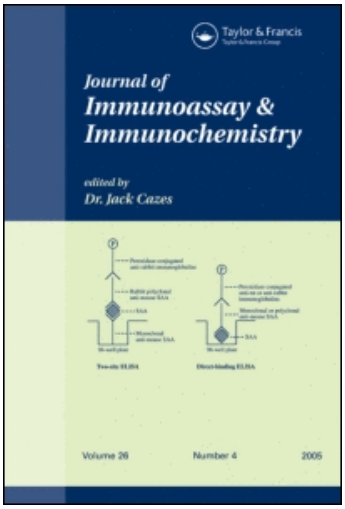

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PREPARATION OF ^{125}I-LABELED HUMAN GROWTH HORMONE OF HIGH QUALITY BINDING PROPERTIES ENDOWED WITH LONG-TERM STABILITY

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ABSTRACT

^{125}I-labeled human growth hormone (^{125}I-labeled.hGH) was prepared by using two variants of the chloramine T labelling procedure and purified by polyacrylamide gel electrophoresis (PAGE) of the reaction mixture.

Variant A produced a tracer with high specific activity (100 ± 10 μCi/μg), high maximal binding capacity to antibodies (93%) and long-term stability (at least 150 days at -20°C). No diiodinated tyrosil residues could be detected in this tracer.

Variant B was devised to obtain higher yields of labeled hormone. The electrophoresis of the iodination mixture revealed two radioactive components with Rm values of 0.49 and 0.55 which result from the iodination of hGH variants preexisting in the starting material. Both tracers had similar specific activities (70 ± 10 μCi/μg), high maximal binding capacity to antibodies or receptors (80-100%, after 80 days of their obtention) and high stability (at least 100 days at -20°C).

It is concluded that the iododerivatives of hGH obtained by either method are adequate to perform radioimmunoassay and receptor studies and have long-term stability.

(KEY WORDS: biological and immunological properties; iodination of proteins; iododerivatives of human growth hormone; radioreceptor assays; stability studies)

INTRODUCTION

Although it has been shown that extensively iodinated human growth hormone retains most of its biological and immunological properties (1, 2), a high incorporation of radioactive iodine to this protein rapidly affects its biological activities probably due to the occurrence of an intense radiation damage (3).

The present practice for radioiodination of hGH is a compromise directed to generate derivatives containing an average of no more than one atom of ^{125}I per molecule of protein. Since the purification of these tracers is usually achieved by gel filtration through a column of Sephadex G-100, they are heterogeneous mixtures with differing proportions of monoiodo and polyiododerivatives plus non-iodinated molecules. The radioligands thus obtained have limited stability mostly due to the decay of the polyiodinated species which originate products with altered biological properties (4). These tracers must be used, in general, within one or two weeks of their obtention or repurified by a new gel filtration.

The purpose of the present work was to obtain and characterize a mono ^{125}I-labeled hGH with enough purity to display an specific activity close to its theoretical value, suitable for immunological assays and receptor studies and endowed with long-term stability (at least 3 months).

MATERIALS AND METHODS

Materials: hGH was prepared by the method of Mills et al. (5). Carrier-free $Na^{125}I$ was obtained from New

England Nuclear, Boston, MA, USA.
Rabbit antiserum against hGH was obtained as indicated by Poskus et al. (6). The mouse monoclonal antibodies (QA68 and NA39) were kindly provided by Dr. J. Ivanyi. Rat liver microsomes were prepared as described by Bonifacino et al. (7).

Methods:

Iodination procedures: Two variants of the Na^{125}I-chloramine T procedure of Greenwood et al. (8), as modified by Roth (9), were employed: in variant A the molar ratios of the before mentioned reagents and hormone were 2:1:1; in variant B they were changed to 1:5:2.

In variant A the iodination was done as follows: 1 mCi of Na^{125}I was added to 5 µg of hGH dissolved in 20 µl of 0.3 M potassium phosphate buffer pH 7.4, followed by 0.22 µg of chloramine T dissolved in 3 µl of 0.05 M potassium phosphate buffer, pH 7.4. The reaction was allowed to proceed at room temperature for 5 min, when 5 µl of a solution of 0.2 mg/ml of potassium thiosulfate were added. Since, under these conditions, the incorporation of iodine to the protein was not greater than 20%, the formation of polysubstituted molecules was minimal but also the yield of useful tracer was low.

Applying variant B the yield of tracer was higher, the percentage of iodination raised to 80% and polyiodination was diminished by increasing the proportion of cold hormone. The reagents were added in the same order as in variant A but in the following amounts: 1 mCi of Na^{125}I, 20 µg of hormone dissolved in 30 µl of buffer and 0.88 µg of chloramine T dissolved in 12 µl of buffer. The length of the reaction and its halting were as described before.

Isolation of the ^{125}I-labeled hGH from the reaction mixtures: It was performed by PAGE as indicated for the preparation of monoiodoinsulin (4, 10,11). After the electrophoretic run the gel was fractionated into 1.5 mm slices with an appropriate cutter, and each slice was extracted overnight at 4°C by immersion in 400 µl of 0.1 M NH_4HCO_3 buffer, pH 8.0, containing 0.5% (w/v) of bovine serum albumin (BSA). The relative mobility values (Rm) were calculated relative to the displacement of the tracking dye (bromophenol blue). The extracts selected for further studies were diluted with 25 mM-Tris/HCl, pH 7.4, to a final radioactivity concentration of 4-5x10^3 cpm/µl and

then kept at -20°C until used. The specific activity (S.A.), determined by self-displacement analysis (12), was 100 ± 10 μCi/μg for the fraction of Rm 0.49 (variant A), and 70 ± 10 μCi/μg for fractions of Rm 0.49 and 0.55 (variant B).

In order to obtain conventional tracers purified by gel filtration for comparative purposes, fractions of the reaction mixtures obtained by either method, were chromatographed in a Sephadex G-100 column (7). The specific activity of these tracers was calculated from the values describing the elution profiles and varied from 20 to 60 μCi/μg.

When conventionally purified tracers of higher specific activity (160 μCi/μg) were required, they were prepared increasing slightly the amount of chloramine T used.

Determination of the maximal binding capacity of the tracers: These values were established with polyclonal antibodies or with rat lactogenic receptors.

The specific binding of the tracers to increasing dilutions of the antiserum was evaluated by liquid phase radioimmunoassay (RIA) (13). When using microsomal proteins from female rat livers the specific binding to the lactogenic receptors was determined as described by Bonifacino et al. (7).

The method used to analyze the data (12), plots the inverse of the counts specifically bound versus the reciprocal dilution of the acceptor used and gives an estimate of the proportion of bioactive hormone present in the tracer.

Stability of the tracers on storage at -20°C: The stability of the tracers obtained by variant A was evaluated by measuring their total binding to an excess of polyclonal antiserum and their non-specific binding in the absence of antiserum, as a function of time. The experiments were carried out as indicated by Peña et al. (13). Since charcoal is used to separate the antibody complexes from non-damaged free tracer, when this method is applied in the absence of antibodies, it evaluates as non-specific binding the presence of aggregates and/or degradation products arising from the tracer (14). As an alternative method to detect molecular damage of the tracer, we also applied high performance liquid chromatography (HPLC) on a Biosil TSK-250 Column (Bio-Rad, U.S.A.). The chromatographies were performed with 20 mM Na_2HPO_4, 50 mM Na_2SO_4, pH 6.8 at a flow rate of 1 ml/min. The elution profiles permitted the quantitative differentiation of aggregates and fragments from the monomeric tracer.

Measurement of the proportion of mono and diiodo tyrosines present in the tracers: An amount of tracer containing approximately 1.10^6 cpm was completely hydrolyzed by incubation for 16 h at 37°C with 0.1 mg of pronase (Sigma). The reaction was carried out in 150 µl of 50 mM Na_2HPO_4 buffer, pH 7.4. The identification of the iodotyrosines was done by gel filtration on a Sephadex G-25 column (15).

Fractionation of unlabeled hGH by PAGE: 400 µg of unlabeled hGH were submitted to PAGE under the same conditions employed for the iodination mixture. Each gel slice was extracted with 200 µl of 50 mM NA_2HPO_4 buffer, pH 7.4. The concentration of immunoreactive hormone in each extract was evaluated by a solid phase RIA as follows: 200 µl of a dilution of the rabbit antiserum in 13 mM sodium borate buffer, pH 8.0, were deposited in the wells of a disposable polyvinyl flex microtiter plates (Dynatech Lab. Inc., Alexandria, VA, U.S.A.) and incubated 16 h at 37°C. The dilution chosen was that required to bind 50% of the labeled hormone. The wells were emptied by aspiration, rinsed twice with saline and then incubated 10 min at room temperature with 13 mM sodium borate buffer, pH 8.0, 0.5% (w/v) BSA. The emptied wells thus prepared were incubated 16 h at 37°C with 100 µl of different dilutions of the gel slices-extracts and 1.10^4 cpm of ^{125}I-labeled hGH. At the end of the incubation period, the wells were washed twice with saline and counted in a γ-counter. A standard curve prepared with a solution of known concentration of hGH was run in parallel. The concentration values were interpolated in a logit-log plot (16).

RESULTS

^{125}I-Labeled hGH Prepared by Variant A

Purification procedure: Figure 1, bottom panel, shows the distribution of radioactivity after PAGE of the iodination mixture. The specific binding to a polyclonal antiserum against hGH of the different eluted fractions, as well as the tracer damage, are shown in the top panel of Figure 1.

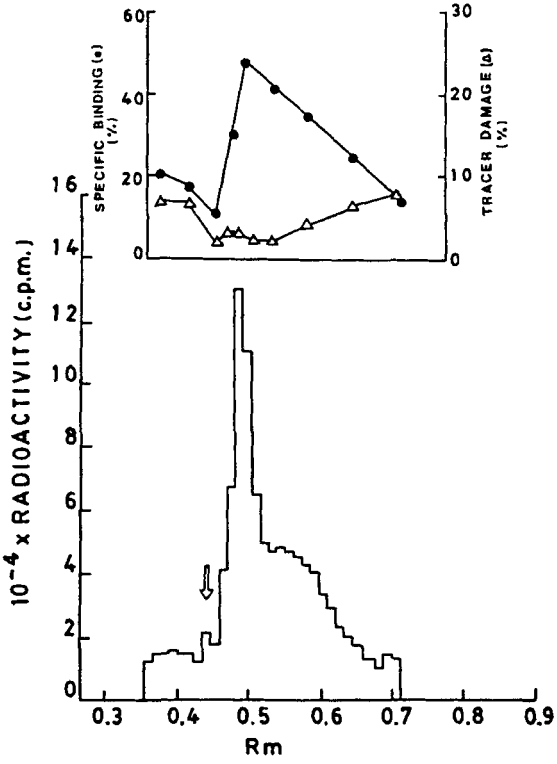

FIGURE 1: PAGE of the iodination mixture prepared according to variant A for labeling hGH. Binding properties of the different fractions.
The bottom panel shows the distribution of radioactivity in the gel after the electrophoresis. To this end, 1.5 mm slices of gel were eluted as indicated in the Materials and Methods section. The position of the unlabeled hormone (arrow) was determined in a separate gel run in parallel and stained with Coomasie Brilliant Blue. The unreacted $Na^{125}I$ runs ahead of the tracking dye (bromophenol blue). In the top panel it is shown the specific binding to polyclonal antibodies (●) of the fractions aligned below measured by a solid phase RIA (17) as well as the tracer damage (Δ) evaluated with charcoal (see Materials and Methods).

The results obtained indicate that the fractions comprised between Rm 0.49 and 0.52 show the highest binding to the antibodies and the lowest damage. For fractions with higher Rm values, the specific binding curve rapidly decreases while the damage of the tracer increases steadily.

The maximal binding capacity to polyclonal antibodies was also measured for fractions of Rm 0.49 and 0.60. The values obtained were 93% and 38% respectively. The specific activity of the Rm 0.49 fraction was 100 ± 10 μCi/μg, determined by self displacement analysis.

According to these results, fractions of Rm 0.49-0.52 were selected for further study.

Specific binding properties: The results collected in Figure 2 compare the binding properties of two labeled hormone preparations with high specific activity. A tracer purified by PAGE (S.A. 100 μCi/μg), displays a higher B/F ratio than a conventional tracer obtained by gel filtration (S.A. 160 μCi/μg), for all the acceptor systems studied.

Stability at -20°C: As shown in Figure 3 the fractions of tracer with Rm comprised between 0.49 and 0.52 were remarkably stable at -20°C for more than 150 days. The total binding to an excess of polyclonal antiserum remained high (between 80-90% of the total counts added) while the non-specific binding increased slightly up to approximately 25%. The differences between both values allow an extended use of these tracers for RIA and binding studies.

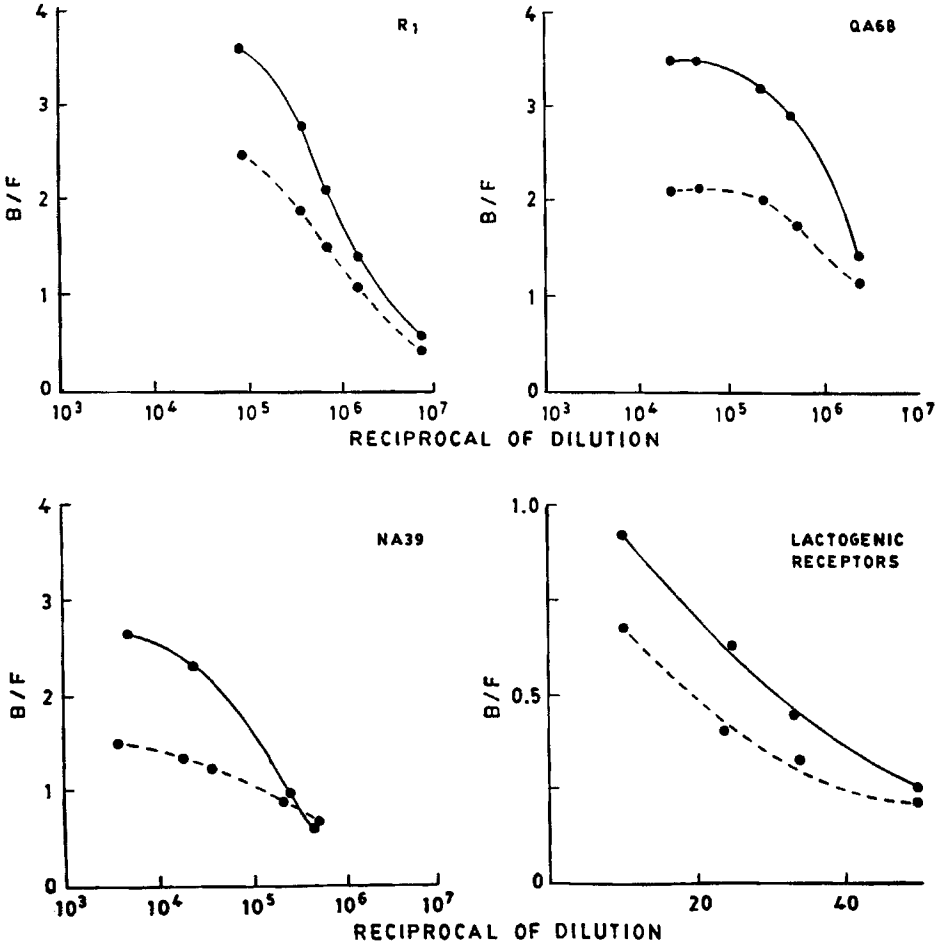

FIGURE 2: Specific binding of the Rm 0.49 tracer (see Figure 1) to a polyclonal antiserum, two monoclonal antibodies and lactogenic receptors, compared to that of a conventional tracer purified by gel filtration.
The variation of the B/F ratios at different dilutions of a rabbit polyclonal antiserum (R_1), monoclonal antibodies (QA68, NA39) and lactogenic receptors from female rat liver, were measured for the ^{125}I-labeled hGH prepared by variant A (——) and purified by PAGE (S.A.: 100 μCi/μg) and for a conventional tracer (---) purified by gel filtration (S.A.: 160 μCi/μg). Immunological assays were performed by liquid phase RIA (13). Receptor assays were carried out as described by Bonifacino et al. (7). The experiments were completed within 5 days of preparation of the conventional tracer.

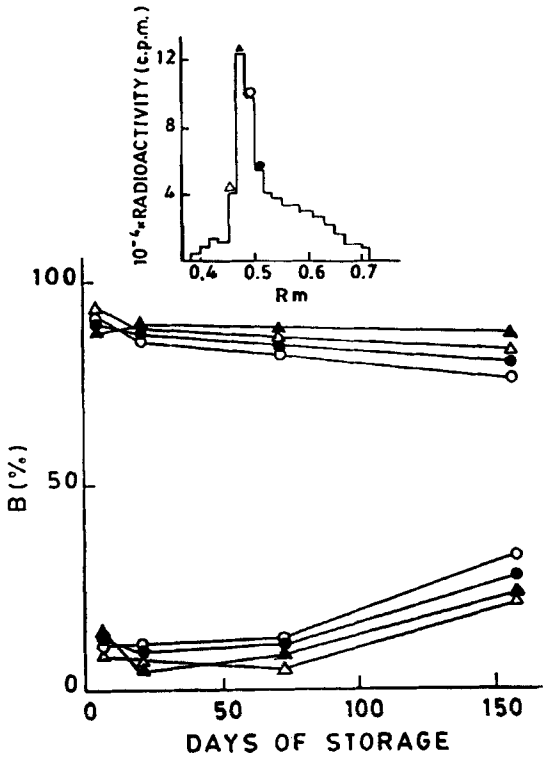

FIGURE 3: Effect of storage at -20°C on the stability of the tracers Rm 0.49 to 0.52 (variant A). The tracers indicated on the top diagram were kept at -20°C until assayed by liquid phase RIA (13). The upper curves in the bottom graph show the total binding of the tracers to an excess of polyclonal antiserum while the lower ones correspond to the non-specific binding determined in the absence of antiserum. B (%) indicates the percentage of radioactivity bound with respect to the total amount of radioactivity initially placed in each tube.

TABLE 1

Content of Iodo Amino Acids and ^{125}I Present in the ^{125}I-Labeled hGH Prepared by Variant A and Purified by PAGE or by Gel Filtration.

The tracers were completely hydrolyzed by digestion with pronase, as indicated in the Materials and Methods section. The enzymatic products were filtered through a Sephadex G-25 column (1.2 x 110 cm) (15). Results are expressed as percentage of total radioactivity in the eluate.

^{125}I-labeled hGH Purification Method	Fraction	^{125}I-	Content of MIT %	DIT
PAGE	Rm 0.49	1.54	95.20	0
	Rm 0.50	2.90	95.88	0
	Rm 0.60	9.07	82.30	5.46
Filtration through Sephadex G-100	Monomeric Peak	5.67	88.01	2.89

Content of monoiodotyrosine (MIT) and diiodotyrosine (DIT) in different tracer preparations: It was established as indicated in Table 1. The only iodo amino acid detected in the tracers purified by PAGE, with Rm 0.49 and 0.50, was MIT whereas a significant proportion of DIT is present in the fraction with Rm 0.60, as well as in the tracer conventionally purified. The increasing ^{125}I values along the gel indicate a trailing contamination with the unreacted isotope which runs with a Rm 1.2. A similar effect explains the ^{125}I content in the conventionally purified tracer.

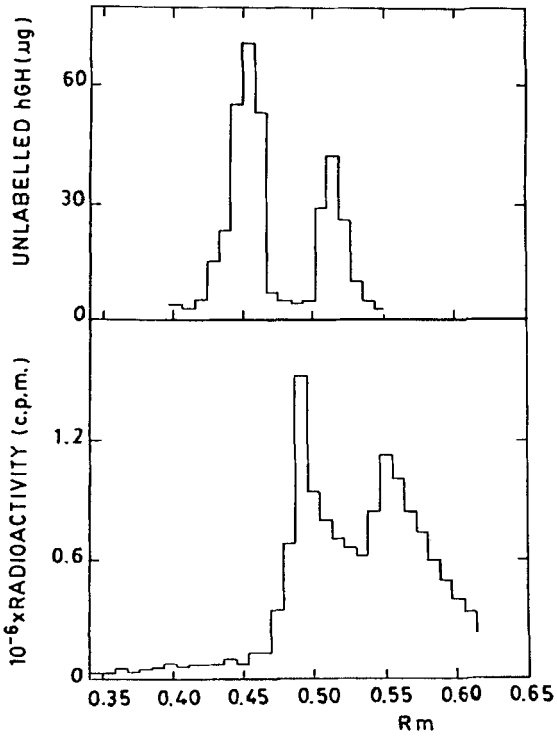

FIGURE 4: PAGE of hGH and of the incubation mixture prepared according to variant B of labeling the hormone.
The distribution in the gel of 400 µg of unlabeled hormone is shown in the top panel. The gel was screened by a solid phase RIA as indicated in the Materials and Methods section. Two components with Rm 0.45 and 0.51 were detected. The distribution of radioactivity in the gel, corresponding to the labeling incubation mixture is shown in the bottom panel. Two components with Rm 0.49 and 0.55 were evident.

^{125}I-Labeled hGH Prepared by Variant B

Purification procedure: The distribution of the different radioactive components (with the exception of ^{125}I) present in the iodination mixture and separated by PAGE is shown in Figure 4 (bottom panel). Two main components, with Rm 0.49 and 0.55, were evident and both had a similar specific activity (70 μCi/μg) determined by self displacement analysis. Since the unlabeled hormone also gave two components by PAGE (Figure 4, top panel), the origin of both radioactive species was suspected to arise from the iodination of variants forms present in the starting hormone. This hypothesis was proved true by separate iodination and PAGE of each native species as well as of their mixture. The pattern obtained with the mixture (not shown) was indistinguishable from that displayed in Figure 4 (bottom panel).

Maximal binding capacity of the Rm 0.49 and 0.55 tracers: The maximal amount of labeled hormone that an antibody or receptor system was able to recognize is shown in Table 2. Independently of the acceptor used both tracer showed maximal binding capacities in the upper attainable level which remained constant for at least 80 days of storage at -20°C.

Stability at -20°C: This property was studied by establishing the presence of monomeric or aggregated hormone and degradation products, during prolongued storage of the tracers at -20°C (Figure 5). The analysis was carried out by a HPLC-gel filtration procedure which showed that the tracers of Rm 0.49 and

TABLE 2

Maximal Binding Capacity of the Tracers of Rm 0.49 and 0.55 (Fig.4).

Polyclonal antiserum or lactogenic receptors from female rat liver were employed for these measurements which were carried out as indicated in the Materials and Methods section. The results express the percentage of the total radioactivity added that an infinite acceptor concentration is able to recognize.

Tracer assayed (Fig.4)	Acceptor System	Maximal Binding Capacity %		
		Days of storage at -20°C		
		9	25	80
Rm 0.49	polyclonal antibodies	113	85	81
	lactogenic receptors	108	89	97
Rm 0.55	polyclonal antibodies	102	95	100
	lactogenic receptors	80	94	102

0.55 were remarkably stable, at least during 90 days at -20°C. In contrast, a conventional tracer also obtained by variant B (50 μCi/μg), in spite of its low specific activity, had only a 50% of monomeric labeled hormone after the same time.

DISCUSSION

The purification by PAGE of hGH iodinated by variant A led to the isolation of a tracer with high

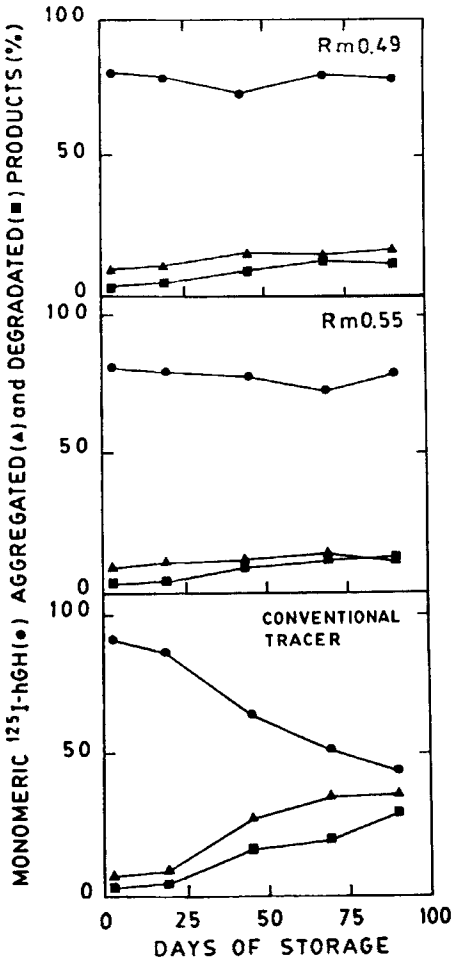

FIGURE 5: Study of the stability at -20°C of the tracers, prepared by variant B, purified by PAGE, compared to that of a conventional tracer purified by gel filtration. Samples of each tracer of Rm 0.49 (S.A. 70 µCi/µg) or Rm 0.55 (S.A. 70 µCi/µg) and a conventional one (S.A. 50 µCi/µg) were submitted to HPLC through a Bio-Sil TSK250 column, as indicated in the Materials and Methods section. The percentage of monomeric hormone (●), aggregated hormone (▲) or degradation products (■) was evaluated. This analysis was repeated several times during a total period of 90 days, in which the tracers were kept at -20°C.

specific activity (100 ± 10 μCi/μg), high maximal binding capacity to antibodies (93%), long-term stability (150 days) (Figure 3) and absence of DIT (Table 1). Since the theoretical specific activity of a ^{125}I-labeled hGH containing 1 atom of ^{125}I per molecule is 110 μCi/μg (9), the forementioned results suggest that the tracer obtained is a monoiodo-derivative of the hormone. However, it is doubtful that this tracer is an homogeneous species since there are, at least, 6 tyrosines equally accessible for iodination in hGH (1).

The fractions eluted from the gel corresponding to zones of high mobility (Rm ≥ 0.60) showed a low specific binding to polyclonal antibodies, a high tracer damage (Figure 1), a low maximal binding capacity (38%), and had DIT in their molecules (Table 1). These data suggest the presence of polyiodinated derivatives and are in accordance with the higher values of specific activity obtained by Hughes et al. (3) for species of labeled hGH migrating close to the anode in PAGE.

The gel filtration method usually employed to purify the crude iodinated hormone is not effective to separate among themselves the species with different degrees of iodination. Hence, all the species coexist in these tracers. The effect of this heterogeneity is clearly shown when the specific binding to different acceptors of one of these tracers (containing an average of 1.45 atoms of ^{125}I per molecule of hormone) is compared to that of a tracer purified by PAGE (0.90 atoms of ^{125}I per molecule) (Figure 2). Our results are not in accordance with those reported by Hughes et al. (3) who have demonstrated that hGH and prolactin retain their biological and immunological activities

after iodination to high specific activity (corresponding to an average of 1.5 atoms of ^{125}I per molecule of hormone). Furthermore, these authors assayed the tracer within six days of their preparation and no information is given in their long-term stability. Data from our laboratory (not shown) indicate that the conventionally purified tracer (S.A. 160 μCi/μg) stored 15 days at -20°C originates 70% of aggregated hormone and degradations products, evaluated by HPLC-gel filtration. This finding supports the idea that tracers with high specific activity, purified by gel filtration, are not convenient for long-term use even if they retain full biological potency immediately after their obtention. Although it is known the higher stability of conventional tracers with low specific activity (Figure 5), it should be emphasized that this kind of radioligands are unsuitable when radioimmunoassays or radioreceptor-assays of high sensitivity are needed.

The tracer prepared by variant A has good binding properties, high specific activity, and is useful for, at least, 150 days. However, it is produced in low yield since the incorporation of ^{125}I is only 20% of the total and the iododerivatives obtained represent only 15% of the labeled molecules. Hence, the iodination procedure was modified by varying the molar ratios of the reagents (Variant B), whereby it was possible to obtain a higher yield of two radioligands with similar specific activities, stabilities and quality. These labeled species are the same present in the gels obtained with variant A although in this case the tracer with Rm 0.55 is only seen as a shoulder (compare Figures 1 and 4).

The Rm 0.49 and 0.55 tracers obtained with variant B are originated from the iodination of forms of hGH preexisting in the native hormone (Figure 4). The decrease in specific activity of these tracers, compared to that of the tracer prepared by variant A, can be explained by the greater concentration of unlabeled hormone used which probably contaminates the fractions studied. The higher maximal binding capacity (Table 2) and the long-term stability (Figure 5) of both tracers suggest that they are monoiododerivatives.

The two forms of native hGH detected by PAGE were also found in other preparations of the hormone obtained in our laboratory and in the lot HS2243E from N.I.H., N.I.A.M.D.D. Hormone Distribution Program. When the first form (Rm 0.45) isolated by PAGE was stored at -20°C for 1 month, and then submitted again to electrophoresis, the second form (Rm 0.51) was detected (not shown). This result suggests that the component of Rm 0.51 could be a deamidated form arising from that of Rm 0.45 as indicated by Lewis et al. (18). Both their chemical differences as well as the way in which they affect the binding of the tracers (Rm 0.49 and 0.55) to different acceptors systems are now in study.

Although the various ^{125}I-labeled GHs obtained by the usual procedures in the literature have been extensively used, in many instances they are products of either low specific activity and low sensitivity or high specific activity with poor stability. The iododerivatives described in this paper can be reproducibly obtained with high specific activity and long-term stability. These properties ensure high quality and sensitivity in their binding behaviour and

a considerable saving in ^{125}I costs. This last consideration is important in countries where this isotope is not locally produced.

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REFERENCES

1. Goodman, H.M. and Levy, L.K. Preparation and biological reactivity of polyiodinated human growth hormone. Endocrinology 1983; 113: 2017-2023

2. Bartolini, P. and Ribela, M.T. Influence of chloramine T iodination on the biological and immunological activity on the molecular radius of the human growth hormone molecule. J. of Immunoassay 1986; 7: 129-138

3. Hughes, J.P.; Tanaka, T.; Gout, P.W.; Beer, C.T.; Nobel, R.L. and Friesen, H.G. Effect of iodination on human growth hormone and prolactine: characterized by bioassay, radioimmunoassay, radioreceptor assay, and electrophoresis. Endocrinology 1983; 111: 827-832

4. Linde, S., Hanse, B., Sonne, O., Hoest, J.J. and Gliemann, J. Tyrosine A14 [^{125}I] monoiodoinsulin. Preparation, biological properties, and long-term stability. Diabetes 1981; 30:1-8

5. Mills, J.B., Ashworth, R.B., Wilhelmi, A.E. and Hartree, A.S. Improved method for the extraction and purification of human growth hormone. J. Clin. Endocrinol. Metab. 1969; 29: 1456-1459

6. Poskus, E., Zakin, M.M., Fernández, H.N. and Paladini, A.C. Detection of immunologically active zones in equine growth hormone. Eur. J. Immunol. 1976; 6: 409-417

7. Bonifacino, J.S., Sánchez, S.H. and Paladini, A.C. Characterization of human somatotropin binding to detergent-solubilized lactogenic receptor from rat liver. Biochem. J. 1981; 194: 385-394

8. Greenwood, F.C., Hunter, W.M. and Grover, J.S. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem. J. 1963; 89:114-123

9. Roth, J. Methods for assessing immunologic and biologic properties of iodinated peptide hormones. In: O'Malley, B.W. and Hardman, J.G., eds. Methods in Enzymology, vol. XXXVII, Hormone Action, part B. New York: Academic Press 1975: 223-228

10. Linde, S. and Hansen, B. Preparation and characterization of monoiodoinsulin. Int. J. Peptide Protein Res. 1974; 6: 157-163

11. Linde, S., Hansen, B. and Lernmark, A. Preparation of stable radioiodinated polypeptide hormones and proteins using polyacrylamide gel electrophoresis. In: O'Malley, B.W. and Hardman, J.G., eds. Methods in Enzymology, vol. XCII, Immunochemical Techniques, Part E, New York; Academic Press 1983: 309-335

12. Calvo, J.C., Radicella, J.P. and Charreau, E.H. Measurement of specific radioactivities in labelled hormones by self-displacement analysis. Biochem. J. 1983; 212: 259-263

13. Peña, C., Poskus, E. and Paladini, A.C. A relevant antigenic site in human growth hormone localized in sequence 98-128. Mol. Immunol. 1980; 17: 1487-1491

14. Odell, W.D. Use of charcoal to separate antibody complexes from free ligand in Radioimmunoassay. In: O'Malley, B.W. and Hardman, J.G., eds. Methods in Enzymology, vol. LXX, Immuchemical Techniques, Part A, New York: Academic Press 1980; 274-279

15. Mattera, R., Turyn, D., Fernández, H.N. and Dellacha, J.M. Structural characterization of iodinated bovine growth hormone. Int. J. Peptide Protein Res. 1982; 19: 172-180

16. Feldman, H. and Rodbard, D. Mathematical theory of radioimmunoassay. In: Odell, W.D. and Daughaday, W.H., eds. Principles of Competitive Protein Binding Assay, Lippincott, Philadelphia and Toronto 1971; 158-203

17. Vita, N. Poskus, E., Peña, C., Pérez, A.R. and Paladini, A.C. Epitopes in human growth hormone and chorionic somatomammotropin studied with monoclonal antibodies. Arch. Biochem. Biophys. 1983; 225:436-445

18. Lewis, U.J., Singh, R.N.P., Bonewald, L.F. and Seavey, B.K. Altered proteolytic cleavage of human growth hormone as a result of deamidation. J. Biol. Chem. 1981; 256: 11645-11650