CONVERSION OF EXOGENOUS INSULIN INTO HIGH MOLECULAR WEIGHT FORMS IN VIVO

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SUMMARY

[^{125}I]-insulin, injected in rats, was converted into high molecular weight forms as judged by gel filtration of blood serum samples collected at various intervals. These forms represented 26% (10 min. after injection) to 81% (240 min. after injection) of the total immunoprecipitable radioactivity. Their molecular weights were not affected by rechromatography in 0.1 M borate buffer (pH 8) or in 8 M urea-1 M acetic acid (pH 2.4). On incubation of [^{125}I]-insulin with blood serum in vitro, no high molecular weight forms could be observed.

The presence of high molecular weight forms of endogenous immunoreactive insulin (IRI)* in peripheral bovine sera has been reported by Konijnendijk and Bouman (1). Bouman (2) has also reported the production of considerable quantities of high molecular weight IRI by incubating normal serum with crystalline insulin in the presence of small amounts of liver homogenates at 37°C, an effect significantly reduced in the absence of serum. More recently, Yalow and Berson (3) reported the presence of high molecular weight endogenous IRI in patients with insulinoma. The major component of IRI in the sera of these patients exhibited a molecular weight greater than that of albumin.

The present studies were undertaken in order to establish whether exogenous insulin injected into rats is converted in vivo into high molecular weight forms.

*The following abbreviations are employed: Alb, albumin; [^{125}I], proinsulin; α-CHT, α-chymotrypsin; α-Glob, α-globulin; GPAS, guinea pig anti-insulin antiserum; IRI, immunoreactive insulin; proins, [^{125}I]-proinsulin.

MATERIALS AND METHODS

Porcine [^{125}I]-insulin (specific activity: 45.8 to 58.1 mCi/mg insulin) containing an average of 1 atom iodine per molecule was purchased from Abbott Laboratories. Guinea pig anti-insulin antiserum (GPAS) was obtained from Peter Wright (Indianapolis, Ind.); neutralizing potency was 1.11 units porcine insulin per ml antiserum. The antiserum was diluted 220-fold with 5% bovine serum albumin in borate buffer, pH 8.0. The neutralizing capacity of the diluted GPAS was about 500 microunits insulin per 0.1 ml. The diluted preparations were stored at -15°C and were used for the immunoprecipitation studies of [^{125}I]-insulin described below. Rabbit anti-guinea pig antisera were purchased from Sylvana, Millburn, N.J.

Gel filtration on Biogel P-150 (100-200 mesh) was performed in a plastic column (1x60 cm, bed volume about 35 ml) in 0.1 M borate buffer (pH 8.0), containing 1% human serum albumin, at 4°C or in 8 M urea-1 M acetic acid (pH 2.4) at room temperature. Albumin was added in the borate buffer in order to eliminate nonspecific adsorption of insulin to the Biogel columns. 0.25 ml serum samples were applied on the column and 1.0 to 1.2 ml fractions were collected. In studies with 8 M urea-1 M acetic acid the samples were incubated for 24 hours at room temperature before chromatography. γ-globulin (γ-Glob), albumin (Alb), α-chymotrypsinogen (α-CHT) and [^{125}I]-proinsulin (Proins) were used as molecular markers.

Immunoprecipitation of the [^{125}I]-insulin fractions collected in borate buffer was performed as follows: 0.5 ml of each fraction was added to glass rimmed tubes (13x100 mm) containing 0.4 ml 5% bovine serum albumin (BSA) in borate buffer (pH 8.0). 0.1 ml of diluted GPAS was added to each tube and stored at 4°C for 48 hours. Following the incubation with GPAS, 0.2 ml of undiluted rabbit anti-guinea pig antiserum was added followed by the addition of 0.1 ml normal guinea pig serum diluted 1:50 with 5% BSA in borate buffer (pH 8.0). The tubes were incubated for 72 hours at 4°C and then centrifuged at 4°C. The supernatant fluid was decanted into rimmed glass tubes (13x100 mm) and

both supernatant fluids and precipitates were counted for five minutes each in a Nuclear Chicago automated well-type scintillation counter. The radioactivity in the precipitate is referred to as the immunoprecipitable radioactivity and that in the supernatant fluid as the nonimmunoprecipitable radioactivity.

In vivo experiments. 0.25 ml samples of the commercial [^{125}I]-insulin, in 1% human serum albumin, were chromatographed on the Biogel P-150 column, as described above, and the pooled fractions corresponding to the single component insulin were lyophilized, dissolved in a small volume of distilled water and dialyzed for 24 hours at 2°C against 1,000 volumes of 0.15 M NaCl with two volume changes. Groups of four Charles River Laboratories male CD fed rats (120-130 g) were injected into the jugular vein with 0.5 ml of the dialyzed porcine [^{125}I]-insulin. Each rat received about 6 milliunits of [^{125}I]-insulin. The animals were narcotized lightly during injection with 50% CO_2:50% O_2. Blood samples (0.2 ml) were obtained from the jugular vein of each rat at various intervals after injection. The sera were separated by centrifugation and pooled. 0.25 ml pooled serum samples obtained at the various intervals after injection were subjected to filtration on Biogel P-150. The fractions were examined for the presence of immunoprecipitable and nonimmunoprecipitable radioactivity as described above.

Separation of high molecular weight forms. Rat sera obtained five minutes after the injection of porcine [^{125}I]-insulin were subjected to Biogel P-150 filtration in borate buffer, pH 8.0, as described above. The tubes containing the high molecular weight fraction were pooled and dialyzed for 24 hours against 1 liter cold 0.15 M NaCl at 4°C. The dialyzed preparations from several runs were pooled, 0.25 ml aliquots were placed in plastic tubes and stored at -15°C.

RESULTS AND DISCUSSION

Fig. 1 shows the distribution of immunoprecipitable and nonimmunoprecipitable radioactivity in Biogel P-150 fractions of blood sera of rats following injection of single component [^{125}I]-insulin. Within 10 minutes of injection about

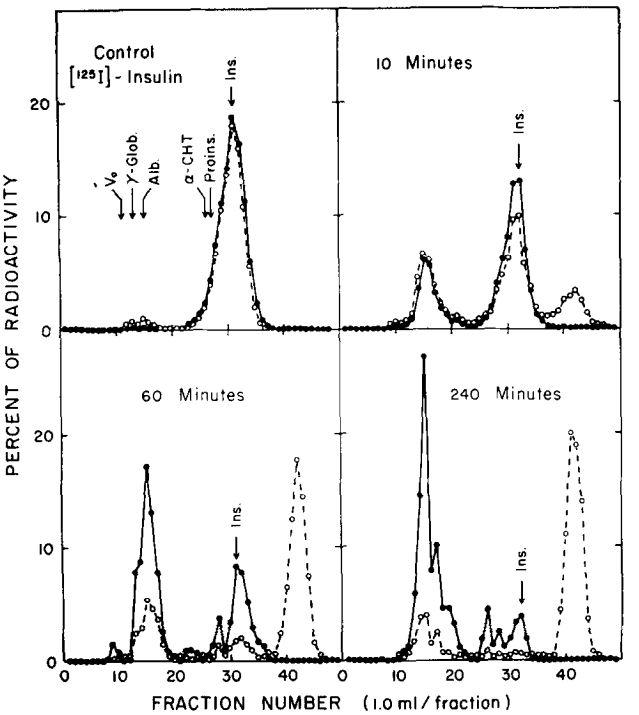

Figure 1. Distribution of immunoprecipitable (●——●) and nonimmunoprecipitable (o----o) radioactivity in Biogel P-150 fractions of rat sera obtained at various intervals after the injection of single component [^{125}I]-insulin in rats. Control [^{125}I]-insulin was chromatographed in the presence of 0.25 ml rat serum. Values are expressed as percentage of total immunoprecipitable or total nonimmunoprecipitable radioactivity.

26% of the total immunoprecipitable radioactivity of serum appeared in a high molecular weight form (70,000 to over 100,000 daltons). At 60 minutes the high molecular weight form of immunoprecipitable radioactivity was about 69% and at 240 minutes about 81% of the total immunoprecipitable radioactivity. With progression of time, multiple immunoprecipitable fractions of [^{125}I]-insulin appeared in the sera ranging from over 100,000 to 24,000 daltons (Fig. 1). Nonimmunoprecipitable radioactivity in the small molecular weight region following the insulin fraction may represent degradation products of [^{125}I]-insulin. Control porcine [^{125}I]-insulin, used for the *in vivo* injection, was also subjected to gel filtration after *in vitro* incubation with 0.25 ml rat serum. The *in vitro* incubation of the control [^{125}I]-insulin with rat serum did not affect its molecular weight (Fig. 1), suggesting that the conversion of insulin to higher molecular weight forms occurs *in vivo*.

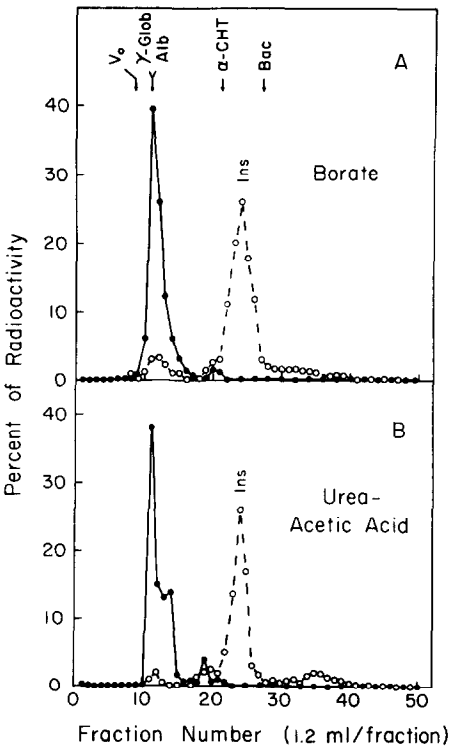

Figure 2. Rechromatography of the high molecular weight form of [^{125}I]-insulin (●——●) on Biogel P-150 in borate buffer, pH 8.0 and in 8 M urea-1 M acetic acid. Control single component porcine [^{125}I]-insulin (o----o) was chromatographed under the same conditions. Values are expressed as percentage of total radioactivity.

Over 95% of the radioactivity of the control single component [^{125}I]-insulin was immunoreactive with guinea pig anti-insulin antisera. The high molecular weight fractions exhibited reduced immunoreactivity compared to single component insulin. Between 50 and 65% of the radioactivity of these fractions was recovered in the immunoprecipitate.

As shown in Fig. 2, the molecular weight of the high molecular weight form of [^{125}I]-insulin was not affected by rechromatography in borate buffer, pH 8.0 (Fig. 2A) or in 8 M urea-1 M acetic acid (Fig. 2B), indicating that it does not represent products of nonspecific adsorption of insulin to serum proteins.

These studies provide direct evidence for the conversion of [^{125}I]-insulin into high molecular weight forms *in vivo*.

ACKNOWLEDGEMENTS

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