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A NEW SUPPORT FOR POLYPEPTIDE SYNTHESIS IN COLUMNS

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An automated synthesis of polypeptides by the solid phase method (1) would be much easier if the support would always stay in a column. With the resins presently used, this is not possible and a batch process has to be applied because the different solvents necessary in a solid phase synthesis step cause variable swelling of the resin. Since the gel nature of the polystyrene-based resin is responsible for this property, we have tried to overcome these difficulties by using inorganic materials as supports. Alcohols can be esterified to the silanol groups on the surface of any type of silica (e. g. porous glass beads, silicagel, quartz) (2, 3). These modified supports have been successfully used in high speed chromatography (2, 3) and their properties show that the organic groups are located on the surface of the inorganic support, e. g., considered to stick out, somewhat like a brush having the desired functional group at the free end of the bristle. The mass transfer has to be faster than the rate of diffusion in the resins used up to now. In the case of polystyrene resins, it has been demonstrated that statistical failure sequences occur because the coupling of individual amino acids is incomplete (4-6). Every factor that increases the rate of reaction can improve the synthesis and extend the use of the solid phase method for synthesis of longer peptide chains with reasonable purity. The increase in speed of the mass transfer would be an important factor in this view. An increase of diffusion would enable shorter coupling times leading to faster synthesis. A real solid support material additionally prevents any ion exchange mechanism before or after the coupling step, which might be disadvantageous.

In order to obtain such materials suitable for column synthesis of polypeptides, we prepared the mono ester of 1.4-dihydroxy-methyl-benzene of silica (7). The ester bond is chemically stable in all anhydrous solvent systems used during a solid phase synthesis. The carboxylic

group of the first amino acid is connected to the free benzylic alcohol group by 1,1'-carbonyldiimidazole (CDI). Both the N,N'-dicyclohexyl carbodiimide (DCCI) procedure and the activated ester method (ONP) have been successfully used for the further coupling steps.
Brush type supports (Biopak (7)) for the column solid phase synthesis have been prepared with different contents of benzyl alcohol groups varying from 0.006 - 0.06 mMol/g.
The synthesis of various peptides in a specially designed synthesizer for column procedure shows that the reaction time can be reduced by a factor of at least two and that the products can be obtained with the same purity as by the common procedures. The time can most likely be reduced much more than by a factor of two if an instrument is designed allowing higher flow rates. The column synthesizer used in these investigations did not allow faster flow rates. In Table 1, some conditions used in these experiments are compared with the conditions in solid phase synthesis on resins.

TABLE 1 Comparison of Conditions for Peptide Synthesis with Biopak (7) and Resins

	Polystyrene Resin (Bio-Beads S-X2)	Biopak (7)
Esterification	48 hrs, 80 °C, Triethylamine separate reaction vessel	2 hrs, 20 °C, CDI column procedure
Capacities	0.1 - 0.5 mMol/g	0.006 - 0.06 mMol/g
Time required for one cycle	DCCI : 4 hrs ONP : 10 hrs	2 hrs 6 hrs
Automation	Batch procedure	Column procedure
Cleavage	HBr-CF_3COOH, 90 min	HBr-CH_3COOH, 20 min or HBr-CF_3COOH

For the synthesis of the dodecapeptide $(leu\text{-}ala)_6$ on Biopak (7), the same derivatives and reagents have been used as described (4) for the synthesis on resins. 4 g each of each three supports with different capacities (I. 0.006 mMol/g; II. 0.02 mMol/g; III. 0.04 mMol/g) were utilized and the following yields obtained after hydrolysis from the support: I. 12 mg, II. 85 mg, III. 210 mg. These peptides were purified by precipitation from trifluoroacetic acid with ether. The products obtained have been found to be identical to samples prepared by common resin procedure (4). The partial hydrolysate was checked for ala-ala and leu-leu sequences indicative of any failure sequences by the described combined gas chromatography-

mass spectrometry procedure (8). They were well beyond the figures found for resin synthesis (4, 8).

The tetrapeptide leu-leu-gln-gly, a partial sequence of secretin, was synthesized by the p-nitrophenyl ester method using a Biopak support with 0.05 mMol/g capacity.

These results suggest that the new support material for the solid phase method shortens the reaction time and enables an easier operation in a continuous or discontinuous column procedure. These real solid supports enable an easier scaling up of the synthesis in comparison to a batch procedure. Further advantages are: 1) no shaking is required, consequently no mechanical corrosion of the solid phase appears; and 2) a considerably smaller excess of the reagents is necessary to obtain the same equilibrium. Also, monitoring of the reaction might be possible by inserting thermistors into the column in order to measure the reaction heat.

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REFERENCES

(1) R.B. Merrifield, Federation Proc. 21, 412 (1962)

(2) I. Halász, I. Sebestian, Lecture at the 5th Int. Symposium on Advances in Chromatography, Las Vegas, Nev., Jan. 1969

(3) I. Halász, I. Sebestian, Angewandte Chemie, Internatl. Ed., 8, 453 (1969)

(4) E. Bayer, H. Eckstein, K. Hägele, W. König, W. Brüning, H. Hagenmaier, and W. Parr, J. Amer. Chem. Soc. 92, 1735 (1970)

(5) E. Bayer, H. Hagenmaier, W. Parr, H. Eckstein, G. Jung and P. Hunzicker, in E. Scoffone, Peptides 1969, North Holland Publ., in print

(6) E. Bayer, Chemie f. Labor u. Betrieb 3, (1969)

(7) The material is available under the name Biopak from Waters Associates, Framingham, Mass

(8) E. Bayer, and W. König, J. Chrom. Sci. 7, 95 (1969)