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A high molecular weight form of pancreatic polypeptide was present in USP insulin and is absent in a more recent insulin preparation

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The concentrations and molecular forms of pancreatic polypeptide (PP) and glucagon were determined in USP XX and single-peak insulin preparations. In USP insulin the concentration of PP was 7 ng mg^{-1} insulin; on gel chromatography the PP immunoreactivity fractionated into two peaks of about equal size. One of the PP peaks was in the region of 4200 dalton PP, and the other PP peak eluted earlier in the region of a 7000–10 000 dalton peptide. The concentration of PP in single-peak insulin was 0·8 ng mg^{-1} insulin; the immunoreactivity eluted in a single peak in the region of [^{125}I]PP. The concentration of glucagon in each preparation was 55 ng mg^{-1} insulin. On gel chromatography the immunoreactive glucagon in each insulin preparation eluted in the region of [^{125}I]glucagon.

Precursor forms of pancreatic polypeptide (PP) have recently been described (Leiter et al 1984; Takeuchi & Yamada 1985). Before the advent of chromatographic purification methods, proinsulin and other high molecular weight forms of insulin contributed as much as 6% to the total immunochemical reactivity of USP insulin (Tantillo et al 1974). These earlier insulin preparations were also contaminated with non-insulin peptide hormones, including pancreatic polypeptide, glucagon and somatostatin; antibodies to these peptides, especially PP, were noted in the most insulin-treated patients (Fitz-Patrick & Patel 1981). We have previously reported that patients treated with USP insulin had a greater incidence of, and higher titre of, antibodies to PP than those who had received the newer more purified single-peak insulin preparations (Meryn et al 1986). In this report we examine the concentrations and molecular forms of PP and glucagon in USP and single-peak insulin preparations.

Materials and methods

Representative lots of USP insulin from 1963 (836550) and single-peak insulin from 1972 (615-D63-5) were obtained in crystalline form as gifts from Dr Ronald Chance, Lilly Research Laboratories. These preparations were reconstituted in acid water, pH 2·3, before use. The concentration of insulin and glucagon in each of these insulin preparations was determined by radioimmunoassay (RIA) according to Yalow & Berson (1970, 1973). The concentration of PP was determined as previously described (Meryn & Bauman 1984) using a rabbit anti-PP antiserum (615 R110 146-10) and bovine PP standard obtained from Lilly Research Laboratories.

The amounts of the contaminants in insulin were determined and an amount of insulin containing approximately 1 ng of PP or glucagon was fractionated at a flow rate of 20 mL h^{-1} at 4 °C on a fine Sephadex G 50 column with a length of 50 cm. PP was fractionated using an alkaline eluent (0·02 M veronal, pH = 8·6) in the absence or presence of 4 M urea. Glucagon was chromatographed using an acid eluent (0·1 M HCl). Marker molecules were used to indicate the void volume and iodide peak. The immunoreactive PP content of successive 1 mL eluate fractions was determined by RIA. Before assay, the glucagon column fractions were evaporated to dryness in a vortex evaporator at 37 °C and −30 mmHg. The elution pattern of each hormone was compared with that of [^{125}I]PP or [^{125}I]glucagon.

Results

The concentration of PP in USP insulin (lot 836550) was 7 ng mg^{-1} insulin, and in single peak insulin (lot 615-D63-5) 0·8 ng mg^{-1} insulin. The concentration of glucagon in each lot was 55 ng mg^{-1} insulin. Sephadex gel filtration resolved the PP immunoreactivity in the USP insulin into 2 peaks of approximately equal size (Fig. 1A). One of the PP peaks was in the region of elution of [^{125}I]PP; the other PP peak eluted earlier corresponding to a higher molecular weight peptide. The PP in single-peak insulin eluted in a single peak in the region of [^{125}I]PP (Fig. 1C). On Sephadex fractionation, immunoreactive glucagon in both insulin preparations eluted in the region of [^{125}I]glucagon (Fig. 1B, 1D).

To exclude the possibility that the larger molecular form was due to non-covalent bonding of PP to itself or other proteins, the preparation was eluted with a buffer containing a high concentration of urea (4 M). Under these conditions, the PP immunoreactivity again eluted in 2 peaks of similar size and elution region (Fig. 2).

Discussion

This is the first report demonstrating that a precursor form of PP was present in USP insulin preparations. Initially noted as a contaminant of insulin preparations,

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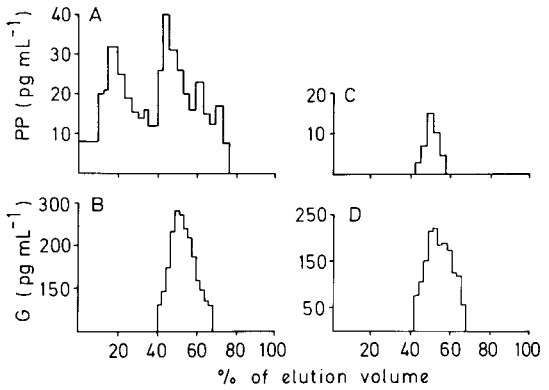

FIG. 1. Gel filtration pattern (Sephadex G50) of pancreatic polypeptide (PP) immunoreactivity (upper panels) and glucagon (G) immunoreactivity (lower panels) in USP insulin (left panels) and single peak insulin (right panels). The abscissa is the percent of elution volume between γ-globulin and $^{125}I^-$.

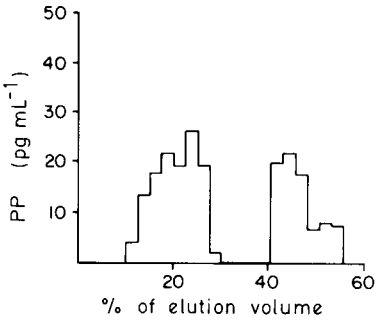

FIG. 2. Gel filtration pattern (Sephadex G50) of pancreatic polypeptide (PP) immunoreactivity in USP insulin eluted in a buffer containing 4 M urea. The abscissa is the percent of elution volume between γ-globulin and $^{125}I^-$.

PP is a 36 amino acid hormone that is a major pancreatic secretory peptide. PP, like most other pancreatic hormones, is known to be synthesized as part of a large precursor protein, pre-propancreatic polypeptide of molecular weight 10 432 daltons (Leiter et al 1984). By gel filtration analysis, the size of the precursor peptide isolated from canine pancreatic islets has been estimated to be between 7200 and 9000 daltons.

In our studies of USP insulin, the high molecular weight peak on gel chromatography was in the 7000 to 10 000 dalton region. Since the propancreatic polypeptide molecule has a C-terminal extension that would not be well-recognized by a C-terminal antiserum (O'Hare et al 1985), the PP antiserum we used is most likely to be N-terminal directed. Extracts of dog pancreas contain only a small fraction of total PP content in a precursor form (Schwartz et al 1980). As such it was quite unexpected that about one-half of the PP immunoreactivity in USP insulin chromatographed as a high molecular weight species. The USP and single-peak insulin preparations we studied were representative of other lots of each of these insulins. The question arises as to whether the crystallization purification procedure employed for USP insulin possibly enhanced the yield of the precursor form in relation to the 4200 dalton PP. However, we did not investigate other lots of USP insulin and, as such, might have been afforded the opportunity to investigate a lot that had an enriched content of the precursor form. The single-peak insulin preparation had an order of magnitude less PP in it than in the USP preparation. Furthermore, there was no high molecular weight species of PP evident on gel chromatography in this preparation.

In a study of 21 patients treated exclusively with USP insulin versus 21 patients treated with single-peak insulin, 12 of the USP-treated patients had antibodies to PP, 1 had a PP antibody titre of less than 1:50, 2 of 1:50, 5 of 1:100 and 4 of 1:1000 dilution of serum (Meryn et al 1986). This is in contrast to the patients treated with single-peak insulin of whom 5 had PP antibodies, with only 1 patient with an antibody titre as high as 1:50 dilution of serum. The striking difference between these groups may have been on the basis of exposing the USP-treated patients to a more antigenic precursor form of PP, as well as to an increased amount of total PP in the insulin preparation. The incidence and titre of antibodies to glucagon was similar in these two patient groups. Only one molecular form of glucagon was demonstrable in these insulin preparations, and the concentration of glucagon was the same in both.

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