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Synthesis of C-Terminal Peptides of the β-Subunit of Human Chorionic Gonadotropin (hCG)[1)]

The hexadecapeptide (A), dotriacontapeptide (B), hexadecapeptide (C) and triacontapeptide (D) related to the carboxyl-terminal region of the β-subunit of human chorionic gonadotropin (hCG) have been synthesized by the fragment condensation method in a conventional manner in order to study their immunological properties.

Keywords——β-subunit of hCG; C-terminal peptides; chemical synthesis; fragment condensation; conjugation to BSA; production of antisera; reactivity with ^{125}I-hCG

Recently, the C-terminal portion of the β-subunit of human chorionic gonadotropin (hCG) became the focus of increasing attention for the immunologic studies because about 30 amino acids of that portion are not present in β-subunits of other human glycoprotein hormones (hLH, hFSH and hTSH). Two slightly different structures for the β-subunit of hCG have been reported, one by Carlsen *et al.*[2)] and the other by Morgan *et al.*[3)] The former contains 147 amino acid residues and the latter has 145 amino acid residues. To date, the solid phase synthesis of C-terminal peptides of the β-subunit of hCG based on the both structures[2,3)] and the formation of antibodies against the synthetic peptides have been described.[4,5)]

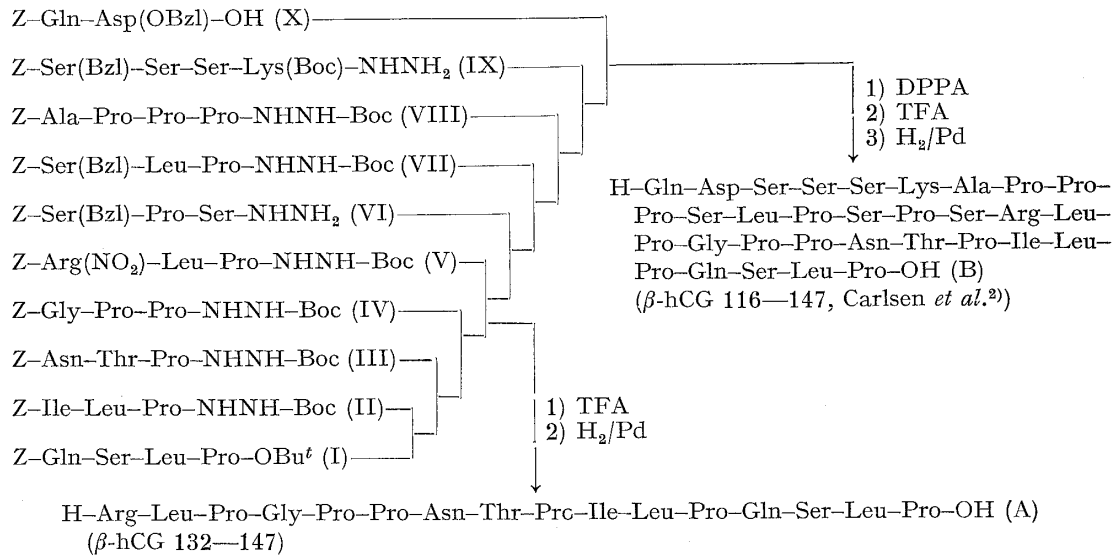

Fig. 1. Synthetic Scheme for the Peptides, (A) and (B)

1) Amino acids, peptides and their derivatives in this communication are of the L-configuration except for glycine. Solvent systems for thin-layer (silica gel, Merck, Kieselgel G) chromatography are: Rf^1=*n*-butanol–AcOH–H_2O (4:1:5), Rf^2=*n*-butanol–pyridine–AcOH–H_2O (4:1:1:2), Rf^3=$CHCl_3$–MeOH–AcOH (90:8:2), Rf^4=$CHCl_3$–MeOH–H_2O (8:3:1), Rf^5=*n*-butanol–AcOEt–AcOH–H_2O (1:1:1:1), Rf^6=*n*-butanol–pyridine–AcOH–H_2O (30:20:6:24), Rf^7=*n*-butanol–pyridine–AcOH–H_2O (2:1:1:2). DPPA=diphenylphosphorazidate, Z=benzyloxycarbonyl, *t*-Boc=*tert*-butyloxycarbonyl, Bu^t=*tert*-butyl, Bzl=benzyl

2) K.B. Carlsen, O.P. Bahl, and N. Swaminathan, *J. Biol. Chem.*, **248**, 9810 (1973).
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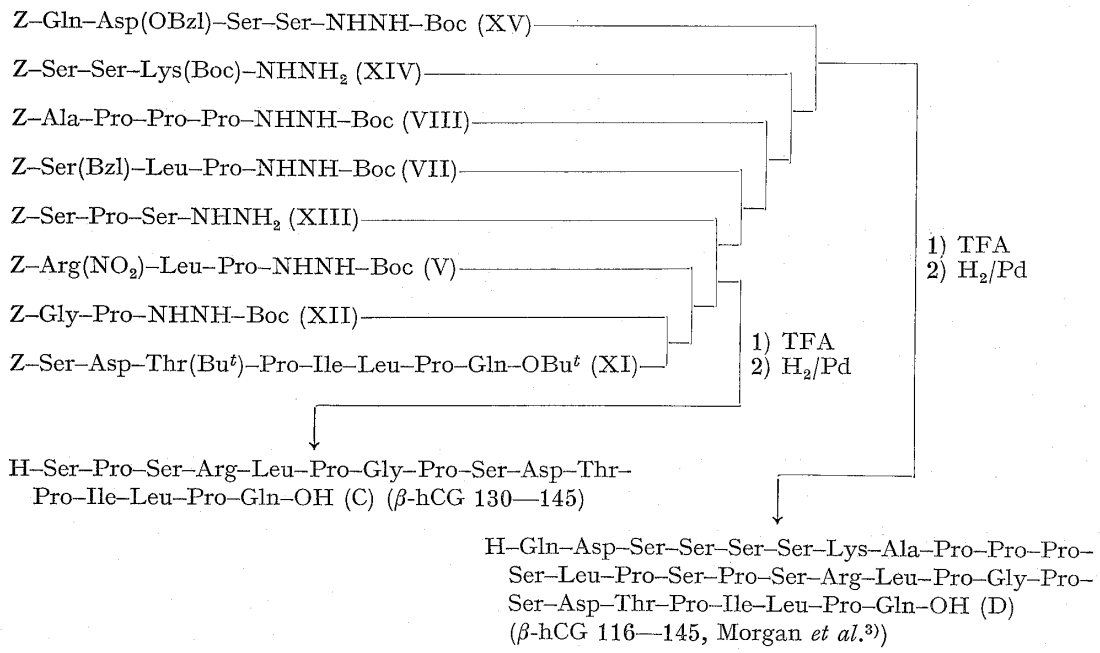

Fig. 2. Synthetic Scheme for the Peptides, (C) and (D)

We wish to report the synthesis of a hexadecapeptide (A) and a dotriacontapeptide (B) corresponding to sequences 132—147 and 116—147 of the β-subunit (Fig. 1, Carlsen *et al.*[2]) respectively and a hexadecapeptide (C) and a triacontapeptide (D) corresponding to sequences 130—145 and 116—145 of the β-subunit (Fig. 2, Morgan *et al.*[3]) respectively as well as some immunological properties of these peptides. The α-amino function of amino acids was protected by the Z group which is cleaved by hydrogenation over a palladium catalyst. For the protection of side chain's functional groups, *t*-butyl derivatives removable by TFA were used during the synthesis, if necessary. For the temporary protection of side chain's functional groups, benzyl group which is removed by hydrogenation was used. Most of the coupling of the intermediates were achieved by the azide coupling procedure[6] and the resulting peptides were purified by gel-filtration on Sephadex LH-20 and/or silica gel column chromatography. The *t*-Boc group on hydrazide nitrogen of the intermediates was removed by TFA or HCl prior to the preparation of azides.

The synthetic scheme for A and B is illustrated in Fig. 1. H–Gln–Ser–Leu–Pro–OBu^t derived from the peptide (I), was condensed successively with Z–Ile–Leu–Pro–NHNH–Boc (II), Z–Asn–Thr–Pro–NHNH–Boc (III), Z–Gly–Pro–Pro–NHNH–Boc (IV) and Z–Arg(NO$_2$)–Leu–Pro–NHNH–Boc (V) by the azide procedure to afford Z–Arg(NO$_2$)–Leu–Pro–Gly–Pro–Pro–Asn–Thr–Pro–Ile–Leu–Pro–Gln–Ser–Leu–Pro–OBu^t (mp 165—171°; $[\alpha]_D^{22}$ −133.0° (c= 1.0, MeOH); *Anal.* Calcd for $C_{90}H_{142}N_{22}O_{28}\cdot 3H_2O$: C, 53.1; H, 7.3; N, 15.2; Found: C, 53.1; H, 7.3; N, 15.1). The hexadecapeptide (A) was derived from the protected peptide by TFA treatment followed by hydrogenation and purification by gel-filtration on Sephadex G-25 ($[\alpha]_D^{25}$ −158.7° (c=0.5, H_2O); Rf^1 0.05, Rf^2 0.45; Amino acid ratios in an acid hydrolysate: Asp 1.00; Thr 0.88; Ser 0.94; Glu 1.08; Pro 6.13; Gly 0.95; Ile 0.98; Leu 3.06; Arg 0.92, average recovery 79%). The protected hexadecapeptide was hydrogenated over a palladium catalyst and the resulting amine was coupled successively with Z–Ser(Bzl)–Pro–Ser–NHNH$_2$ (VI), Z–Ser–(Bzl)–Leu–Pro–NHNH–Boc (VII), Z–Ala–Pro–Pro–Pro–NHNH–Boc (VIII) and Z–

6) J. Honzle and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).

Ser(Bzl)–Ser–Ser–Lys(Boc)–$NHNH_2$ (IX) by the azide procedure and Z–Gln–Asp(OBzl)–OH (X) by the diphenylphosphorazidate (DPPA) method[7] to give Z–Gln–Asp(OBzl)–Ser–Ser–Ser–Lys(Boc)–Ala–Pro–Pro–Pro–Ser–Leu–Pro–Ser–Pro–Ser–Arg–Leu–Pro–Gly–Pro–Pro–Asn–Thr–Pro–Ile–Leu–Pro–Gln–Ser–Leu–Pro–OBu^t (amorphous powder; $[\alpha]_D^{25}$ −75.9° (c=0.4, DMF); *Anal.* Calcd for $C_{169}H_{263}N_{39}O_{50}\cdot HCl\cdot 4H_2O$: C, 54.1; H, 7.3; N, 14.6. Found: C, 54.4; H, 7.2; N, 14.3). It was treated with TFA, hydrogenated over a palladium catalyst and then purified by gel-filtration on Sephasex G-25 to afford the peptide (B) ($[\alpha]_D^{23}$ −173.4° (c=0.2, H_2O); Rf^2 0.05, Rf^7 0.53; Amino acid ratios in an acid hydrolysate: Asp 2.09; Thr 0.86; Ser 6.63; Glu 2.11; Pro 11.45; Gly 1.00; Ala 0.97; Ile 0.90; Leu 3.86; Lys 0.95; Arg 0.90, average recovery 79%).

In order to construct the peptides, (C) and (D) (Fig. 2), the C-terminal octapeptide (XI), after removal of the Z group, served as an amino component, with which relatively small peptides, (XII), (V), (XIII), (VII), (VIII), (XIV) and (XV) were successively condensed by the azide method. The octapeptide (XI) was synthesized in the stepwise manner by the *p*-nitrophenyl ester procedure except for Thr residue which was introduced by the N-hydroxysuccinimide ester method and Ser by the azide procedure starting with H–Gln–OBu^t. The peptide (XI) was hydrogenated over a palladium catalyst and the resulting amine was coupled successively with Z–Gly–Pro–NHNH–Boc (XII), V and Z–Ser–Pro–Ser–$NHNH_2$ (XIII) by the azide procedure to afford Z–Ser–Pro–Ser–Arg–Leu–Pro–Gly–Pro–Ser–Asp–Thr(Bu^t)–Pro–Ile–Leu–Pro–Gln–OBu^t (mp 240° (dec.) with sintering at 165°; $[\alpha]_D^{23}$ −107.8° (c=1.0, MeOH); *Anal.* Calcd for $C_{89}H_{142}N_{20}O_{26}\cdot HCl\cdot 2H_2O$: C, 54.0; H, 7.48; N, 14.1. Found: C, 53.8; H, 7.49; N, 14.1.). This peptide was treated with TFA, hydrogenated over a palladium catalyst and purified by gel-filtration on Sephadex G-25 to give the hexadecapeptide (C) ($[\alpha]_D^{23}$ −163.0° (c=0.3, H_2O), Rf^1 0.05, Rf^2 0.38; Amino acid ratios in an acid hydrolysate and an APM digest (number in parentheses): Asp 0.95 (0.93); Thr 1.05; Ser 2.93 (3.05); Glu 1.02; Pro 5.17 (4.97); Gly 1.00 (1.02); Ile 0.86 (1.00); Leu 1.81 (1.96); Arg 0.91 (0.92); Thr+Gln (1.93, calcd as Thr) average recovery 95% and 90% respectively). The protected hexadecapeptide was converted to the corresponding amine *via* catalytic hydrogenation and condensed with VII, VIII, Z–Ser–Ser–Lys(Boc)–$NHNH_2$ (XIV) and Z–Gln–Asp(OBzl)–Ser–Ser–NHNH–Boc (XV) by the azide method to give Z-Gln-Asp(OBzl)–Ser–Ser–Ser–Ser–Lys(Boc)–Ala–Pro–Pro–Pro–Ser–Leu–Pro–Ser–Pro–Ser–Arg–Leu–Pro–Gly–Pro–Ser–Asp–Thr(Bu^t)–Pro–Ile–Leu–Pro–Gln–OBu^t (mp 245° (dec.) with sintering at 160°; $[\alpha]_D^{23}$ −77.0° (c=0.2, DMF); Rf^2 0.76, Rf^3 0.12; Rf^4 0.10; *Anal.* Calcd for $C_{160}H_{250}N_{36}O_{50}\cdot HCl\cdot 5H_2O$: C, 53.3; H, 7.30; N, 14.0. Found: C, 53.1; H, 7.15; N, 14.3). It was deblocked by TFA treatment and hydrogenation. The resulting deblocked peptide was purified by column chromatography on Sephadex G-25 equilibrated and eluted with 5% AcOH and the location of the eluted material was determined by the method of Lowry *et al.*[8] to afford the peptide (D) ($[\alpha]_D^{25}$ −176.5° (c=0.2, H_2O); Rf^5 0.10, Rf^6 0.33; Amino acid ratios in an acid hydrolysate and an APM digest (number in parentheses): Asp 2.12 (1.81); Thr 0.91; Ser 7.36 (6.93); Glu 2.19; Pro 9.07 (7.16); Gly 1.00 (1.00); Ala 1.04 (0.85); Ile 0.98 (1.08); Leu 3.02 (2.85); Lys 1.00; Arg 0.92; Thr+Gln (2.85 calcd as Thr) average recovery 95% and 87% respectively).

The peptides, A, B, C and D were converted to the corresponding hydrochlorides and conjugated to bovine serum albumin (BSA) in the usual manner.[9] Antisera were produced in New Zealand White rabbits with multiple intradermal injection of these conjugates in Complete Freund's adjuvant. Little binding of the labeled hCG (^{125}I-hCG) was observed with antisera to the peptides, A, B and C. About 1% binding of ^{125}I-hCG was observed with an antiserum to the peptide, D, at a 1: 500 dilution.

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GC-MS Identification of Active Metabolite of Oxacillin in Man

The structure of active metabolite of oxacillin excreted in human urine was identified as 5-hydroxymethyl derivative by means of trimethylsilylation followed by GC–MS analysis.

Keywords——gas chromatography-mass spectrometry; metabolite; oxacillin; isoxazolylpenicillin; trimethylsilyl derivative; high performance liquid chromatography; β-lactam antibiotics

Oxacillin is one of isoxazolylpenicillins effective in treating infections caused by β-lactamase producing S. aureus. In the course of pharmacokinetic studies on this drug, we have found an unknown metabolite peak appearing on the high performance liquid chromatogram of human urine excreted after oral administration of oxacillin (peak 2 in Fig. 1). This peak had the retention time between those of unchanged oxacillin and penicilloic acid, the latter being known as an inactive metabolite common to most of penicillins.

It has been known that isoxazolylpenicillins undergo biotransformation to active metabolites,[1] the structure of the metabolite for dicloxacillin being identified as 6-[3-(2,6-dichlorophenyl)-5-hydroxymethyl-4-isoxazolecarboxamido]-penicillanic acid.[2] It has been left uncertain, however, if the active metabolite of oxacillin may have a similar structure, although the retention behavior on a reversed phase thin layer chromatography implied the possibility of analogy.[3]

While we were preparing this paper, Thijssen manifested by means of mass spectrometry that the active metabolites for isoxazolylpenicillins in the rat are 5-hydroxymethyl analogues common to oxacillin, cloxacillin, dicloxacillin, and flucloxacillin.[4] This paper describes gas chromatographic–mass spectrometric (GC–MS) identification of the unknown metabolite of oxacillin found from human urine.

Oxacillin tablets were orally dosed to human subjects (250 mg×2 for each), and urine was collected 2—2.5 hours after administration. The urine specimen was acidified to pH 2.5 with use of ammonium sulfate/surfuric acid solution and extracted with dichloroethane.

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