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Multiple peptide synthesis on cotton carriers: Elucidation and characterization of an antibody binding site of CRF

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Introduction

The segmental carrier approach [1–4] has remarkably advanced the throughput in solid phase peptide synthesis, thus facilitating the preparation of large numbers of sequences required as tools for the investigation of protein-ligand interactions at the molecular level.

We have found cotton fabric a well suited cellulose segmental carrier with appropriate mechanical and chemical stability and functional groups for modification [5]. As alternative to DMAP we used N-methylimidazole (NMI) as catalyst for the acylation of hydroxyl groups.

For the determination of the 41 residue hypothalamic peptide hormone CRF in body fluids we have established a CRF-RIA. In order to elucidate the sites of the peptide which are recognized by the obtained antibodies we have synthesized seven overlapping CRF fragments altogether representing the whole sequence. Furthermore, we have prepared the alanine replacement set of the only sequence recognized by the antibodies in order to identify essential residues.

Results and Discussion

As carrier for all syntheses we used disks of cotton fabric which were first acylated by Fmoc-Gly (1.5–3.5/μmol/cm^2 or 0.08–0.18 mmol/g), then deprotected and acylated with an acid labile handle of p-alkoxybenzyl ester type [6]. The OH groups of the handle were then acylated with the starting amino acids (Fig. 1) using NMI instead of DMAP as catalyst. NMI was found to be superior to DMAP concerning both Fmoc-stability (no deprotection in 50% NMI/DMF during 20 h) and racemization (NMI: <1%, DMAP: 5–8%). Peptide syntheses were carried out following Fmoc/Bu^t strategy using bromophenol blue monitoring [7] to check course and completion of couplings.

Seven overlapping fragments of CRF were prepared: 1–10, 6–15, 11–20, 16–25, 21–30, 26–35 and 31–41, the C-terminal undecapeptide having been found the only sequence recognized by an anti-CRF-antiserum obtained by immunization

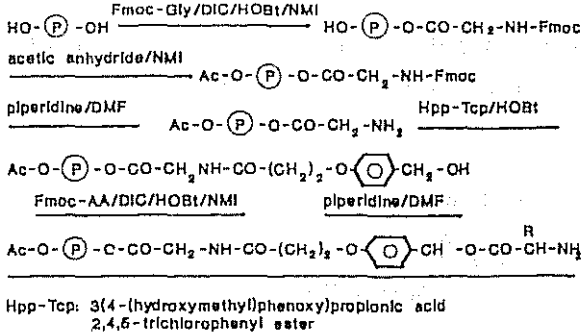

Fig. 1. Modification of the carrier.

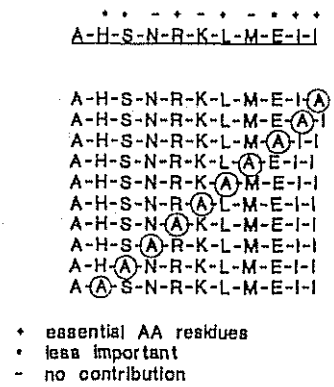

Fig. 2. Alanine replacement set of CRF (31–41).

of rabbits with human CRF coupled to porcine thyroglobulin byglutaraldehyde [8]. Furthermore, we have prepared the alanine replacement set of CRF (31–41) (Fig. 2) in order to elucidate residues essential for the antibody binding. Interestingly, the substitution of hydrophobic amino acids as Leu^{37}, Ile^{40} and Ile^{41} and of Arg^{35} drastically decreases the cross reactivity with the native sequence (based on the relation of the ED_{50} values, tracer: ^{125}I-Tyr^0-CRF) thus indicating the importance of these residues for the antibody binding. Glu^{37}, Ser^{33} and His^{32} are of lower influence and Asn^{34}, Lys^{36} as well as Met^{38} do not contribute at all.

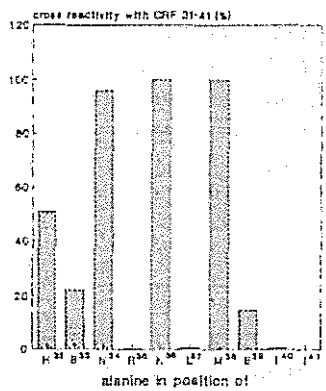

Fig. 3. Contribution of individual AA of CRF (31–41) to the antibody binding.

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