SYNTHESIS OF AN ENCEPHALITOGENIC FRAGMENT OF THE PROTEIN OF MYELIN AND ITS 6-GLYCINE ANALOG

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The encephalitogenic nonapeptide with the structure Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg (I) is a fragment of the main protein of myelin and, like the protein itself, possesses unique immunological properties – the capacity for inducing in animals experimental allergic encephalomyelitis (EAE) [1]. In view of this, the encephalitogenic peptide and some of its analogs and fragments have been synthesized in a number of laboratories [2-4]. It has been shown that for the manifestation of activity, i.e., the excitation of EAE in animals the primary structure of the peptide must include such amino acids as tryptophan, glutamine, and arginine (or lysine); a modification of the amino acids mentioned or their replacement by others causes a complete loss of activity.

For a further study of the dependence of the biological properties of the peptide (I) on its primary structure and conformation, we have performed the synthesis of the nonapeptide (I) and its 6-glycine analog (II) [5]. Both compounds were synthesized by the classical method of condensing fragments. The protected nonapeptide Z-Phe-Ser-Trp-Gly-Ala-Glu(OBzl)-Gly-Gln-Arg(NO_2)-ONB (III) was obtained with a yield of 60% by the azide method in Rudinger's modification [6] by the union of two fragments: the N-terminal benzyloxycarbonylpentapeptide and the C-terminal tetrapeptide. The protected 6-glycine analog (IV), Z-Phe-Ser-Trp-Gly-Ala-Gly-Gly-Gln-Arg(NO_2)-ONB was also synthesized by the azide method through the union of the N-terminal tetrapeptide and the C-terminal pentapeptide. The protective groups of compounds (III) and (IV) were eliminated by catalytic hydrogenolysis in the presence of palladium. The free peptides were purified by ion-exchange chromatography on carboxymethylcellulose and also by preparative ionophoresis on FN-17 paper, pH 1.7, voltage gradient 70 V/cm, and pH 3.5, voltage gradient 40 V/cm.

The nonapeptide (I) [3] with the composition $C_{46}H_{64}N_{14}O_{14}$ was obtained with a yield of 50%, mp 155-164°C, $[\alpha]_D^{25}$ −30.5° (c 0.8; water), R_f 0.18 [butanol–acetic acid–water (4 : 1 : 1)] and 0.21 (isoamyl alcohol–pyridine–water (35 : 35 : 30)]. The electrophoretic mobility on FN-16 paper at pH 3.5 (relative to lysine) was 0.33.

The 6-glycine analog of (II), with the composition $C_{43}H_{60}N_{14}O_{12} \cdot 2CH_3COOH \cdot 5H_2O$ was synthesized with a yield of 40%, decomp. 153°C $[\alpha]_D^{25}$ −9.1 (c 0.5; water), R_f 0.79 [butanol–acetic acid–water (4 : 1 : 1)] and 0.83 [isoamyl alcohol–pyridine–water (35 : 35 : 30)]. Its electrophoretic mobility at pH 3.5 (relative to lysine) was 0.40.

The results of tests on guinea pigs in the laboratory of the biochemistry of the nervous system of the Institute of Biochemistry of the Academy of Sciences of the Uzbek SSR showed that compounds (I) and (II) possess a high encephalitogenic activity.

Analogs of an 11-membered fragment of the main protein of myelin differing from the nonapeptide (I) by the presence of C-terminal lysine in place of arginine and by two additional amino acids at the N-end have been synthesized previously [2]. The authors concerned showed that the replacement of glutamic acid by isoleucine does not affect the EAE activity of this undecapeptide.

It follows from the results that we have obtained that the complete elimination of the side chain of the amino acid present in position 6 of peptide (I) does not lower the encephalitogenic acitivy of this peptide.

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