THE PREPARATION OF THE [Arg-B^5], [Gly-B^6], AND [Gly-B^5] ANALOGS OF BOVINE INSULIN

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In a study of the structural-functional relationships in the insulin molecule we have obtained previously-unknown semisynthetic analogs of bovine insulin – [Arg-B^5]insulin (I), [Gly-B^6]insulin (II), and [Gly-B^5]insulin (III) – by coupling the A chain of natural bovine insulin with the corresponding synthetic analogs of the B chain [1]. The protected analogs of the B chain were first demasked by treatment with sodium in liquid ammonia in the presence of sodium amide [2] and were then subjected to oxidative sulfitolysis [3], as the result of which the following bis-S-sulfonate analogs of the B chain were prepared: (IV) with the histidine-B^5 residue replaced by an arginine residue; (V) with the leucine-B^6 residue replaced by a glycine residue; and (VI) with the histidine-B^5 residue replaced by a lysine residue. The amino-acid analyses of the bis-S-sulfonates obtained were as follows:

	His	Arg	Lys	Gly	Leu	Ala	Val	Phe	Glu	Asp	Pro	Ser	Thr	Tyr
IV	1,1	2,0	1,0	3,0	4,1	2,1	3,1	3,3	3,4	1,1	1,0	0,7	0,7	2,1
V	2,0	0,8	0,7	4,1	3,4	1,6	3,5	3,0	3,0	0,9	1,1	0,7	0,3	2,0
VI	1,0	1,0	1,0	4,0	4,2	1,6	3,1	3,1	3,0	1,3	1,1	0,4	0,4	1,6

The bis-S-sulfonates (IV) and (V) were purified by ion-exchange chromatography on a column of SP-Sephadex C-25 using 0.5 M acetate buffer (pH 3.3) in 8 M aqueous urea; elution was carried out with the same solvent with an exponential gradient of 0.35 M sodium chloride. The electrophoretic mobilities of the bis-S-sulfonates (IV) and (V) corresponded to that of the bis-S-sulfonate of the B chain obtained from natural insulin (electrophoresis on "Khromatograficheskaya M" paper, pH 2.6, 720 V, 10 mA).

The purified bis-S-sulfonates (IV) and (V), and also the bis-S-sulfonate (VI) (without preliminary purification) were brought into combination with a fourfold excess of the tetramercapto form of the A chain of bovine insulin prepared by treating the tetra-S-sulfonate of the A chain with 2-mercaptoethanol [4].

The biological activity of the analog (I) was determined in the combination solution from the convulsive effect in mice – it amounted to 10%. Under the same conditions, combinations of synthetic B chain of bovine insulin with the natural A chain gave an activity of 25%.

The analogs (II) and (III) were isolated by ion-exchange chromatography on a column containing CM-Sephadex C-25 by elution with 0.04 M acetate buffer (pH 4.0) in 8 M urea solution with an exponential gradient of 0.35 M sodium chloride. Amino-acid analyses: (II) – His 2.0; Arg 1.0; Lys 1.0; Gly 5.0; Leu 5.0; Ala 3.0; Val 5.0; Phe 3.1; Glu 5.5; Asp 1.8; Pro 1.0; Ser 1.2; Thr 0.7; Tyr 3.6; Ile 0.7; (III) – His 1.2; Arg 1.1; Lys 1.1; Gly 5.1; Leu 6.4; Val 5.0; Ala 2.8; Phe 3.2; Glu 6.5; Asp 2.7; Pro 1.1; Ser 1.4; Thr 1.1; Tyr 2.9; Ile 0.8. The electrophoretic mobility of the analog (2) corresponded to that of natural insulin, while for the analog (3) it was somewhat smaller.

The biological activities on testing by means of the convulsive effect in mice were 27% for (II) and 30% for (III) (of the activity of the international standard for insulin).

The results obtained show that the replacement of the amino-acid residues histidine-B^5 and leucine-B^6, which are invariant in the insulins of different species, enables insulin analogs to be obtained that retain a fairly high specific hormonal activity.

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