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Brief technical note

Human growth hormone radioiodination using different batches of ^{125}I of various ages

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Summary

The reproducibility and the influence of the batch and decay level of $Na^{125}I$ on the radioiodination of human growth hormone (hGH) were examined by a polyacrylamide gel electrophoresis (PAGE) technique. The between-day coefficient of variation (CV) exhibited a value of 11.9% for labelling yields and 14.3% for antibody specific binding. The within-day variation for different shipments and isotope decay-levels was similar to that for the control experiment, carried out under the same conditions, using $Na^{125}I$ from a single lot. The results indicate that the decay-level and batch do not significantly influence either the yields or the immunological properties of the labelled product. The suitability of this PAGE technique as a control test for radioiodinated proteins is established by comparison with gel chromatography on Sephadex G-100.

Introduction

It is generally believed that one of the main factors responsible for the low degree of reproducibility (and often total failure) in protein radioiodination by both ^{131}I and ^{125}I is the quality of the radioisotope. Thus, poor reproducibility has been attributed to noxious substances present in the radiochemical reagent [1–5], to variable specific activity [6] or isotopic abundance [7,8], to uncontrollable variability from batch to batch [2,4,7] and from manufacturer to manufacturer [2,4,9] and to the age of the isotopic preparation [6,8,10]. Many of these authors have noted that the existence of this capricious behaviour which is directly related to the so-called 'preparation damage' and which hampers the attainment of reproducible specific activities, requires the development and testing of more adequate quality control methods [4,8,11].

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We [12] and others [1,5,8,10] have found that the variability and the success of the labelling seems to depend mainly on the type and heterogeneity (presence of aggregates, isohormones, etc.) of the hormonal preparation. As quantities of fresh extracts were available, we were in a position to examine the between- and within-day reproducibility of the chloramine T labelling reaction with different batches of $Na^{125}I$ of various ages in order to define the length of time over which a given lot could provide comparable labelling results.

For this purpose, seven shipments of radioiodide, each received in a different month, were used. A PAGE technique, whose performance in this type of analysis is compared to the routinely used Sephadex G-100 gel filtration technique, was utilized to determine labelling yields and antibody specific bindings [13,14] of hGH preparations simultaneously labelled with three or more different batches of $Na^{125}I$ of various ages.

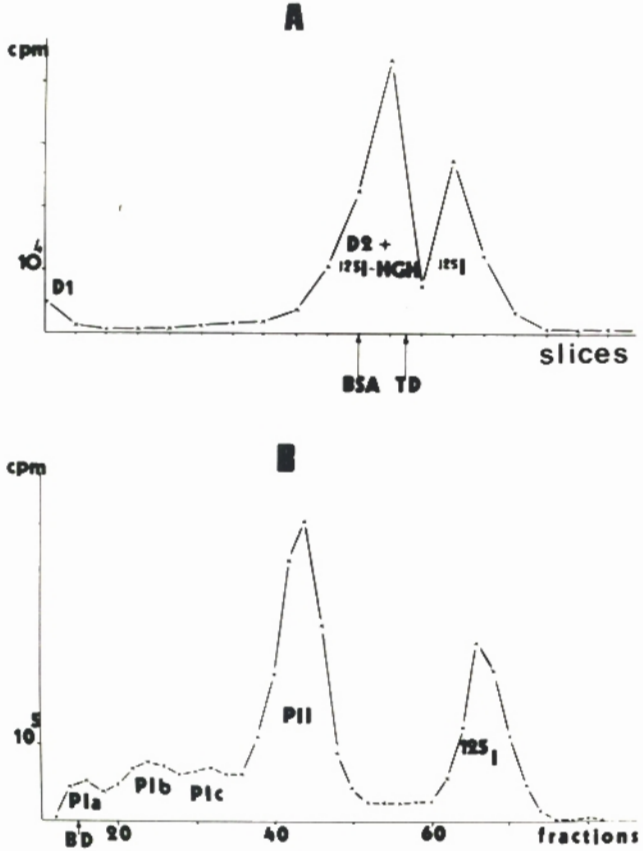

Fig. 1. A: Typical 7% polyacrylamide gel electrophoresis in 15-cm tube of a labelling mixture of hGH. Marker: bovine serum albumin (BSA) and tracking dye (TD) bromophenol blue. B: Sephadex G-100 chromatography of the same labelling mixture. Column size: 2 cm × 40 cm; flow rate: 12 ml/h; fraction volume 2 ml. Void volume marker: Blue Dextran (BD). See 'Results' for further explanations.

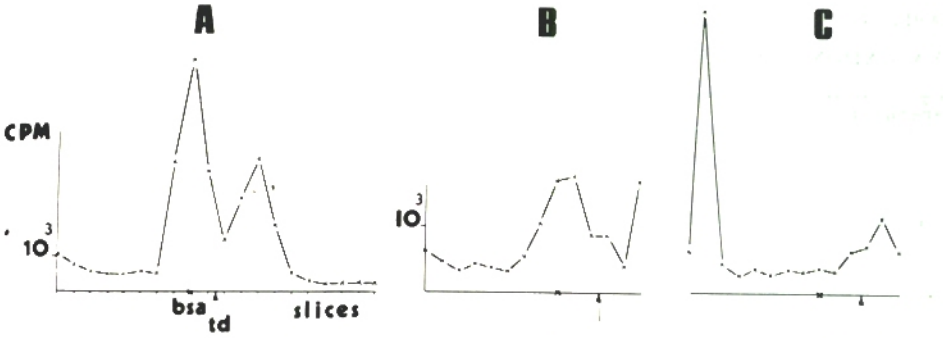

Fig. 2. Typical example of PAGE analysis of the products of a labelling reaction. A, Labelling mixture; B, one-day incubation blank; C, one-day incubation with antiserum anti-hGH, diluted 1 : 2000.

Materials and methods

Human growth hormone (lots 12 and 13) used in the labelling, was prepared in this laboratory (IPEN) as previously described [13]. $Na^{125}I$, free of carriers, of high specific radioactivity (from 450 to 550 $\mu Ci/\mu l$), was purchased from New England Nuclear (Boston, MA, USA). Rabbit antiserum against hGH was prepared at the IPEN, and used at a dilution of 1 : 2000; its performance had been previously compared to that of antiserum kindly provided by the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), Bethesda, MD, USA. PAGE, as well as the incubation technique were performed as described elsewhere [13]. The labellings were performed according to the method of Greenwood [11], using 50 μg of chloroamine T and 200 μg of metabisulfite for 5 μg of hGH in a final volume of approximately 70 μl.

Results

The performance of 7% PAGE, shown in Fig. 1A, can be compared to the simultaneous chromatographic purification of the same labelling mixture (Fig. 1B).

TABLE I

CORRESPONDENCE BETWEEN SEPHADEX G-100 AND PAGE ANALYSIS

Sephadex G-100			7% Page	
Component	cpm (% of total)		Component	cpm (% of total)
PIa	6.6		D1	4.6
PIb + PIc	17.4	64.1	D2 + [^{125}I]hGH	62.7
PII	46.7			
^{125}I	29.2		^{125}I	32.7

TABLE II

EVALUATION OF IMMUNOACTIVITY OF IMPURE TRACER IN 6 EXPERIMENTS

Experiment	Yield				Binding to excess of antibody			
	n	$\bar{X}$ (%)	SD	CV (%)	n	$\bar{X}$ (%)	SD	CV (%)
Control	4	60.0	4.6	7.7	4	67.7	9.5	14.1
1	4	56.7	6.6	11.7	3	74.1	7.2	9.7
2	3	51.1	4.0	7.8	4	51.1	9.5	18.7
3	4	69.6	5.9	8.4	4	66.0	5.3	8.0
4	4	58.1	4.7	8.1	4	74.6	2.7	3.6
5	3	50.9	2.2	4.4	3	(25.2)	3.2	12.7
Between-day	6	57.7	6.9	11.9	5	66.7	9.5	14.3

According to the nomenclature used for the Sephadex G-100 fractions in previous work [12,14], in a regular hGH radioiodination we usually obtain, in addition to monomeric [^{125}I]hGH, presumably dimeric (PIc) and aggregate (PIa) forms of [^{125}I]hGH, and free ^{125}I. On a PAGE, PIa (refered to as D1) is better resolved, while PIb (an immunologically inactive form, called D2 in this system) and PIc migrate unresolved and indistinguishable from the monomeric form. The peak of free ^{125}I always migrates faster than the tracking dye bromophenol blue. The reasonable correspondence between the two analytical systems is exemplified by the data in Table I, which refers to the separations presented in Fig. 1.

In this type of quality control, a sample of each labelling mixture is analyzed on PAGE as in Fig. 2A, following addition of metabisulfite and buffer with carrier KI and bovine serum albumin (BSA). As a first approximation, the percentage of total counts corresponding to [^{125}I]hGH (monomer and dimer) + D2, was calculated as labelling yield.

In order to confirm that reproducibility in the total yields of labelling was accompanied by a comparable reproducibility in the amount and immunoactivity of the resultant [^{125}I]hGH, samples of the labelling reaction were incubated for 24 h with antibody diluted 1 : 2000 and then analyzed on the same PAGE system (Fig. 2B and C). Since free ^{125}I is eliminated during electrophoresis and D1 can easily be discounted, the method permits rapid evaluation of the immunoactivity of our impure tracer.

Table II presents the results of this type of analysis for six separate experiments. A series of four simultaneous labellings using the same fresh batch of ^{125}I served as the control. In the other experiments, 6 monthly batches, ranging in age from less than 1 to 4 months at the time of the experiment, were employed. Approximately the same radioactivity (814.7 $\pm$ 70.6 μCi) was used in each of the 22 labellings. One average binding value (experiment 5) was rejected as being outside of the control limits on the basis of the between-day variance. An F-test, comparing the within-day variances of the control test to the variance of each separate experiment showed that the differences, in yields and specific bindings, were never significant ($P = 0.05$).

Discussion

The results presented here indicate that efficiency and reproducibility in ^{125}I-labelling is not remarkably variable from batch to batch or day to day. In contrast to other reports, we find no marked influence due to the age of the radioisotopic preparation, whose deterioration had been attributed, among other causes, to tellurium build up, derived from ^{125}I decay. Our results therefore imply that Na^{125}I can be stored, even in high concentration, for times up to almost two half-lives.

In our hands labelling reproducibility and extent of 'preparation damage' have not been proven to be as variable as has often been stated. Our labelling yields presented a CV less than 12%. The percentage of specific binding, a more direct indication of the extent of immunological damage, approached 67% with a maximum CV of 18.7%. Furthermore, the contribution from D1 (aggregate forms), in all these experiments was always less than 9% ($4.85 \pm 2.42\%$ for $n = 22$) of the total radioactivity.

We believe, on the basis of our present and previous results, that the nature, purity and storage conditions of the hormonal preparation are the primary factors responsible for many cases of poor and/or irreproducible iodination. Although we employed two different extraction lots of hGH in this study, without noting any appreciable differences, it should be emphasized that the hormone solutions were freshly prepared immediately prior to each labelling and no extract was older than 6 months.

The present method of quality control, which takes advantage of the versatility and flexibility of the PAGE technique, can be readily adapted to the analysis of reproducibility, yields and specific bindings in these and other radioactive tracer labelling experiments.

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