INTERACTION OF THE GLUCOSE TOLERANCE FACTOR (GTF) WITH INSULIN

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Summary

Partially purified glucose tolerance factor (GTF) which had been extracted from Brewer's yeast was mixed with ^{125}I-insulin, and the solution was chromatographed on Sephadex G-50. Similarly, ^{125}I-insulin which had not been reacted with GTF was chromatographed. Insulin reacted with GTF produced a significantly greater effect on glucose uptake in epididymal tissue than that of native insulin. When GTF, exclusive of insulin, was chromatographed, the fraction which potentiated insulin activity had an elution volume greater than that of insulin. These results demonstrate that GTF binds to insulin. When insulin was reacted with acetic anhydride under conditions which acetylate the α and ε amino groups, GTF binding to insulin was inhibited. These results suggest that the α and ε amino groups of insulin may be involved in the binding of GTF to insulin.

In 1957, Schwartz and Mertz (1) first described the glucose tolerance factor (GTF), a dietary ingredient which contains chromium and is required to maintain normal glucose tolerance in rats. Subsequent experiments have demonstrated that GTF potentiates the effect of insulin on glucose metabolism in epididymal adipose tissue (2,3). Attempting to elucidate the mechanism whereby GTF facilitates glucose metabolism, we have examined the interaction between GTF and insulin.

Methods

The GTF preparations used in the experiments described below were obtained from Brewer's yeast following extraction with ethanol

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and subsequent chromatography on activated charcoal. These purification procedures increase the specific activity of GTF approximately 80-fold and result in a preparation which contains 60 µg Cr/g extract.

Labeled ^{125}I-insulin was purchased from Amersham Searle* and the preparations were diluted to a concentration of 85 mU/ml with cold insulin prior to experimentation. A fraction (0.1 ml) of this preparation was removed for use as a standard.

To examine the interaction of GTF with insulin by chromatography, 40 mg of the GTF preparation was mixed with 20 mU of the insulin preparation in a volume of 0.5 ml. The contents were incubated at room temperature for 2 hours after which the sample was applied to a 0.9- x 60-cm column packed with Sephadex G-50 which had been swollen and equilibrated with 0.025 M KH_2PO_4, pH 7.4. The same buffer was used to elute the sample, and 1.0 ml-fractions were collected and monitored for radioactivity in a gamma well scintillation counter. Similarly, labeled insulin which had not previously been mixed with the GTF preparation was applied to an identical column and eluted as described above.

Following chromatography, the labeled insulin fractions were combined, freeze-dried and diluted to a concentration of 3.0 mU insulin/ml. The insulin and insulin-GTF samples were assayed for biological activity in epididymal adipose tissue from chromium-deficient rats by using the method described by Mertz et al. (4).

The elution volume of the insulin-potentiating fraction from the yeast extract was determined by dissolving 40 mg of the GTF preparation in 0.5 ml KH_2PO_4 and chromatographing the sample on a

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*Trade names and Company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product by the United States Department of Agriculture.

0.9- x 60-cm Sephadex G-50 column as described above. The eluted samples were combined into three fractions, freeze-dried and diluted with 1.0 ml distilled water. The pooled fractions obtained from chromatography of GTF were assayed for biological activity in the epididymal fat pad assay system (4).

Acetylinsulin (Ac Insulin) was prepared by dissolving 2 mg insulin in 20 ml 0.2 M $NaHCO_3$, pH 8.6. Acetic anhydride was added to the insulin solution over a period of 12 hours until the mole ratio of acetic anhydride to insulin was 10:1. The acetylated insulin preparation was then chromatographed on Sephadex G-25 and freeze-dried. Acetylinsulin-GTF was prepared by mixing 40 mg of the GTF extract (vide supra) with 20 mU of acetylinsulin.

Results and Discussion

As illustrated in Figure 1, chromatography of labeled insulin results in three radioactive peaks. The first peak, fractions 14-20, coincides with the void volume of the column and probably represents either ^{125}I, which reacted with albumin during the labeling process, or polymeric insulin. The third peak of radioactivity is eluted at the total volume of the column and probably consists of unreacted ^{125}I. The major peak of radioactivity, fractions 31-41, is eluted at a volume which coincides with the molecular weight of monomeric insulin. The elution volume of labeled insulin was unaffected by the addition of the GTF preparation.

When the insulin preparations were assayed following chromatography, insulin which had been reacted with the yeast extract produced a significantly greater effect on glucose uptake in epididymal tissue than that of native insulin (Figure 2). Moreover, when the GTF preparation, exclusive of insulin, was chromatographed and subsequently assayed in the presence of added insulin, only that fraction which was eluted at the total volume of the column demonstrated insulin-

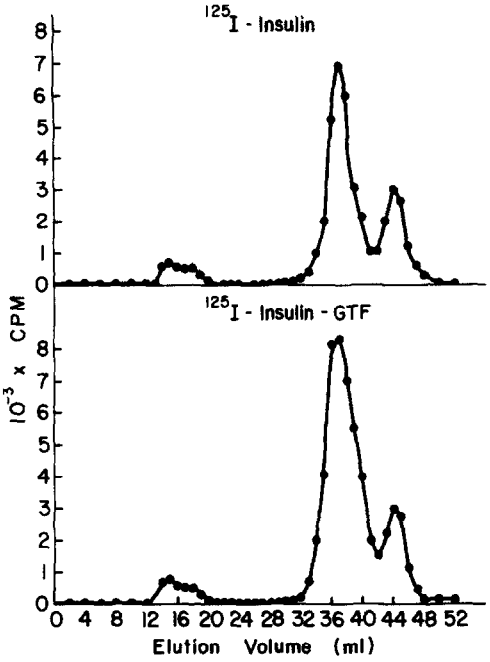

Fig. 1.

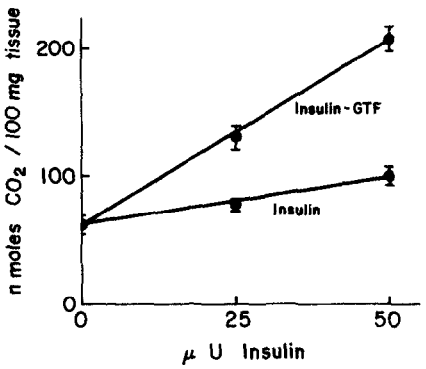

Fig. 2.

Figure 1. Elution of labeled insulin and insulin-GTF from a Sephadex G-50 column. For details, see text.

Figure 2. Response of epididymal adipose tissue to insulin and insulin-GTF. Following chromatography of insulin and insulin-GTF on Sephadex G-50, fractions 31-40 (Figure 1) were pooled and prepared for assay as described in the text. Each point represents the mean and standard deviation of three assays.

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potentiating activity (Figure 3). These results indicate that the glucose tolerance factor is a low molecular weight compound which potentiates insulin activity after binding to the insulin molecule.

Experiments with acetylated insulin give some insight into the nature of binding between GTF and insulin. As shown in Figure 4, acetylation of insulin under the conditions described above had no effect on insulin activity. However, acetylation of insulin inhibited the potentiating effect of GTF. At alkaline pH, acetic anhydride, reacts readily with the α amino groups of glycine in the A chain and

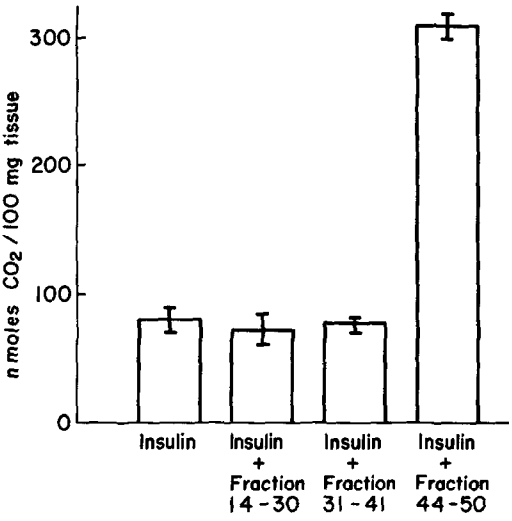

Fig. 3.

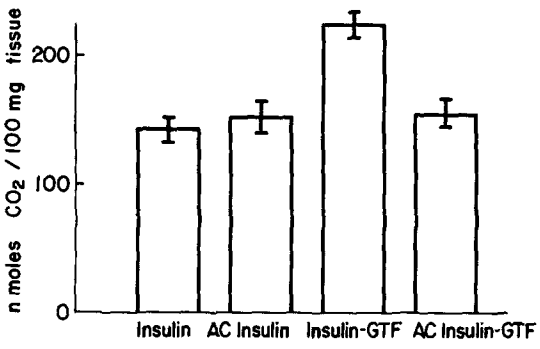

Fig. 4.

Figure 3. Assay of GTF fractions following chromatography on Sephadex G-50. Following gel filtration chromatography of the GTF preparation (for details, see text), 0.01 ml of each pooled, concentrated fraction was assayed in the presence of 100 μU insulin. The illustrated values represent the mean and standard duration of four assays.

Figure 4. Effect of acetylation of insulin on GTF potentiation of glucose metabolism in epididymal adipose tissue. Insulin-GTF acetylated insulin (Ac Insulin) and Ac Insulin-GTF were prepared as described in the text and 125 μU of each preparation was assayed for biological activity. The illustrated values represent the mean and standard duration of three assays.

phenylalanine in the B chain as well as with the ε amino group of lysine in the B chain of porcine insulin (5). Thus, the α and ε amino groups of insulin are apparently involved in the binding of GTF to insulin.

References

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