it a better choice of bulk gas than others to be present for electron-impact ionization of organic compounds.

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Studies on the Synthesis of Proinsulin. III. Synthesis of Polypeptides Related to the Connecting Peptide Segment of Bovine Proinsulin[1,2]

Sir:

The species variations in the primary structure of porcine,[3,4] bovine,[5-7] and human[8,9] proinsulin are located mostly in the connecting peptide portions and give rise to their unique immunological determinants.

We have previously prepared porcine proinsulin connecting peptide derivatives[10] which were as immunologically active as porcine proinsulin or the connecting peptide of natural origin when measured on an equimolar basis.[1,11] In addition, a rather small peptide fragment located in the central region of the connecting peptide was found to embody the full immunological activity of the complete connecting peptide.[1,11]

This communication describes the syntheses and immunological properties of [59-formyllysine]-bovine proinsulin$_{31-60}$ (I), possessing the entire sequence of the bovine connecting peptide portion, and [50-glycine, 52-alanine,59-formyllysine]-bovine proinsulin$_{31-60}$ (II), possessing the sequence based on an early publication.[5] Differences between I and II are substitutions of amino acids only at positions 50 and 52. Immunological activities of I and II were determined by two different methods with essentially the same results (Chart I).

Using the ethanol precipitation immunoassay[12] with [^{125}I]proinsulin as tracer and a guinea pig antiserum to bovine proinsulin, I cross-reacted with natural bovine proinsulin on an equimolar basis, while II reacted rather poorly (Figure 1). Similar results also were obtained using the double antibody method[13] with ^{125}I synthetic tyrosinated bovine connecting peptide[14] as tracer and a different guinea pig antiserum against bovine proinsulin. In this system, I cross-reacted

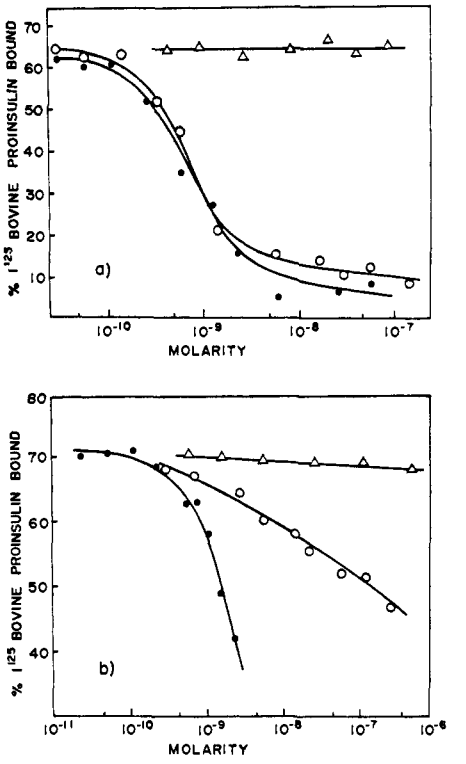

Figure 1. Ethanol precipitation immunoassays: (a) (●) bovine proinsulin, (○) synthetic peptide I, and (△) synthetic peptide III; (b) (●) bovine proinsulin, (○) synthetic peptide II, and (△) synthetic peptide IV.

Chart I

H-Arg-Arg-Glu-Val-Glu-Gly-Pro-Gln-Val-Gly-Ala-Leu-Glu-
31
Leu-Ala-Gly-Gly-Pro-Gly-Ala-Gly-Gly-Leu-Glu-
Gly-Pro-Pro-Gln-Lys(F)-Arg-OH
60

I

H-Arg-Arg-Glu-Val-Glu-Gly-Pro-Gln-Val-Gly-Ala-Leu-Glu-
31
Leu-Ala-Gly-Gly-Pro-Gly-*Gly*-Gly-*Ala*-Leu-Glu-
50 52
Gly-Pro-Pro-Gln-Lys(F)-Arg-OH
60

II

H-Gly-Pro-Gly-Ala-Gly-Gly-Leu-Glu-Gly-Pro-Pro-Gln-
47
Lys(F)-Arg-OH
60

III

H-Gly-Pro-Gly-*Gly*-Gly-*Ala*-Leu-Glu-Gly-Pro-Pro-Gln-
47 50 52
Lys(F)-Arg-OH
60

IV

(1) For part II see N. Yanaihara, T. Hashimoto, C. Yanaihara, M. Sakagami, and N. Sakura, *Diabetes*, **21** (Suppl. 2), 476 (1972).

(2) The amino acids except glycine are of the L configuration: DMSO, dimethyl sulfoxide; DMF, dimethylformamide; Z, benzyloxycarbonyl; OBu^t, *tert*-butyl ester; Boc, *tert*-butoxycarbonyl; F, formyl. R_f^1 and R_f^{11} values refer to the solvent systems: 1-BuOH–AcOH–H_2O (4:1:5) (upper layer) and 1-BuOH–pyridine–AcOH–H_2O (30:20:6:24), respectively. Samples for acid hydrolyses were dried *in vacuo* over P_2O_5 at room temperature, and acid hydrolyses were performed in constant boiling HCl at 110° for 48 hr in sealed tubes. Peptide contents are expressed by average recoveries of amino acids in acid hydrolysates based on formula weight.

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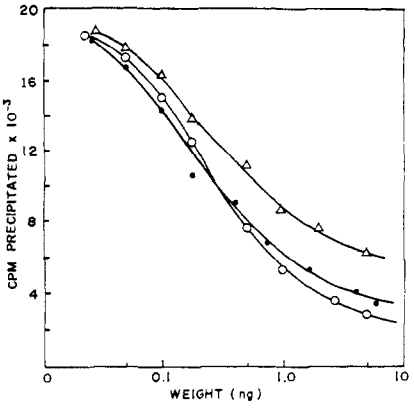

Figure 2. Double antibody immunoassays: (●) natural bovine C-peptide; (○) synthetic peptide I; and (△) synthetic peptide II. ^{125}I synthetic tyrosinated bovine connecting peptide was used as a tracer.

almost identically with natural bovine connecting peptide, while II failed to displace the tracer completely (Figure 2). Accurate evaluation of the cross-reactivity of II was difficult because of nonparallelism in the displacement curves of the synthetic peptide and the natural bovine proinsulin or connecting peptide standard. Formyl protection of lysine residue at position 59 was shown to have no effect on the immunological reactivity of the connecting peptide. Both peptide III and IV, which were used as the amino components in the fragment condensations, showed no cross-reaction with bovine proinsulin or the connecting peptide. Detailed immunological evaluation of the synthetic bovine connecting peptide and the related peptides will be published elsewhere.

It was clearly shown that full cross-reactivity of the bovine connecting peptide is obtained with the revised amino acid sequence. The distinct difference of immunological reactivity observed which was caused by an inversion of residues 50 and 52 seems to indicate that the 47–60 sequence is indeed involved in the immunological activity tested. The fact that synthetic peptide III was immunologically inactive could mean that only part of the antigenic determinant resides in the 47–60 sequence or that the whole 31–60 sequence is necessary for the determinant to acquire the conformation in which it is immunologically active with the particular antisera used. In addition, the present demonstration shows that the structural change in the sequence in which the antigenic determinant is located can be detected by the immunological technique when homogeneous synthetic peptides of definite structure are used. Further synthetic studies on the antigenic determinant of bovine connecting peptide are now under way.

The syntheses of I and II were accomplished by the Rudinger azide modification.[15] For the synthesis of I, the azide derived from Z-Arg(NO_2)-Arg(H^+)-Glu-Val-Glu-Gly-Pro-Gln-Val-Gly-Ala-Leu-Glu(OBu^t)-Leu-Ala-Gly-N_2H_2-Boc acetate trihydrate (V) [mp 228–230°; $[\alpha]^{25}D$ −35.1° (*c* 1.1 DMSO); R_f^I 0.45; R_f^{II} 0.75; amino acid ratios in acid hydrolysate, Arg + $Orn_{1.89}Glu_{4.04}Pro_{0.96}Gly_{2.91}Ala_{2.01}Val_{2.09}Leu_{1.98}$. *Anal.* Found: C 50.4; H, 7.2; N, 17.0] was coupled with III [$[\alpha]^{28}D$ −112.0° (*c* 1.0, 10% acetic acid); R_f^I 0.02; R_f^{II} 0.30; amino acid ratios in acid hydrolysate, $Lys_{0.94}Arg_{0.97}Glu_{1.92}Pro_{2.91}Gly_{5.18}Ala_{0.98}Leu_{1.09}$; peptide content 88%], which had been obtained by catalytic hydrogenolysis, followed by gel filtration on Sephadex G-10, of Z-Gly-Pro-Gly-Ala-Gly-Gly-Leu-Glu-Gly-Pro-Pro-Gln-Lys(F)-Arg(H^+)-OH acetate pentahydrate [mp 184–192°; $[\alpha]^{28}D$ −44.0° (*c* 1.0, DMF); R_f^I 0.19; R_f^{II} 0.59; amino acid ratios in acid hydrolysate, $Lys_{0.91}Arg_{0.93}Glu_{1.86}Pro_{3.09}Gly_{5.18}Ala_{1.06}Leu_{0.98}$. *Anal.* Found: C, 49.1; H, 7.0; N, 16.8].

The resulting crude protected triacontapeptide was hydrogenated to give a crude preparation of I, which was purified by chromatography on CM-Sephadex C-25 using ammonium acetate buffer as an eluent and desalted by gel filtration on Sephadex G-25 [$[\alpha]^{28}D$ −100.0° (*c* 1.0, 10% acetic acid); R_f^I 0.08; R_f^{II} 0.54; amino acid ratios in acid hydrolysate, $Lys_{0.96}Arg_{3.09}Glu_{5.70}Pro_{4.22}Gly_{8.21}Ala_{3.09}Val_{1.85}Leu_{2.88}NH_{3(2.41)}$; peptide content 87%].

The synthesis of II was carried out in the same manner as described above. The azide derived from V was coupled with IV [$[\alpha]^{25}D$ −117.5° (*c* 0.3, 10% acetic acid); R_f^I 0.05; R_f^{II} 0.28; amino acid ratios in acid hydrolysate, $Lys_{0.96}Arg_{0.92}Glu_{1.92}Pro_{3.08}Gly_{5.16}Ala_{1.07}Leu_{0.99}$; peptide content 91%], which was prepared by hydrogenation of Z-Gly-Pro-Gly-Gly-Gly-Ala-Leu-Glu-Gly-Pro-Pro-Gln-Lys(F)-Arg(H^+)-OH acetate trihydrate [mp 166–168°; $[\alpha]^{28}D$ −51.8° (*c* 1.0, DMF); R_f^I 0.17; R_f^{II} 0.53; amino acid ratios in acid hydrolysate, $Lys_{0.96}Arg_{0.92}Glu_{1.94}Pro_{3.03}Gly_{5.16}Ala_{1.02}Leu_{0.99}$. *Anal.* Found: C, 50.5; H, 7.2; N, 16.2]. The resulting mixture of crude materials was hydrogenated, and in the manner as described for I pure triacontapeptide II was isolated [$[\alpha]^{28}D$ −104.9° (*c* 0.5, 10% acetic acid); R_f^I 0.09; R_f^{II} 0.55; amino acid ratios in acid hydrolysate, $Lys_{0.94}Arg_{2.91}Glu_{6.07}Pro_{3.89}Gly_{8.10}Ala_{3.15}Val_{2.03}Leu_{2.91}NH_{3(2.46)}$; peptide content 90%].

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An Electron Spin Resonance Study of Some Group IVb Organometallic Peroxy Radicals

Sir:

Electron spin resonance spectroscopy (esr) has proved to be a useful technique for the study of the chain carrying peroxy radicals (ROO·) involved in the autoxidation of organic compounds.[1] For example, absolute

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