Synthesis of a Tetratriacontapeptide corresponding to Sequence 112—145 of the β-Subunit of Human Chorionic Gonadotropin

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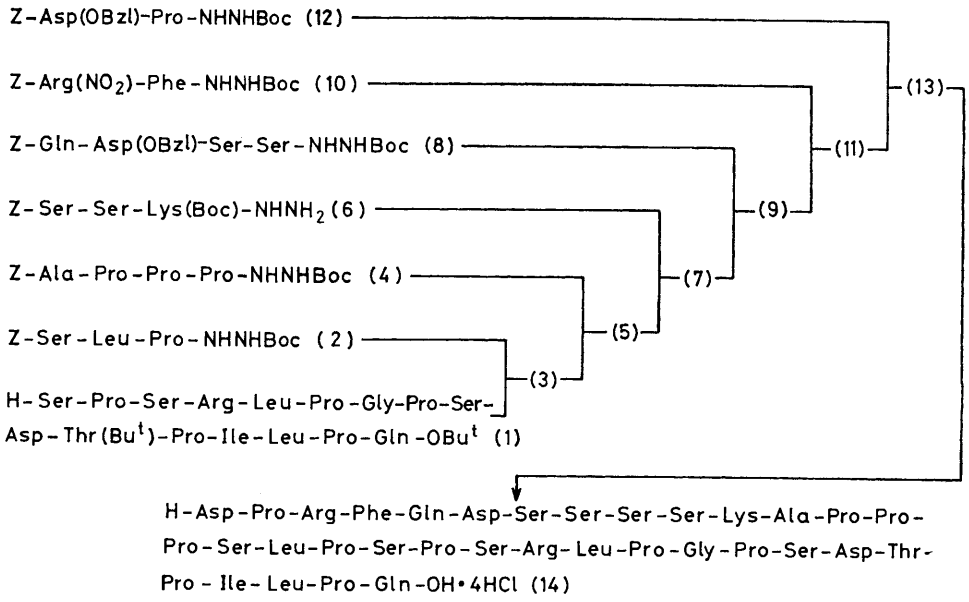
Summary A tetratriacontapeptide corresponding to sequence 112—145 of the β-subunit of human chorionic gonadotropin (hCG), which was synthesized by assembling peptide fragments by the azide procedure, was then conjugated to bovine serum albumin; injection of the conjugated antigen into rabbits produced antisera which exhibited 30% specific binding with [^{125}I]hCG at a 1 : 1000 dilution.

ANTISERA specific to human chorionic gonadotropin (hCG) without cross-reaction with other glycoprotein hormones, especially human luteinizing hormone (hLH), have been required not only for diagnostic use but also for fertility regulation. Synthesis of the *C*-terminal peptide of the β-subunit of hCG would make possible the production of such antisera because the unique *C*-terminal portion[1] (approximately 30 amino-acid residues) is not present in the β-subunit of other human glycoprotein hormones [hLH,[2] human follicle-stimulating hormone (hFSH),[3] and human thyroid-stimulating hormone (hTSH)[4]]. To date, the solid-phase syntheses[5] and liquid-phase synthesis[6] of *C*-terminal peptides of the β-subunit of hCG and the formation of antisera against the synthetic peptides have been described. We report the synthesis of a tetratriacontapeptide corresponding to sequence 112—145 of the β-subunit of hCG, as well as its immunological properties.

Starting with the *C*-terminal hexadecapeptide (**1**),[7] the seven peptide fragments in the Scheme were coupled successively by the azide procedure[8] in order to minimize racemization and avoid the need for protection of side-chain functional groups of the amino-acid residues as much as possible during the synthesis. (Scheme). The α-amino functions of the amino-acids were protected by the Z group. The Bzl protecting group of the β-carboxy function of Asp and the NO_2 protecting group of Arg were removed by catalytic hydrogenation over palladium immediately after the introduction of the corresponding oligopeptide fraction. The regenerated guanidino-group of Arg was protected as its hydrochloride during the synthesis.[9] The hydroxy-groups of the Ser residues were not protected. The carboxy-group of the *C*-terminal Gln residue and the hydroxy-group of the sole Thr residue were protected as their t-butyl ester and t-butyl ether, respectively, and the ε-amino group of the Lys residue was protected as its t-butyloxycarbonyl (Boc) derivative during the synthesis. The Boc group on the hydrazide nitrogen of the peptides (**2**), (**4**), (**8**),[7] and (**10**) was removed by trifluoroacetic acid (TFA) and that of (**12**) by HCl in dioxan prior to the preparation of the corresponding azide. After azide coupling, the protected peptide products were isolated and purified by column chromatography on (i) Sephadex LH-20 using EtOH (method A) or dimethylformamide (DMF) (method B) as eluant and (ii) silica gel with the following



SCHEME. Synthesis of the tetratriacontapeptide (**14**).

solvent systems: $CHCl_3$–MeOH–H_2O (16:3:1, lower phase, method C); (8:3:1, lower phase, method D) or Bu^nOH–acetic acid–H_2O (4:1:5, upper phase, method E) to afford purified peptides as follows: nonadecapeptide (**3**) [purified by methods A and D, yield 1·85 g (76%)], tricosapeptide (**5**) [methods A and D, yield 1·71 g (92%)], hexacosapeptide (**7**) [methods B and D, yield 1·72 g (88%)], triacontapeptide (**9**)[7] [methods B and E, yield 1·31 g (66%)], dotriacontapeptide (**11**) [methods B and E, yield 1·10 g (90%)], and finally the protected tetratriacontapeptide (**13**) [methods B and E, yield 0·9 g (70%)], $[\alpha]_D^{27}$ −69·5° (DMF), R_f 0·53 (t.l.c., silica gel, Bu^nOH–pyridine–acetic acid–H_2O 4:1:1:2). The polypeptide (**13**) was treated with TFA containing anisole for 3 h, hydrogenated over a palladium catalyst, and purified by gel-filtration on Sephadex G-25 using 5% aqueous acetic acid as eluant. The eluted material was detected by the method of Lowry *et al.*;[10] work-up afforded the desired peptide as its tetrahydrochloride (**14**) after lyophilization from water containing 0·1 N HCl (2 ml) [yield 190 mg (68%)], $[\alpha]_D^{27}$ −153·8° (H_2O), R_f 0·27 (silica gel, Bu^nOH–pyridine–acetic acid–H_2O 30:20:6:24). The amino-acid analyses of an acid hydrolysate (6 N HCl, 20 h, 110 °C) were as follows: Asp 3·01, Thr 0·92, Ser 6·55, Glu 2·04, Pro 10·14, Gly 0·95, Ala 1·00, Ile 0·95, Leu 3·01, Phe 1·08, Lys 1·00, and Arg 2·02; (average recovery 95%).† The peptide (**14**) was conjugated to bovine serum albumin (BSA) by dicyclohexylcarbodi-imide (DCC) in the usual manner.[11] New Zealand White rabbits were immunized with the conjugated antigen in Freund's complete adjuvant by multiple intradermal injection. After eight weeks from the first immunization, antiserum capable of 30% binding of [^{125}I]hCG or [^{125}I]β-subunit of hCG at a dilution of 1:1000 was produced. These bindings were inhibited by hCG as well as the synthetic tetratriacontapeptide (**14**) in a dose-response manner, and at least 500 mI.U. of hCG were needed for this inhibition reaction. The cross-reactivity of this antiserum with the α-subunit of hCG or hLH was negligible. Thus, antiserum against the peptide described above was proved to be specific to hCG, as expected theoretically. Furthermore, this antiserum did not inhibit the biological activity of hCG in a mouse uterine weight bioassay, although Stevens reported that an antiserum against a synthetic peptide (residues 111—145 of hCG) inhibited the biological activity of CGs from man and baboon in the same test as described above.[12]

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† A longer acid hydrolysis (6 N HCl, 48 h, 110 °C) gave the following results: Asp 2·99, Thr 0·95, Ser 4·72, Glu 2·05, Pro 9·79, Gly 0·95, Ala 1·00, Ile 0·89, Leu 2·87, Phe 1·02, Lys 0·91, and Arg 1·92; (average recovery 85%). Some serine was destroyed during the hydrolysis.

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