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Isolation and amino acid sequences of squirrel monkey (*Saimiri sciurea*) insulin and glucagon

(New World primate/owl monkey/marmoset monkey)

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ABSTRACT **It was reported two decades ago that insulin was not detectable in the glucose-stimulated state in *Saimiri sciurea*, the New World squirrel monkey, by a radioimmunoassay system developed with guinea pig anti-pork insulin antibody and labeled pork insulin. With the same system, reasonable levels were observed in rhesus monkeys and chimpanzees. This suggested that New World monkeys, like the New World hystricomorph rodents such as the guinea pig and the coypu, might have insulins whose sequences differ markedly from those of Old World mammals. In this report we describe the purification and amino acid sequences of squirrel monkey insulin and glucagon. We demonstrate that the substitutions at B29, B27, A2, A4, and A17 of squirrel monkey insulin are identical with those previously found in another New World primate, the owl monkey (*Aotus trivirgatus*). The immunologic cross-reactivity of this insulin in our immunoassay system is only a few percent of that of human insulin. Squirrel monkey glucagon is identical with the usual glucagon found in Old World mammals, which predicts that the glucagons of other New World monkeys would not differ from the usual Old World mammalian glucagon. It appears that the peptides of the New World monkeys have diverged less from those of the Old World mammals than have those of the New World hystricomorph rodents. The striking improvements in peptide purification and sequencing have the potential for adding new information concerning the evolutionary divergence of species.**

Mann and Crofford (1) reported two decades ago that insulin was not detectable in the glucose-stimulated state in *Saimiri sciurea*, the New World squirrel monkey, by a radioimmunoassay (RIA) system developed with guinea pig anti-pork insulin antibody and labeled pork insulin. The same system detected reasonable levels of insulin in rhesus monkeys and chimpanzees (1), which suggested that New World monkeys, like the New World hystricomorph rodents such as the guinea pig (2) and the coypu (3), might have insulins whose sequences differ markedly from those of Old World mammals. It is of interest that pancreatic glucagon, which is identical among all Old World mammals studied to date (4–8), also differs in the New World hystricomorphs (9–11). In this report we describe the purification and amino acid sequences of squirrel monkey insulin and glucagon and examine the relationship between these peptides and the corresponding peptides of Old World and New World mammals.

METHODS

Two pancreata were obtained from the Yerkes Regional Primate Research Center at Emory University (Atlanta, GA). The tissues were collected shortly after death and were maintained frozen until extracted. Both animals had died from natural causes, one from diabetes and the other from chronic nephritis.

Steps in the purification of insulin and glucagon were monitored using in-house RIA procedures generally used in the laboratory. The insulin assay does not distinguish among beef, pork, and human insulins (12). The guinea pig antiserum generally used in the laboratory (13) does not detect guinea pig glucagon, but an antiserum that does detect guinea pig glucagon was also available if needed (10). Insulin and glucagon contents in all solutions were monitored by RIA using beef insulin and pork glucagon standards.

Each pancreas (weights of 0.5 and 1 g) was processed separately by extraction with 5 volumes of acid alcohol (0.2 M HCl in 75% ethanol) with a Teflon grinder. The extracts were centrifuged, the precipitate was discarded, and the supernatant was precipitated with 8 volumes of acetone following overnight storage at −30°C. After centrifugation the precipitate was dissolved in 10 ml of 1 M acetic acid. The peptides were then purified by HPLC using an MB C_{18} Radial-Pak column (Waters) and elution with a linear gradient of 20–60% acetonitrile in 0.13% heptafluorobutyric acid. Following the single HPLC step the insulin and glucagon peak fractions were pure.

A portion of the purified insulin (5 nmol) was reduced with 2-mercaptoethanol and alkylated with 4-vinylpyridine. The A and B chains were separated by HPLC and sequenced using an automated gas-phase sequencer with on-line PTH amino acid analyzer (Applied Biosystems).

The insulin A chain was fully sequenced and the N-terminal portions of the insulin B chain and of glucagon were also determined. The C-terminal sequences of the insulin B chain and glucagon were determined following the generation of overlapping C-terminal peptide fragments from the parent peptides by proteolytic cleavage. Portions of the insulin B chain (1 nmol) and glucagon (100 pmol) were digested with endoproteinases Glu-C (1 μg) and Arg-C (0.1 unit), respectively, in 100 μl of 0.1 M Tris·HCl (pH 8) for 16 hr at 20°C. The C-terminal fragments were then purified by HPLC on a Nova-Pak C_{18} column (Waters) prior to amino acid sequencing.

RESULTS

In the RIA using the usual antiserum employed in the laboratory (13), dilutions of extracts containing squirrel monkey glucagon gave a curve that was superposable on the curve of pork glucagon standards. However, squirrel monkey insulin was much less cross-reactive than beef insulin in the RIA employed (Fig. 1). Therefore an extract of the monkey pancreas was used as an arbitrary standard throughout the insulin purification. Following the purification the actual

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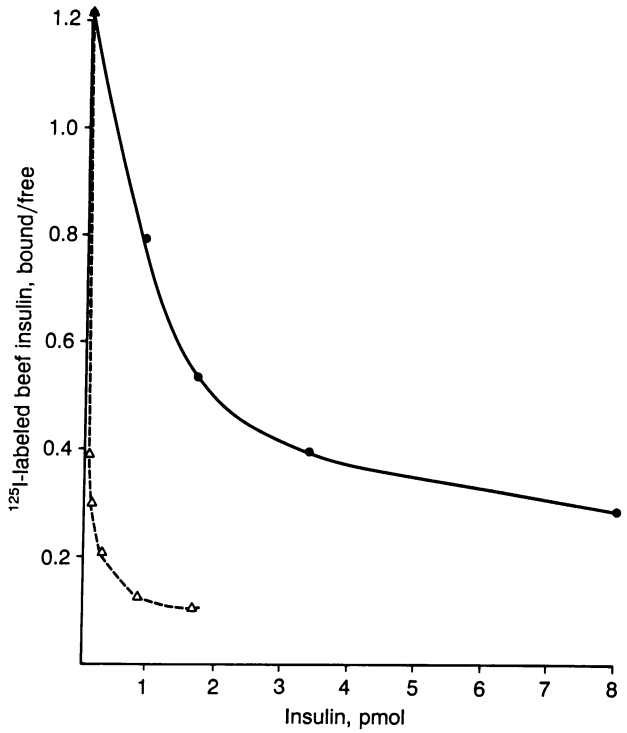

FIG. 1. Standard curves for beef insulin (△) and purified squirrel monkey insulin (●).

concentrations of insulin in the various steps were determined. The insulin and glucagon contents of the 1-g pancreas were 2.7 nmol and 0.8 nmol, respectively, and those of the 0.5-g pancreas were 6.6 nmol and 1.7 nmol. Shown in Fig. 2 is the single-step purification of one of the pancreatic extracts. The insulin and glucagon were pure after the single HPLC step. After sequencing it was observed that squirrel monkey glucagon was identical with the usual mammalian glucagon (4–7). The amino acid sequence of the squirrel monkey insulin is shown in Fig. 3. It differs from human insulin at three sites in the A chain and two sites in the B chain.

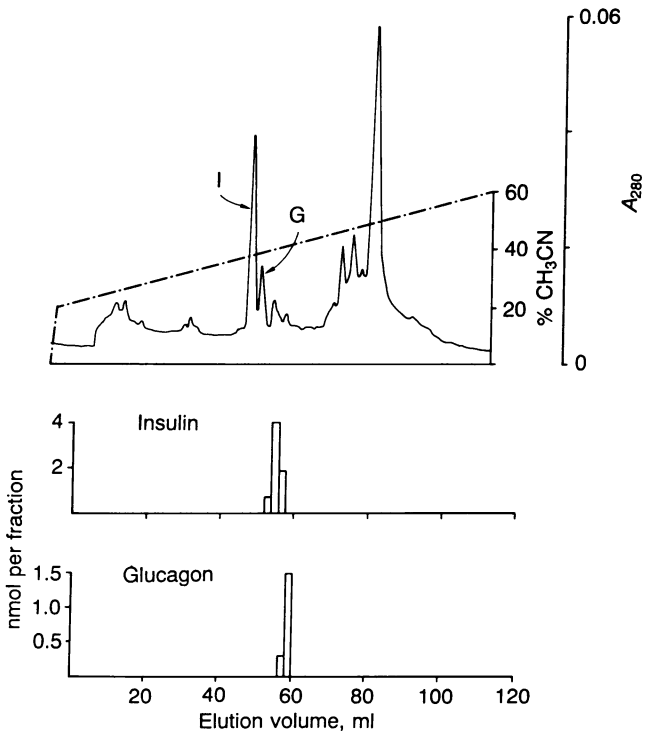

FIG. 2. HPLC (C_{18} Radial-Pak) purification of insulin and glucagon in an acid extract of a single squirrel monkey pancreas. A single step was sufficient to resolve the insulin (I) and glucagon (G) peaks. Recovery of insulin was 79% and recovery of glucagon was 106% as determined by RIA.

DISCUSSION

It is of considerable interest that the amino acid sequence of squirrel monkey insulin is identical with the sequence described by Seino *et al.* (14) of another New World primate, the owl or night monkey (*Aotus trivirgatus*). Although these monkey insulins differ from the human insulin sequence at only five positions, three in the A chain and two in the B chain, the monkey insulins cross-react weakly with anti-porcine insulin antibodies. In the Mann and Crofford study (1), circulating insulin was not detected in the glucose-stimulated squirrel monkey, suggesting that the cross-reactivity in their RIA system was less than 10% that of the insulins of the Old World monkeys. Seino *et al.* (14) reported that the cross-reactivity of owl monkey insulin in their assay system was only 1% that of human insulin, not significantly different from the cross-reactivity of squirrel monkey insulin in our RIA system. The metabolic potency of owl monkey insulin is only about 20% that of the human hormone (14). Since owl monkey and squirrel monkey insulins have identical sequences, it is likely that they have identical biologic properties.

An earlier study (15) of steroid hormones in New World and Old World primates demonstrated interesting differences in the concentrations of total and free plasma cortisols. In general the cortisol concentrations in New World monkeys are higher than in Old World monkeys. The total plasma cortisol in Old World monkeys ranged from 21 to 29 μg/dl and free cortisol ranged from 0.8 to 1.2 μg/dl. The corresponding levels in the owl monkey were 30 μg/dl and 4.3 μg/dl. However, the levels in the squirrel monkey were strikingly higher, 199 μg/dl and 31 μg/dl, respectively. These differences presumably occurred after the bifurcation of Old and New World primates and before the divergence of the New World primates from each other (15). In spite of the divergence of glucocorticoid receptor characteristics of the squirrel and owl monkeys, it appears that there was no further mutation of their insulins. Since the circulating cortisol levels of the New World marmoset monkey resemble those of the squirrel monkey (15), it can be predicted that its insulin will not differ from that of the owl and squirrel monkeys.

Squirrel monkey glucagon does not differ from the usual glucagon found in Old World mammals (4–8), although guinea pig and chinchilla glucagons (9–11) differ in five and three sites, respectively, from the usual Old World mammalian

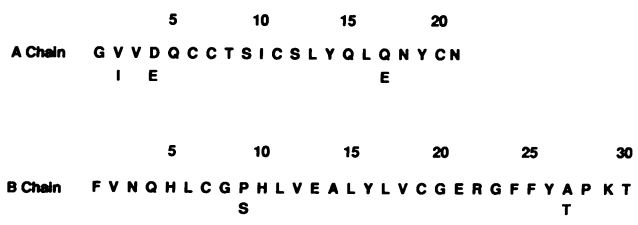

FIG. 3. Amino acid sequence of squirrel monkey insulin. One-letter amino acid notation is used. The sites that differ in the corresponding human peptide are shown below the squirrel monkey sequence. The amino acid sequence of glucagon is not shown but it is identical with the highly conserved sequence of the Old World mammalian peptide.

glucagon. It is likely that glucagon is conserved among all the New World primates.

It appears that the peptides of the New World primates have diverged less from those of the Old World mammals than have those of the New World hystricomorph rodents. Much has been learned about evolutionary biology through a variety of morphologic and biochemical studies. The striking improvements in peptide purification and sequencing have the potential for adding important new information concerning the divergence of species.

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