

Insulin-stimulating protein from human plasma

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An insulin-stimulating protein was isolated from human plasma by a procedure involving Sephadex G-100 chromatography and reverse-phase HPLC. The isolated material gave a single band on SDS-polyacrylamide gel electrophoresis. This protein itself had no insulin-like activity, but enhanced fatty acid synthesis from glucose in rat adipose tissue explants in the presence of suboptimal concentrations of insulin. It also stimulated the effect of insulin on CO₂ liberation from glucose by isolated rat adipocytes and increased the maximal response to insulin.

Insulin Insulin stimulation Adipocyte Fatty acid synthesis CO₂ production

1. INTRODUCTION

It is well accepted that the action of insulin is greatly influenced by alterations in sensitivity of responsiveness of target tissues to the hormone [1]. However, little is known about the mechanisms of these phenomena. There are reports that insulin fragments [2-4] or fragments from human growth hormone [5] potentiate the action of insulin in vitro. Previously [6], we found that treatment of bovine serum albumin with trypsin produced an insulin-stimulating peptide and we determined the structure of this peptide. These findings suggest the presence of a substance(s) that controls the action of insulin of target tissues in vivo. Subsequently, we isolated a protein from human plasma that stimulates the action of insulin on adipose tissue systems in vitro. Here, we report the isolation and some biological activities of this protein from human plasma.

Abbreviations: H-ISP, insulin-stimulating protein from human plasma

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2. MATERIALS AND METHODS

2.1. Assay of H-ISP activity

H-ISP activity was assayed by measuring fatty acid synthesis and CO₂ production from glucose in rat adipose tissue explants and isolated fat cells, respectively, in the presence of suboptimal concentrations of insulin (porcine, crystallized, 24.3 IU/mg, Sigma).

For fatty acid synthesis, 10 explants (total wet wt, 8-10 mg) were prepared from epididymal fat pads of male Wistar rats, weighing about 200 g, that had been fed ad libitum. The explants were cultured on siliconized lens paper [7] in a sterile petri dish containing 3 ml of medium 199 supplemented with 0.05 μ Ci/ml [U-¹⁴C]glucose (Radiochemical Centre), 1.5 mg/ml NaHCO₃ and antibiotics. After incubation for 20 h at 37°C under 3% CO₂ in air, unless otherwise specified, the explants were weighed and hydrolyzed in 1 ml of 2 N KOH in 50% ethanol for 2 h at 100°C. Fatty acids were extracted from the acidic hydrolyzate with 3 ml of petroleum ether [8]. This assay method was used for the purification of H-ISP.

Production of CO₂ was assayed by measuring conversion of [1-¹⁴C]-glucose to ¹⁴CO₂ by rat adipocytes, essentially as described [9]. Reaction mixtures contained (0.75-1) $\times 10^6$ cells/ml, [1-¹⁴C]-

glucose (0.2 mM, 0.3 μ Ci/ml) and 10 mg/ml crystallized bovine serum albumin (Sigma) in a total volume of 0.4 ml Krebs-Ringer phosphate buffer. 14 CO $_2$ was collected and counted as described [10].

2.2. Analytical gel electrophoresis

Analytical gel electrophoresis was performed as described in [11], except that the final concentrations of acrylamide and bisacrylamide were 20.86 and 0.096%, respectively.

3. RESULTS

3.1. Purification of H-ISP

Chilled ethanol (2 l) was added to 1 l human plasma with stirring for 30 min in the cold. Insoluble material was removed by centrifugation at $10000 \times g$ for 30 min, and the resulting supernatant concentrated to about 300 ml at 37°C under reduced pressure. The concentrated supernatant was shaken with an equal volume of chloroform, and the aqueous phase concentrated to about 200 ml under reduced pressure, dialyzed overnight against two changes of distilled water (5 l each) in a Spectrapor 3 (Spectrum Medical; 3500 Da cutoff) and lyophilized.

The dried material (about 200 mg protein) was dissolved in 15 ml 10 mM Tris-HCl buffer containing 0.1 M NaCl (pH 7.40) and passed through a Sephadex G-100 column (4.4 \times 90 cm) in the same buffer at a flow rate of 20 ml/h. Fractions containing H-ISP activity were pooled, dialyzed, and lyophilized.

The lyophilized material was dissolved in distilled water and fractionated on a TSK gel ODS-120A column (7.8 \times 300 mm, Toyo Soda) with 0.1% trifluoroacetic acid (solvent A) as the mobile phase, and 90% acetonitrile containing 0.1% trifluoroacetic acid (solvent B) as a mobile-phase modifier. Elution was carried out with a linear gradient of solvent B as follows: 53% B for 20 min, 53–66% B in 30 min and 66–100% B in 10 min at a flow rate of 1 ml/min at 22–25°C. H-ISP activity was eluted from the column after 42–50 min under these conditions. Final purification was achieved on a Hi-Pore RP 304 column (4.6 \times 250 mm, Bio-Rad) with solvent A as the mobile phase, and solvent B as the mobile-phase modifier. Elution was effected with a linear gradient of solvent B as

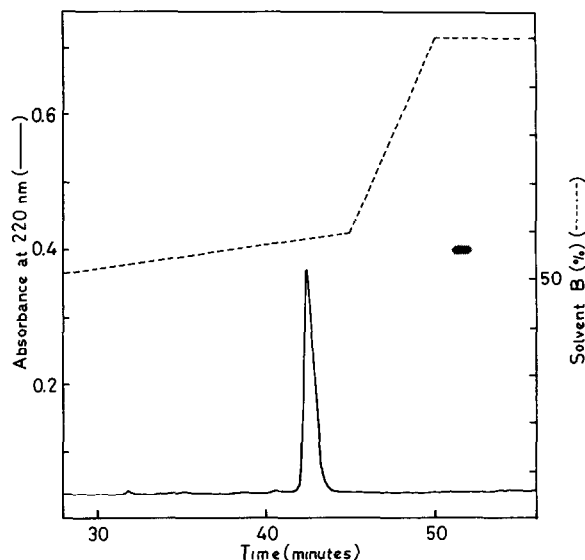


Fig.1. HPLC of H-ISP on a Hi-Pore RP-304 column. The horizontal bar indicates the fractions of H-ISP pooled and used for further experiments. The inset shows the SDS-polyacrylamide gel electrophoretic profile of purified H-ISP.

follows: 0–40% B in 5 min, 40–60% B in 40 min and 60–100% B in 5 min at a flow rate of 1 ml/min at 22–25°C (fig.1). As shown (fig.1, inset) the purified material gave a single band with an M_r of about 16 000 on SDS-polyacrylamide gel electrophoresis. The isoelectric point of H-ISP was estimated to be about 4.7. Purified H-ISP was stable on heating at 60°C for 10 min and no loss of activity was observed on storage as an aqueous solution for at least 6 months at –20°C or for 24 h at 4°C at pH 2.0.

3.2. Biological activities of H-ISP

As can be seen from fig.2A, H-ISP markedly stimulated the action of insulin added at a suboptimal concentration (0.2 mU/ml) on fatty acid synthesis in the adipose tissue explant system. Stimulation was maximal with more than 15 μ g/ml of H-ISP under the experimental conditions. H-ISP alone had no insulin-like activity in the concentration range tested (fig.2A). Next, the effect of H-ISP was tested as a function of the concentration of insulin. H-ISP shifted the concentration-response curve of insulin-dependent fatty acid synthesis to the left (fig.2B).

As shown in table 1, H-ISP also stimulated CO $_2$

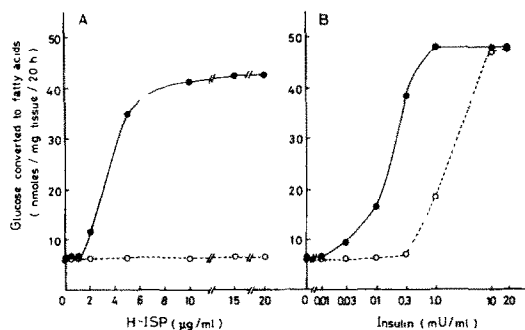


Fig.2. (A) Effect of the concentration of H-ISP on fatty acid synthesis from glucose in the presence of insulin. Adipose tissue explants were incubated at 37°C for 20 h with the indicated concentrations of H-ISP in the absence (○) or presence (●) of insulin (0.2 mU/ml). Values are means for duplicate determinations in two separate experiments. (B) Dose-response curves for the effects of insulin with and without H-ISP. Adipose tissue explants were incubated with the indicated concentrations of insulin alone (○), or with H-ISP (15 μg/ml) (●). Values are means for duplicate determinations in two separate experiments.

Table 1

Effect of H-ISP on CO₂ liberation from glucose by rat adipocytes

Insulin (μU/ml)	H-ISP (10 μg/ml)	¹⁴ CO ₂ liberated from D- [1- ¹⁴ C]glucose (nmol/10 ⁶ cells per h)	
		Total	Insulin- stimulated
0	—	9.93 ± 0.31	
	+	9.90 ± 1.13	
5	—	16.62 ± 2.46	6.69
	+	22.87 ± 3.71	12.97
10	—	29.70 ± 3.77	19.77
	+	39.42 ± 3.70	29.52
30	—	52.29 ± 1.88	42.36
	+	69.81 ± 5.44	59.91
100	—	53.50 ± 2.36	43.57
	+	72.29 ± 6.90	62.39

Experimental conditions were as described in the text.
Values are means ± SE (n = 4)

production from [1-¹⁴C]glucose by isolated rat adipocytes in the presence of suboptimal concentrations of insulin. Again, H-ISP had no effect in the absence of added insulin, but significantly increased the maximal response to insulin to about 140% of the maximal value obtained with insulin-alone (100 μU/ml) (table 1).

4. DISCUSSION

This paper describes the presence of an insulin-stimulating principle, H-ISP, in human plasma for the first time. The results suggest that H-ISP plays a role in controlling the insulin sensitivity and/or insulin responsiveness of target tissues. Authors in [12] reported that glucose ingestion was accompanied by a marked increase in insulin sensitivity of human fat cells and they suggested that oral glucose stimulates the release of a circulatory factor(s) that potentiates the insulin effect. Authors in [13] also observed an increase in insulin sensitivity of rat adipocytes after intravenous glucose infusion. However, little is known about the cellular processes that could enhance insulin sensitivity. Therefore, it might be of interest to examine the participation of H-ISP in these processes and also at the clinical level to investigate the relationship between H-ISP and the etiology of non-insulin-dependent diabetes mellitus.

REFERENCES

- [1] Kahn, C.R. (1978) *Metabolism* 27, 1893-1902.
- [2] Weitzel, G., Eisele, K., Schulz, V. and Stock, W. (1973) *Hoppe-Seyler's Z. Physiol Chem.* 354, 321-330.
- [3] Fujino, M., Wakimasu, M., Taketomi, S. and Iwatsuka, H. (1977) *Endocrinology* 101, 360-364.
- [4] Kikuchi, K., Lerner, J., Freer, R.J. and Day, A.R. (1981) *J. Biol. Chem.* 256, 9441-9444.
- [5] Ng, F.M. and Bornstein, J. (1979) *Diabetes* 28, 1126-1130.
- [6] Ueno, A., Arakaki, N., Inoue, H., Oribe, T. and Takeda, Y. (1984) *Biochem. Biophys. Res. Commun.*, in press.
- [7] Topper, Y.J., Oka, T. and Vonderhaar, B.K. (1975) *Methods Enzymol.* 39, 443-454.
- [8] Dittmer, J.C. and Wells, M.A. (1969) *Methods Enzymol.* 14, 482-530.
- [9] Fain, J.N., Reed, N. and Saperstein, R. (1967) *J. Biol. Chem.* 242, 1887-1894.

- [10] Ono, M., Inoue, H., Suzuki, F. and Takeda, Y. (1972) *Biochim. Biophys. Acta* 284, 285-297.
- [11] Ito, K., Date, T. and Wickner, W. (1980) *J. Biol. Chem.* 255, 2123-2130.
- [12] Arner, P., Bolinder, J. and Östman, J. (1983) *J. Clin. Invest.* 71, 709-714.
- [13] Livingston, J.N., Kingston, W.J., Finocchiaro, L. and Moxley, iii, R.T. (1984) *Endocrinology* 115, 55-59.