Ribonuclease segments A, S + core RBM 09/09

Additional Narrative for the Narrator: (pages follow the expanded version II E from 9/7/01; Frames follow Jim Pfeiffer's Version 1.0, from May 14). Bold typing indicates additional narration.

(10/09A version).

Yu Cong Du, one of the original young members of the insulin group in Shanghai, summarizes the early work of these Chinese laboratories.

The insulin story was a landmark chapter in the history of peptide chemistry.

The research on insulin has continued up to the present day.

Bruce Merrifield in the spring of 1962 reported at a Federation Meeting the general principle of carrying out peptide synthesis on a solid support.

In recent years Stewart has made effective antagonists of bradykinin that may become important drugs.

In 1964 Garland Marshall joined Merrifield laboratory as his first graduate student. He synthesized the hypertensive octapeptide, angiotensin, which extended the solid phase method to include new amino acids. He was the first to realize that a fragment synthesis was possible for the Solid Phase Peptide Method. And he felt strongly about the generality of the solid phase synthesis principle.

If significant amounts of uncoupled amino groups were still present, the machine would go on hold until the problem was corrected. Eventually this instrument laid the groundwork for later commercial machines. All these were discontinuous, batch machines like the originals. Soon, continuous flow machines were constructed. Initially they had the same problems that Merrifield encountered due to resin compression. But Shepard and Atherton overcome this difficulty by putting their polyamide resin inside a rigid porous zeolite cage. They built a photometric monitoring system to estimate the extent of coupling and deprotection. It was based on the uptake or release of the Fmoc group. This instrument was marketed by Pharmacia and by Millipore and became quite popular.

At this same time the exploratory research group of Merck, Sharp and Dome, under Robert Denkewalter, and Ralph Hirshman with the strong support of Max Tishler

undertook the synthesis of Rinonuclease S-protein 21 to 124 and obtained enzyme activity after combination with the S-peptide.

The Solid Phase approach enabled more detailed studies of the enzymatic properties of Ribonuclease A. Berndt Gutte, Michael Lin, and later Bob Hodges showed that the carboxyl terminal synthetic tetradecapeptide could be used to activate shortened Ribonuclease 1 to 118. They studied the role of different residues at the carboxyl terminus, much as Hoffman and Scoffone had done with the S-peptide at the amino terminus. They also constructed the first non-covalently bound 3-component protein ~~consisting of segments from Ribonuclease A sequence spanning amino acids 1 to 20, 21 to 118 and 111 to 124, which had good enzymatic activity.~~ consisting of ribonuclease A segments corresponding to S-peptide 1-20, C-peptide 111 to 124 and core protein 21 to 118. The complex had good enzymatic activity

This is considered to be the first *de novo synthesis* of a designed protein.

Fodor and his collaborators used photolithography to produce all sequences of a set of amino acids in a given peptide, each with a defined address on a plate.

Furka reported in 1990 the design of the "divide, couple, and mix" strategy for the combinatorial synthesis on beads of a mixture containing all possible combinations of a given size and fixed number of amino acids.

At about the same time Kit Lam independently devised a similar "split synthesis" strategy to solve the problem of making combinatorial libraries containing equimolar amounts of peptides.

It took another 25 years before major developments along these lines in organic synthesis were accomplished. An early example is a benzodiazopine library. In recent years K.C. Nicolau has applied the solid phase combinatorial synthesis techniques to the synthesis of more structurally complex, natural product-based libraries He points out: "Merrifield's pioneering work in solid phase chemistry revolutionized the field of peptide synthesis. The same philosophy of solid phase chemistry is now being implemented in the latest revolution in organic synthesis".

Eberle expressed the following opinion.

The current situation has been evaluated by Teresa Kubiak.

According to Arno Spatola:

Murray Goodman comments on the following:

According to Daniel Veber:

Charles Deber extends these ideas:

The importance of physical chemical methods was extended by Robert Hodges.

But Robin Offord points out

Tom Muir continues along these lines

As the last speaker on the panel, Victor Hruby, offers the final summary of the future of peptide science.

Kit Lam
video #

Lapsed time	Time from start	Text
	omit 2:05	
3min 2	11:57	2:07 Made a solution-phase library of peptides. 1987
3		2:08 They bound to an anti-peptide antibody, but couldn't retrieve a peptide. Probably because they
4		2:09 were not equimolar.
4:15	11:58	2:09:15 In 1989 while in his rocking chair he had (about) a Eureka (not) to get equal amounts
6		2:11 Use a split synthesis approach
7	12:01	2:12 about 30 minutes later I had another Eureka. I suddenly realized that each bead had only one peptide sequence.
8	12:02	2,13 after 9 months we had a proof of concept and
9		2:14 presented a paper at the peptide symposium in 1991
9:45 or 14	12:07 out of order →	2:14:45 or 2:19 Pleased to say that the origin of combinatorial chemistry was due to peptide chemists and solid phase peptide synthesis.
9:15 or 3	12:03	2:14:15 or 2:18 Over the last 10 years split synthesis was applied
0:30		2:15:30 to multiple receptors. Now developing assay method
1:52 15		2:17 or 2:20 can also be applied to proteomics.

James Team Video tape #

after Kent + Tam panel

TCR

(18:44) Because peptides and proteins (in their native state) contain many functional groups such as amines and carboxylic acids, it is difficult to form such a specific bond between two peptides.

(16:54) 9:47 Peptide ligation is a controlled chemical reaction to couple two peptides together to form at a specific site to form a specific bond.

17:34 It is a conceptually different method than the conventional (20:38) peptide synthesis methods

17:44 In conventional methods protecting groups, (20:55) and coupling methods have been used.

(19:17) Also it requires deprotection and sometimes gives low yields and the conditions are in organic solvents.

(21:04) Peptide ligation does not need any of these. One of the major advantages of peptide ligation is that it can be done in water (under) extremely mild conditions.

(1) Narrator — new narration

(2) as in video

(3) cellulose 6:28 - 7:28 (8:07) video #3

(4) Dow chemical beads 3 photos of beads reel #3 3:09:14

(5) new scheme — describe chemistry
... MANY INSERTS ... 6:9:45

(6) made tetrapeptide 3:10:44

(7) advantages — 3:17:20

(8) need name SPPS

(11) Presented Atlantic city
— LIMITATIONS ~100% reaction

(9) Envision flow system p. 24 of RBH old slide

(10) manual shaker — photo behind manual shaker

(12) Hutby

(13) John Stewart

(14) Garland — new introduction

(15) Manning

Panayotis Katsoyanis