Production of Anti-Glucagon Sera with A C-Terminal Fragment of Pancreatic Glucagon

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Synopsis

The C-terminal region-specific anti-glucagon sera were raised in rabbits using as immunogen, a conjugate of BSA and a C-terminal fragment of pancreatic glucagon. The hapten was prepared by trypsin digestion of the glucagon, which was proved to be a 1:3 mixture of glucagon (18-29) and (19-29). Six rabbits were immunized by subcutaneous injection of an emulsion of the conjugate with complete Freund’s adjuvant and five of the rabbits produced antibodies to the glucagon (GC-1, GC-2, GC-3, GC-5 and GC-6). For comparison, rabbit antisera were also produced against glucagon polymer (GA-10) and syrupy glucagon fibrils (PGA-2). All these antisera as well as the pancreatic glucagon-specific antiserum 30K were characterized with dog gut-extract (gut-GLI) and glucagon-related peptide fragments in the radioimmunoassay systems. The assay systems utilized 125I-monosubstituted pancreatic glucagon as tracer and human mono-component glucagon as standard. All sera of the GC-series crossreacted with the dog gut-extract very weakly and antisera GC-5 and GC-6 exhibited the lowest crossreactivities with the extract, which were shown to be as low as that of 30 k. Characterization of the antiserum GC-5 with purified glucagon-related fragments indicated that the major antigenic determinant located exactly in the C-terminal region of glucagon. The present results clearly showed high efficiency of the use of the glucagon C-terminal fragment as haptenic immunogen in obtaining the C-terminal region-specific, i.e., pancreatic glucagon-specific antisera.

Since Unger et al. (1959) reported the successful production of glucagon antisera and development of a radioimmunoassay specific for pancreatic glucagon, a number of investigators have been engaged in the radioimmunological measurement of glucagon. However, the existence of gut glucagon-like immunoreactivity (gut-GLI) (Sutherland and deDuve, 1964; Makman and Sutherland, 1964) has often rendered difficulty in the specific measurement of pancreatic glucagon, as most of the antisera elicited against pancreatic glucagon exhibited crossreactivity with gut-GLI as well. The antisera obtained by Unger et al. (1968), 30K and G58, and those produced by Heding (1969), K40, K47 and K964, were proved to be highly specific for pancreatic glucagon and have been widely used for its immunological measurement. However, elicitation of the pancreatic glucagon-specific antisera seemed to be by chance.

Assan and Slusher (1972) found that the structure requirements for binding with the pancreatic glucagon-specific antiserum K47 were satisfied with a C-terminal region of the glucagon and not with the N-terminal and/or central portions which were shown

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to be responsible for binding with non-
specific antiserum PVP-8. A similar ob-
servation was reported by Vinik and
Hardcastle (1974).

This paper described the preparation of
pancreatic glucagon-specific antisera using
a C-terminal fragment of glucagon obtained
by tryptic digestion of bovine/porcine glu-
cagon and characterization of the antiser.

Materials and Methods

Preparation of glucagon fragments

Trypsin digestion: Trypsin (bovine/porcine, X2
crystallized, containing 0.5% chymotryptic activity,
Sigma Chem. Co.) was treated with L-1-tosylamide-
2-phenylethylchloromethyl ketone TPCK, Sigma
Chem. Co.) by the method of Wang et al. (1965).
Trypsin digestion of glucagon was carried out by
a slight modification of the method by Bromer et al.
(1957). Crystalline bovine/porcine glucagon (Sigma
Chem. Co.) (100 mg) was dissolved in 0.1 N NaOH
(2 ml) and pH of the solution was adjusted to 9.0
with 1 M acetic acid. To the solution was added
0.1 M triethylamine bicarbonate buffer (pH 9.0) (35
ml), which was used as substrate solution (0.27%
glucagon). The TPCK-treated trypsin (5 mg) in 0.01 N
HCl (2 ml) was combined with the above substrate
solution and the mixture was incubated at 30°C for
4 hr, when the reaction was terminated by acidify-
ing the mixture to pH 2.0 with conc. HCl and the
enzyme was inactivated by heating the solution in
a boiling-water bath at 100°C for 5 min. The solution
was then lyophilized.

Fractionation of tryptic digest: The above lyo-
philized digest was treated with 0.1 M citrate-HCl
buffer (pH 4.0) (10 ml) and the suspension was cen-
trifuged. The supernatant was lyophilized (60 mg),
which was designated as pH 4-soluble fraction. The
sediment was washed five times with the same buf-
fer and dissolved in 0.1 M triethylamine-bicarbonate
buffer (pH 10.0) (10 ml). The insoluble material was
removed by centrifugation and the solution was ad-
justed to pH 4.0 with 0.1 N HCl. The precipitate
was collected by centrifugation, washed with H2O
and dried (22 mg), which was designated as pH 4-in-
soluble fraction.

The pH 4-soluble fraction (60 mg) was dissolved
in 0.1 M pyridine-formic acid buffer (pH 3.1) (2 ml)
and the solution was applied to a column of Dowex
50×2 (200-400 mesh) (1×45 cm), which had previ-
ously been equilibrated with the same buffer.
The column was eluted at a rate of 20 ml/hr with a
linear gradient of pyridine-acetic acid buffer using
its 0.1 M (pH 3.1) (300 ml) and 2 M (pH 5.5) (300 ml)
solutions. Fractions (6 ml) each were collected and
each fraction was examined with fluorescamine re-
agent (Boehlen et al., 1973). The fractions corre-
responding to the major two peaks (peak I: tube Nos.
135-145 and peak II: tube Nos. 175-185) were de-
salted by gel filtration on Bio-Gel P-2 and lyophilized
(peak I: 11 mg and peak II: 25 mg).

The pH 4-insoluble fraction (20 mg) was dissolved
in 0.03 M borate buffer (pH 9.2) (2 ml) and the solu-
tion was applied to a column of DEAE-Sephadex
A-25 (1×35 cm) which had previously been equi-
librated with the same buffer. The column was elu-
ted with a 0-1.0 M linear gradient salt concentra-
tion in the same buffer using the borate buffer (200
ml) and 1.0 M NaCl in the buffer (200 ml). Elution
rate was 10 ml/hr. Fractions (3 ml each) were col-
lected and monitored by UV absorption at 276 nm.
Salt concentration in each fraction was determined
by titrating an aliquot of each fraction with AgNO3.
The fractions corresponding to the major two peaks
(peak III: tube Nos. 22-26 and peak IV: tube Nos.
31-36) were collected, desalted by gel filtration on
Bio-Gel p-2 and lyophilized (peak III: 3.2 mg and
peak IV: 9.5 mg).

The pH 4-insoluble fraction and the four purified
tryptic peptides corresponding to peaks I, II, III and
IV were characterized by dansyl end group analysis
(Gray and Hartley, 1963a and b) and by amino acid
analysis of their acid hydrolysates.

Preparation of immunogens

The pH 4-insoluble fraction-BSA conjugate was
used as immunogen. The pH 4-insoluble fraction (30
mg) and BSA (crystalline, Sigma Chem. Co.) (100 mg)
were dissolved in 0.1 N NaOH (1 ml) and 0.1 M borate
buffer (pH 9.0) (9 ml) was added. To the solution,
0.1 M glutaraldehyde (Wako Pure Chem. Co.) (3 ml)
was added dropwise by constant stirring, while the
pH of the solution was kept at approximately 9.0
with 0.1 N NaOH. The mixture was stirred at room
temperature for 3 hr and then dialyzed against saline
at 4°C for 24 hr. The yield of the conjugation was
estimated by gel filtration of an aliquot of the re-
action mixture on Sephadex G75 using 1% SDS
containing 0.1 M NaCl as eluent. The yield of the
conjugate was about 80% as calculated from the UV
absorption of the eluates and the molar ratio of the
peptide to BSA in the conjugate was assumed to
be 8:1.

For comparative study, glucagon polymer (Hed-
ing, 1969) and syrupy glucagon fibrils (Heding, 1972)
were also prepared as immunogens. To a solution of
crystalline bovine/porcine glucagon (75 mg) in 0.1 N
NaOH (1 ml), 0.1 M borate buffer (pH 9.5) (19 ml)
was added, and then 0.4 M glutaraldehyde (5 ml) was
added dropwise by stirring over a period of 1.5
hr, while the pH of the solution was kept at ap-
proximately 9.0 with 0.1 N NaOH. The reaction
was terminated by adding glacial acetic acid and the
solution was submitted to dialysis against 0.9% NaCl solution and lyophilized to give glucagon polymer (60 mg). For preparation of syrupy glucagon fibrils, crystalline bovine/porcine glucagon (200 mg) was dissolved in 0.01 N HCl (2 ml) and the solution was kept at 4°C for 24 hr and centrifuged. The precipitate was washed twice with 0.01 N HCl and twice with saline and dried (60 mg).

**Immunization**

Six rabbits were immunized by subcutaneously injecting an emulsion with complete Freund’s adjuvant of the pH 4-insoluble fraction-BSA conjugate (0.5-1.0 mg of the insoluble fraction equivalent for each animal) at 3-week intervals for 6 weeks and at a-month intervals for the following 5-10 months. Blood was obtained 10 days after the final injection. Similarly, the glucagon polymer and glucagon fibrils were injected, after being emulsified with complete Freund’s adjuvant, in foot pads of rabbits by following the same schedule as above. Fifteen rabbits were immunized with the polymer and ten with the fibrils.

**Preparation of ¹²⁵I-labelled glucagon**

Glucagon was iodinated with ¹²⁵I Na and chloramine T in the presence of dimethylsulfoxide (DMSO) according to the procedure of Shima et al. (1975). Purification of the iodinated product was carried out by ion-exchange chromatography on QAE-Sephadex A-25 column according to a modification of the method of Jorgensen and Larsen (1972).

Crystalline bovine/porcine glucagon (3 µg) in 0.01 N HCl (10 µl) was mixed with 0.4 M phosphate buffer (PB) (pH 7.2) (20 µl) and DMSO (5 µl). To the mixture were added successively ¹²⁵I Na (New England Nuclear) (1 mCi) and chloramine T (70 µg) in 0.04 M PB (pH 7.4) (20 µl). The mixture was stirred for 15-20 sec, when the reaction was terminated by adding sodium metabisulfite (120 µg) in 0.04 M PB (pH 7.4) (50 µl). The reaction mixture was combined with 2 M Tris solution (5 µl) and Tris-HCl buffer comprising 0.08 M Tris, 0.02 N HCl and 0.08 M NaCl (pH 8.6) (1 ml). An aliquot (1 ml) of the mixture was applied to a column (1.1 x 28 cm) of QAE-Sephadex A-25 which had previously been equilibrated with the Tris-HCl buffer. The column was then eluted with the buffer at a flow rate of 3 ml/hr and fractions (2 ml each) were collected. The largest peak (tube Nos. 39-49) eluting in the middle of two small peaks was proved to be monoradioiodo-glucagon which corresponded to the "Preparation A" described by Jorgensen and Larsen (1972). Specific activity of fraction No. 49 was 565 pCi/µg and fractions Nos. 49 and 50 were used in the present study.

**Radioimmunoassay procedure**

The standard glucagon used was human mono-component glucagon (Novo Research Institute) and the standard diluent was 0.2 M glycine buffer (pH 8.8) containing 0.25% human albumin and 1% normal sheep serum. In each incubation tube, were mixed properly-diluted anti-glucagon serum (200 µl), standard glucagon or unknown sample (200 µl), labelled glucagon (200 µl, ca. 10,000 cpm) and standard diluent (200 µl). The mixture was incubated for 48 hr at 4°C, when dextran-coated charcoal (0.5 ml) was added. After 1 hr at 4°C, the suspension was centrifuged at 3,000 rpm for 15 min at 4°C and both the supernatant and precipitate were counted for 2 min.

**Preparation of dog gut-extract**

Dog gut-extract was prepared according to the methods of Kenny (1955) and Valverde et al. (1970). Dogs were subjected to laparotomy under Nembutal anesthesia and the intestines were removed immediately after their death from Nembutal overdosage. Mucosal scraps were obtained from the jejunum and they were extracted by the acid-alcohol method (Kenny, 1955). The product from the last-step dialysis was subjected to gel filtration on Bio-Gel P-10 using 0.05 M NH₄HCO₃ as eluent (Valverde et al., 1970). The eluates, so-called "peak I", which were located before ¹²¹I-insulin marker and exhibited immunoreactivity to antiserum GA-10 (described in RESULTS AND DISCUSSION section) were pooled and lyophilized.
Results and Discussion

Tryptic peptides of pancreatic glucagon

Table 1 shows amino acid ratios in an acid hydrolysate of the pH 4-insoluble fraction from the tryptic digest of glucagon. The dansyl end group analysis of the fraction indicated the presence of two major peptides that were terminated with Ala and Arg residues at the N-termini, respectively. Bromer et al. (1957) previously reported that trypsin digestion of glucagon yielded four major peptide fragments corresponding to the sequences 1–12, 13–17, 18–29 and 19–29 which were called N, M, C₁ and C₂ fragments, respectively. Since Arg residue is in position 18 and Ala in position 19, the present results of the amino acid analysis and dansyl end group analysis indicate that the pH 4-insoluble fraction consists mainly of glucagon 18–29 and 19–29 fragments corresponding to C₁ and C₂ in a ratio of approximately 1 to 3. This material was satisfactorily used as haptenic antigen for production of anti-glucagon antisera.

The pH 4-soluble and -insoluble fractions responding to the sequences 1–12, 13–17, 18–29 and 19–29 which were called N, M, C₁ and C₂ fragments, respectively. Since Arg residue is in position 18 and Ala in position 19, the present results of the amino acid analysis and dansyl end group analysis indicate that the pH 4-insoluble fraction consists mainly of glucagon 18–29 and 19–29 fragments corresponding to C₁ and C₂ in a ratio of approximately 1 to 3. This material was satisfactorily used as haptenic antigen for production of anti-glucagon antisera.

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from the tryptic digest of glucagon were further purified by ion exchange chromatography on Dowex 50 column of the pH 4-soluble fraction is shown in Fig. 1a. The chromatographic fractionation of the pH 4-insoluble fraction on DEAE-Sephadex column is shown in Fig. 1b. The two major peaks, I and II, in Fig. 1a and two major peaks, III and IV, in Fig. 1b were examined by dansyl end group analysis and amino acid analysis of their acid hydrolysates (Table 2). The analytical data proved that the peaks I, II, III and IV are glucagon 1-12, 13-17, 18-29 and 19-29 fragments, respectively, and that these purified tryptic peptides are able to be used safely as substrates for characterization of the glucagon antiserum.

Production of antisera

Production of antisera was attempted in rabbits using as immunogens, the pH 4-insoluble fraction—BSA conjugate, glucagon polymer and syrupy glucagon fibrils. After six injections, production of anti-glucagon sera was detected in five rabbits given the conjugate (GC-1, 2, 3, 5 and 6), one given the polymer (PGA-2) and one given the fibrils (GA-10). Table 3 indicates the affinity constants (equilibrium

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Peak I</th>
<th>Peak II</th>
<th>Peak III</th>
<th>Peak IV</th>
</tr>
</thead>
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<tr>
<td>Lys</td>
<td>1.05</td>
<td>0.94</td>
<td>0</td>
<td>0</td>
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<tr>
<td>His</td>
<td>0.99</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arg</td>
<td>0</td>
<td>0</td>
<td>1.17</td>
<td>1</td>
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<tr>
<td>Asp</td>
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<td>1</td>
<td>2.12</td>
<td>2</td>
</tr>
<tr>
<td>Thr</td>
<td>1.90</td>
<td>2</td>
<td>0.91</td>
<td>1.06</td>
</tr>
<tr>
<td>Ser</td>
<td>3.29</td>
<td>3</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td>Glu</td>
<td>1.01</td>
<td>1</td>
<td>2.09</td>
<td>2</td>
</tr>
<tr>
<td>Gly</td>
<td>1.00*</td>
<td>1</td>
<td>0.09</td>
<td>0</td>
</tr>
<tr>
<td>Ala</td>
<td>0</td>
<td>0</td>
<td>1.00*</td>
<td>1</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
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<td>1</td>
</tr>
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<td>0.90</td>
<td>0.96</td>
</tr>
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<td>Leu</td>
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<td>1</td>
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<td>0.82</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phe</td>
<td>0.98</td>
<td>1</td>
<td>1.03</td>
<td>1.15</td>
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* Assumed as 1.00

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Affinity constant high ( \times 10^{14} \text{ M}^{-1} )</th>
<th>Affinity constant low ( \times 10^{9} \text{ M}^{-1} )</th>
<th>Number of binding site low ( \times 10^{-14} \text{ mol/mL} )</th>
<th>Number of binding site high ( \times 10^{-14} \text{ mol/mL} )</th>
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<tbody>
<tr>
<td>GC-1</td>
<td>6.3</td>
<td>8.3</td>
<td>2.9</td>
<td>9.3</td>
</tr>
<tr>
<td>GC-2</td>
<td>2.9</td>
<td>5.6</td>
<td>7.3</td>
<td>24.4</td>
</tr>
<tr>
<td>GC-3</td>
<td>1.9</td>
<td>8.3</td>
<td>7.7</td>
<td>10.4</td>
</tr>
<tr>
<td>GC-5</td>
<td>14.3</td>
<td>—</td>
<td>1.3</td>
<td>—</td>
</tr>
<tr>
<td>GC-6</td>
<td>5.3</td>
<td>13.7</td>
<td>2.3</td>
<td>4.6</td>
</tr>
<tr>
<td>GA-10</td>
<td>1.1</td>
<td>—</td>
<td>8.6</td>
<td>—</td>
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<tr>
<td>PGA-2</td>
<td>7.7</td>
<td>14.7</td>
<td>1.1</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Fig. 2. Crossreactivities of dog gut-extract (○) in radioimmunoassay systems using antisera (a) GA-10 (1:10,000), (b) PGA-2 (1:11,000), (c) GC-1 (1:150,000), (d) GC-2 (1:50,000) (e) GC-3 (1:60,000), (f) GC-5 (1:160,000), (g) GC-6 (1:50,000) and (h) 30 K (1:50,000). Labelled antigen: $^{125}$I-pancreatic glucagon and standard: human mono-component glucagon (●). The dilution scale for dog gut-extract is fixed in such way that the dose-response curve of the extract is superimposed on that of the standard glucagon in the GA-10 assay system.
constants) and number of the binding sites to pancreatic glucagon of these seven antisera. All of the antisera exhibited affinities to the glucagon sufficiently high enough to develop sensitive radioimmunoassay for glucagon.

Characterization of antisera

Crossreactivities of antisera with dog gut-extract: Using pancreatic mono-component glucagon as standard and $^{125}$I-glucagon (monoradioiodo form) as tracer, radioimmunoassays were developed with the seven antisera obtained and crossreactivities of the antisera with dog gut-extract (gut-GLI) were examined in the systems. Figs. 2a—2g show the results of the assays. Crossreactivity with the dog gut-extract was also tested, for comparison, in the system using pancreatic glucagon-specific antiserum 30K (Fig. 2h). Antiserum GA-10 exhibited the highest crossreactivity with the extract and its dilution curve was completely superimposable on the dose-response curve of the standard pancreatic glucagon as shown in Fig. 2a. As compared with GA-10, the other six antisera as well as 30K were less reactive with the gut-extract. Relative crossreactivities of the antisera to the extract are listed in Table 4. The crossreactivity of the present extract in the 30K system was comparable to that of the dog gut-extract measured by Eisentraut et al. (1968) in their 30K assay system. The antisera of GC-series showed relative crossreactivities ranging from 0.64 to 1.5% of that of GA-10, which seemed to be satisfactorily low in order to discriminate gut-GLI from pancreatic glucagon. From these results, it may be concluded that protein conjugate of the C-terminal fragment of glucagon is an efficient immunogen for production of antisera specific to pancreatic glucagon.

Crossreactivities of antisera with glucagon-related peptides: Among the five antisera of GC-series, GC-5 showed a linear Scatchard plot and the highest sensitivity in the radioimmunoassay and it could recognize gut-GLI to the least extent. Thus, crossreactivities of glucagon-related peptides were examined in the system with GC-5 in comparison with those in the systems using GA-10, PGA-2 and 30K. The glucagon-related peptides used were pancreatic glucagon (PG) and its fragments, PG(1–12), PG(13–17), PG(18–29) and PG(19–29). The inhibition curves of these peptides in each system are shown in Figs. 3a—3d. And relative crossreactivities of these peptide fragments in the four systems are summarized in Table 5, which were calculated from the amount of a fragment required for 50% inhibition of $^{125}$I-glucagon binding. The non-specific antiserum GA-10 crossreacted with none of the peptide fragments, while relatively high crossreactivities of PGA-2 were observed with the C-terminal peptides PG(18–29) and PG(19–29) rather than with the N-terminal fragment PG(1–12). Crossreactivity of specific-antiserum 30K with PG(18–29) was 48% of that with pancreatic glucagon and the reactivity with PG(19–29) was decreased to approximately half of with that the (18–29) fragment.

Table 4. Crossreactivity of dog gut-extract with various anti-glucagon antisera

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Crossreactivity of dog gut-extract* (ng/ml pancreatic glucagon equivalent)</th>
<th>Relative crossreactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA-10</td>
<td>44.00</td>
<td>Assumed as 100</td>
</tr>
<tr>
<td>PGA-2</td>
<td>3.40</td>
<td>7.70</td>
</tr>
<tr>
<td>30 K</td>
<td>0.29</td>
<td>0.64</td>
</tr>
<tr>
<td>GC-1</td>
<td>0.36</td>
<td>0.82</td>
</tr>
<tr>
<td>GC-2</td>
<td>0.48</td>
<td>1.09</td>
</tr>
<tr>
<td>GC-3</td>
<td>0.66</td>
<td>1.50</td>
</tr>
<tr>
<td>GC-5</td>
<td>0.29</td>
<td>0.64</td>
</tr>
<tr>
<td>GC-6</td>
<td>0.26</td>
<td>0.59</td>
</tr>
</tbody>
</table>

* A solution containing dog gut-extract equivalent to 44.00 ng standard pancreatic glucagon per ml as determined in the GA-10 system was assayed in each system, and the crossreactivity of the extract in each system was expressed by equivalent weight of the standard glucagon.
indicating an important role of the Arg residue in position 18 in interaction of the antigen with antiserum 30K. This antiserum reacted negligibly with PG(1-12) and PG(13-17). These results with 30K are in well agreement with the data reported by Faloona and Unger (1974). Antiserum GC-5 crossreacted with PG(18-29) and PG(19-29) are in agreement with the data reported by Faloona and Unger (1974). Antiserum GC-5 crossreacted with PG(18-29) and PG(19-29).

Fig. 3. Inhibition curves of glucagon-related peptides in radioimmunoassay systems using antisera (a) GC-5, (b) GA-10, (c) PGA-2 and (d) 30K. Labelled antigen: $^{125}$I-pancreatic glucagon and peptides: pancreatic glucagon (PG) (●), PG (18-29) (○), PG (19-29) (▲), PG (13-17) (▲) and PG (1-12) (■).
Table 5. Relative crossreactivities of pancreatic glucagon-related peptides in glucagon radioimmunoassay systems using four different antisera

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Pancreatic glucagon PG (1-29)</th>
<th>PG (18-29)</th>
<th>PG (19-29)</th>
<th>PG (13-17)</th>
<th>PG (1-12)</th>
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<tbody>
<tr>
<td>GC-5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30 K</td>
<td>1</td>
<td>0.48</td>
<td>0.22</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PGA-2</td>
<td>1</td>
<td>0.23</td>
<td>0.15</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GA-10</td>
<td>1</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Relative crossreactivity of a peptide was calculated by dividing the dose of the standard glucagon required for 50% inhibition by that of the corresponding peptide.

PG(19–29) to the same extent as with pancreatic glucagon. Very little but apparent crossreactivities of GC-5 with PG (1–12) and PG (13–17) may be due to trace contamination of glucagon or/and the N-terminal fragment(s) in the pH 4-insoluble fraction that had been used as haptenic antigen. It has become, thus, clear that the C-terminal region-specific antiserum GC-5 obtained in this study can almost specifically react with pancreatic glucagon and the present results have provided an additional support to the proposal that antibodies specific for pancreatic glucagon interact with the hydrophobic C-terminal region in the molecule (Assan and Slusher, 1972).

Utilizing the pancreatic glucagon-specific antiserum GC-5, the study on practical radio-immunological measurement of glucagon in biological fluids is now under way.

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References