BIOSYNTHESIS OF GLUCAGON IN ISOLATED PIGEON ISLETS[1]

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ABSTRACT

Biosynthesis of glucagon was studied using isolated pigeon islets of Langerhans. H^3-leucine and H^3-tryptophan were found to be actively incorporated into glucagon and a large molecular weight glucagon-related component. Incorporation into both components was inhibited by cycloheximide and significantly reduced by glucose. The possibility of a precursor molecule for the biosynthesis of glucagon was suggested.

INTRODUCTION

The biological roles of the polypeptide hormone, glucagon, are well known (1,2). However, no studies have been done on the biosynthesis of glucagon. The present communication provides evidence for the biosynthesis of glucagon and a possible precursor of glucagon in isolated pigeon islets of Langerhans.

EXPERIMENTAL

Avian islets of Langerhans were chosen as biological materials for our study of glucagon biosynthesis because of their high glucagon

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content (3). Islets were prepared by collagenase treatment (4) of pigeon pancreases. Pigeons were obtained from NASCO Company, Fort Atkinson, Wisconsin, and were starved overnight before the experiment. Isolated islets, 20 per incubation vessel, were incubated in 0.25 ml of Hank's medium (5) containing 0.5 mg/ml of bovine serum albumin (Fraction V, Miles Research Laboratories, Kankakee, Illinois), 20 mg/liter of each of the 18 naturally occurring amino acids, and 20 μ Ci of H^3-leucine or H^3-tryptophan (specific activity, 20 Ci/mM, New England Nuclear Corporation, Boston, Massachusetts). Incubations were done in an atmosphere of 95% O_2 - 5% CO_2 at 37°. To terminate the incubation, 1 ml of cold TCA[1], 10% w/v, was added to the incubation mixture. Islets were homogenized and washed three times with cold TCA. The resultant precipitate was extracted with 1 ml of acid-alcohol (6) for 3 hours at 37°. The acid-alcohol was lyophilized and the residue dissolved in 1 ml of 0.05 M ammonium bicarbonate (7) and dialysed in the same solution for 8 hours at 4°. The dialysed extract was acidified with 0.1 ml of glacial acetic acid and submitted to gel-filtration using a Bio-gel, P-10, 100-200 mesh (Bio-Rad Laboratories, Richmond, California) column, dimensions 50 x 1.5 cm. The column was eluted with 3 M acetic acid. Fractions of 1 ml were collected. Absorbance at 275 mμ was determined with a Gilford spectrophotometer. An aliquot of 0.5 ml from each fraction was counted for radioactivity in a toluene-based scintillation fluid using a Tricarb liquid scintillation counter, Model 3380. The columns were calibrated with I^{131}-labeled bovine proinsulin (supplied by Dr. C. Yip, University of Toronto), insulin (Connaught Medical Research Laboratories, Toronto) and I^{125}-labeled glucagon (porcine-bovine glucagon obtained from Sigma Chemicals, St. Louis, Mo.; I^{125} from New England Nuclear Corp., Boston, Mass.).

[1] trichloroacetic acid

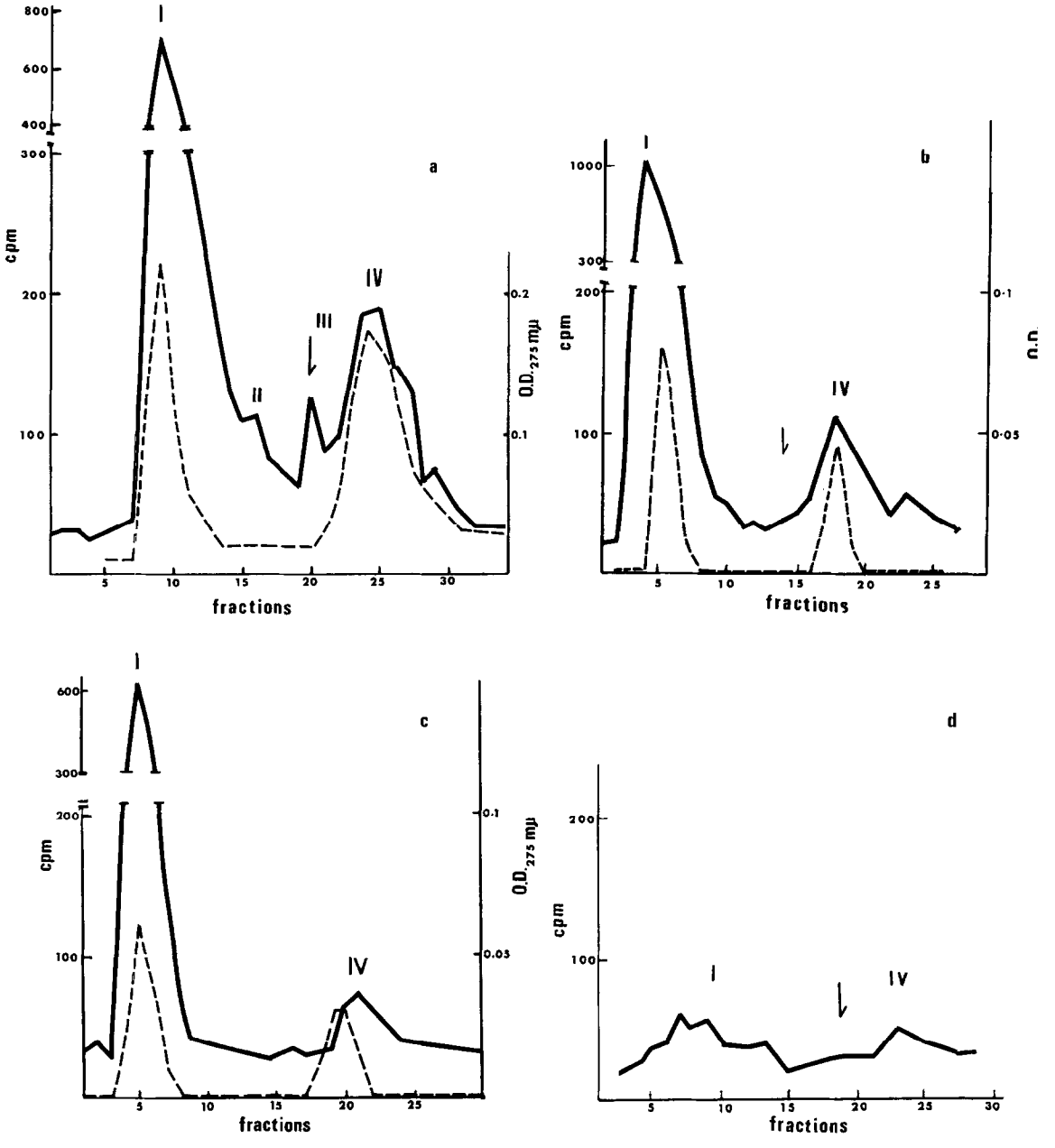

Figure 1. Bio-gel, P-10, gel-filtration of extracts obtained from pigeon islets incubated with (a) H^3-leucine for 4 hours, (b) H^3-tryptophan for 4 hours, (c) H^3-tryptophan for 1.5 hours, and (d) H^3-tryptophan for 4 hours with glucose in incubation medium (3 mg/ml). Radioactivity shown represented 0.5 ml aliquots of 1 ml fractions. The arrow indicates the elution volume of insulin. Discontinuous lines indicate optical density; continuous lines, radioactivity.

Figures Ia and Ib represent the results of experiments in which isolated pigeon islets were incubated with H^3-leucine or H^3-tryptophan for 4 hours. It is evident from Figure Ia that H^3-leucine was incorporated into large molecular weight components, designated I and II, and into lower molecular weight components, III and IV. Component II corresponded in elution volume to that of I^{131}-labeled proinsulin; component III, to that of insulin. Component IV resembled glucagon in size. Islets incubated with H^3-tryptophan showed only two major radioactive components, I and IV, Figure Ib, in contrast to the H^3-leucine labeled experiment. This is expected since tryptophan is known to occur in glucagon and not in insulin, whereas leucine is common to both glucagon and insulin (1).

The incorporation of H^3-tryptophan into components I and IV was found to increase with the time of incubation, Figure Ib and Ic, suggesting that there was an active incorporation of the amino acid into these two components. Cycloheximide completely inhibited incorporation.

To study the effects of glucose on the pigeon islet incorporating system, islets were incubated with H^3-tryptophan in a medium containing 3 mg/ml of glucose for 4 hours. Results, as shown in Figure Id, suggested that glucose reduced the incorporation of H^3-tryptophan into components I and IV.

Fractions containing IV were combined, lyophilized, dissolved in 0.1 ml of 0.01 M HCl, and an aliquot of 50 μl was submitted to polyacrylamide gel electrophoresis, in 15% gels containing 7 M urea, at pH 9.2 (8,9,17). An aliquot, 50 μl, of a glucagon solution, 1 mg/ml in 0.01 M HCl, was applied to a second gel and electroporesed simultaneously. Identical rates of migration of the tracker dye, bromophenol blue, were observed for both gels. The standard gel was stained with amido black, and destained in 7% acetic acid electrophoretically. The radioactive gel was sectioned and the slices solubilized in 30% hydrogen peroxide and counted for radioactivity (10). Similar results were obtained with both H^3-leucine and H^3-tryptophan labeled component IV, showing radio-

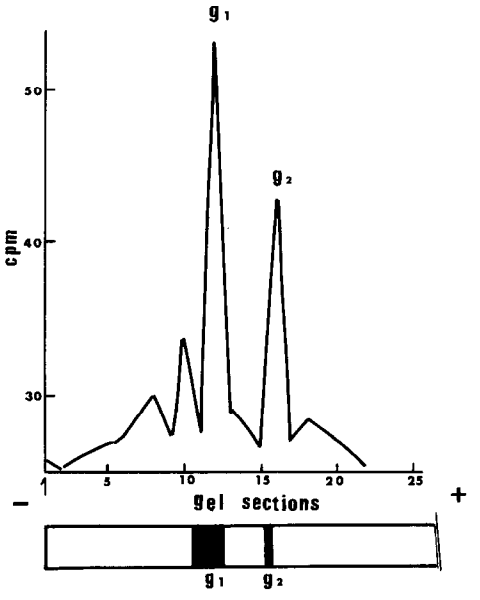

Figure 2. Polyacrylamide gel electrophoresis of H^3-leucine labeled component IV. g1: major component of standard glucagon; g2: minor component. +: anode; -: cathode. Upper panel shows radioactivity profile; lower panel, stained gel containing standard bovine glucagon.

active components corresponding to glucagon and a possible desamido form of glucagon (21,22), Figure 2.

The immunological relationship of component IV to bovine glucagon was studied. Fractions containing IV were combined, lyophilized, and the resultant residue was chemically labeled with radioactive iodide, I^{125}, according to Greenwood and Hunter (11). I^{125}-labeled IV was analyzed on polyacrylamide gel electrophoresis. Results showed an I^{125}-labeled component which coincided with standard glucagon. An aliquot of 0.1 ml of the freshly labeled material was incubated with 0.1 ml of anti-glucagon (goat) serum[1], 1 x 2 diluted, obtained from Miles Research Laboratories, Kankakee, Illinois, for 2 hours at 37° and then for 12 hours at 4°. An identical aliquot of the I^{125}-labeled

[1] anti-serum was tested with I^{125}-glucagon prior to the experiment and was shown to be immunologically active.

TABLE 1

Conditions	Distribution of radioactivity, I^{125}, on Immunochromatogram, F/O ratio
(1) anti-serum	5.61
normal serum	2.34
(2) anti-serum	1.27
normal serum	0.85

(1) Experiment with I^{125}-labeled component IV; (2) With I^{125}-labeled trypsin released product from component I. Total radioactivity per immunochromatogram about 4,000 cpm.

Incubation mixtures were chromatographed on Whatman No. 4 paper (12). Chromatograms were scanned for radioactivity by cutting into 1 centimeter pieces, and counting in a Tricarb liquid scintillation spectrometer.

component was incubated with 0.1 ml of non-immune serum, 1 x 2 diluted. As shown in Table 1, the ratio of the radioactivity located near the front of the chromatogram to that at the origin, F/O ratio, was significantly greater for the anti-serum supplemented incubation than for the control incubation. Thus, component IV was immunologically related to glucagon.

Component I was eluted faster than I^{131}-bovine proinsulin, Figure 1a and 1b, suggesting that it is larger than proinsulin. The possibility of component I being an aggregate of glucagon was considered unlikely, because prolonged storage and repeated gel-filtration in a highly acidic condition, 3 M acetic acid, did not result in dissociation of the component. Combined and lyophilized component I was dissolved in 1 ml of 0.01 M Tris-HCl, pH 7.5. An aliquot containing 50 to 100 µg of protein was incubated with 20 µl of a trypsin (Sigma Chemicals, St. Louis, Mo.) solution, 2 mg/ml in

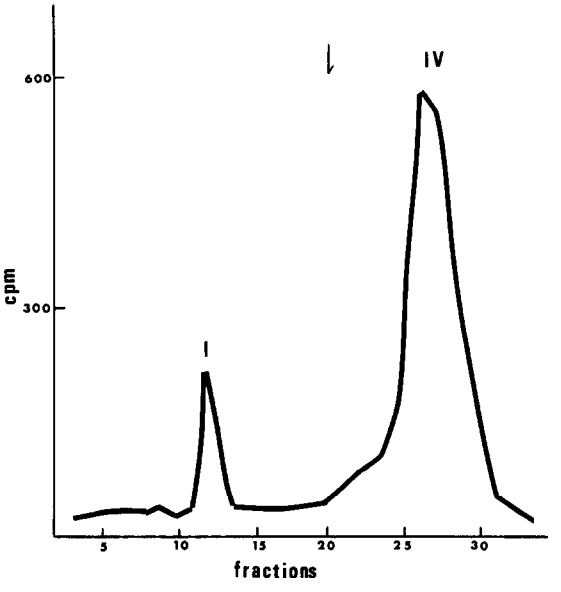

Figure 3. Bio-gel, P-10, gel-filtration of trypsin released product from H^3-labeled component I.

water, for 30 minutes at 37°. Hydrolysis was terminated with the addition of 0.5 ml glacial acetic acid. The hydrolysate was submitted to gel-filtration through a P-10 column, eluted with 3 M acetic acid. Results, as shown in Figure 3, suggested that component I was converted to component(s) similar in size to glucagon. The trypsin released product was recovered by combining the appropriate fractions, lyophilized, labeled with I^{125}, and incubated with anti-glucagon serum or non-immune serum. Results suggested binding of the I^{125}-labeled tryptic product with anti-glucagon serum (Table 1).

DISCUSSION AND CONCLUSIONS

Our data suggested that isolated pigeon islets of Langerhans are active in the incorporation of H^3-leucine and H^3-tryptophan into glucagon and a large molecular weight glucagon-related component. The identification of pigeon glucagon was based on, 1) its correspondence to bovine-porcine glucagon on gel-filtration

and on polyacrylamide gel electrophoresis, and 2) its reactivity with anti-glucagon serum. The large molecular weight component was shown to be related to glucagon in that it was hydrolysed by trypsin to component(s) of the size of glucagon, which, in turn, were reactive against anti-glucagon serum. Glucose, a known regulator of glucagon secretion (13), was found to significantly reduce the incorporation of H^3-tryptophan into both glucagon and the large molecular weight component. These findings suggested the possibility that glucagon, like insulin (14,15,16,17), may be synthesized as a large precursor, which is subsequently converted to glucagon by intracellular proteolyses. Recent reports of large glucagon-like immunoreactivity in pancreatic extracts of various species (7,18,19,20) lend support to this suggestion. Work is in progress to further establish the nature of the large biosynthetic component and its role in the biosynthesis of glucagon.

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