoassayists in predicting the shelf-life of tracer polypeptides.

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Mailing Address:
Gerhard Baumann, M.D.
Center for Endocrinology, Metabolism and Nutrition
Northwestern University Medical School
303 East Chicago Avenue
Chicago, IL 60611

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