chloride (0.78 mmol), and a chloroform-insoluble solid (82.1 mg). The infrared spectrum of this solid had absorptions at 2245 (w), 1050 (vs), 870 (s), and 430 (m) cm^{-1}, in addition to weak bands from $HN(CH_3)_3^+Cl^-$. The X-ray powder photograph of the entire solid sample obtained by heating $HN(CH_3)_3^+SiCl_3^-$ at 100° was essentially identical with that of our authentic sample of $HN(CH_3)_3^+Cl^-$. Therefore, the solid products from the thermal decomposition of $HN(CH_3)_3^+SiCl_3^-$ were $HN(CH_3)_3^+Cl^-$ and a silicon–chlorine polymer.

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Incomplete Deblocking as a Cause of Failure Sequence in Solid Phase Peptide Synthesis

Sir:

The ninhydrin method[1] for measurement of the coupling efficiency of BOC-amino acids with Merrifield resin–peptides showed that the two octapeptides I

b 8 7 b 6 5 4 3 c 2 1
Tyr-Gly-Ser-Leu-Pro-Gln-Lys-Gly-Resin
I

b 8 7 b 6 b 5 4 n 3 2 1
Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Resin
II

13 n12 b11 b10 b9 b8 7 b 6 5 4 3 c 2 1
Ala-Arg-Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys-Gly-Resin
III

b13 b12 b11 10 b9 b8 7 t 6 5 4 c 3 2 1
Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Resin
IV

and II (where b = benzyl, c = carbobenzoxy, n = nitro, and t = tosyl) were synthesized with coupling efficiencies of 98–100%. Amino acid analysis on the octapeptide resins revealed that in both peptides the seventh amino acid was present in 30–50% yield and the eighth amino acid in 5–10% yield. Since the coupling was essentially complete, a possible explanation of these failures is incomplete deblocking of amino acids six and seven. In the examples cited, the Merrifield deblocking reagent,[2] fresh 50% TFA in CH_2Cl_2, was used for 30 min as recommended. Quantitative amino acid analysis on dried weighed resin at different stages of the synthesis showed no loss of peptide during the total synthesis. In any event, cleavage of peptide from the resin cannot explain these results.

The ninhydrin procedure was modified to measure the extent of removal of the BOC group. CH_2Cl_2-washed and dried resin (5–10 mg) was used in the assay. The reaction was carried out as previously described[1] on resin unknown, completely deblocked amino acid resin ester, and a glycine color standard. Solutions were diluted to 20 ml with 50% ethanol and read against a reaction blank at 570 mμ in a DB spectrophotometer. The yield was calculated as per cent of deblocking based upon completely deblocked amino acid resin ester.

When the synthesis of I was repeated, see III, the coupling efficiency was again 98–100%. Deblocking of the first five amino acids with reagent A, 50% TFA–CH_2Cl_2, reagent B, 5.6 *N* HCl–dioxane, or reagent C, an equivolume mixture of A and B, gave yields of 90% or better with one of these reagents, and 100% after deblocking with a second of these reagents. Amino acids which are deblocked completely in one or two trials with reagents A, B, or C will hereafter be referred to as normal. The sixth amino acid, BOC-serine, was deblocked only 70% after four trials with the reagents listed. Use of a new reagent which contained C plus an equivolume of HCl-saturated CH_2Cl_2 raised the yield to 95%. The seventh amino acid, BOC-glycine, was also difficult, but could be deblocked with three trials using the standard reagents A, B, and C.

The eighth amino acid, BOC-tyrosine, deblocked normally. However, the next residue, BOC-histidine, failed to deblock beyond 58% with repeated trials using all of the reagents previously employed. A new reagent, 5.35 *M* HCl–DMSO and CH_2Cl_2 (1:1), for 90 min gave 100% yield. The tenth residue, BOC-threonine, again required three trials using reagents A, B, and C. The next residue, BOC-threonine, deblocked normally.

The failure sequence II was also resynthesized, but for reasons not important here, it was decided to elongate the peptide C terminal by three residues. See IV. Coupling efficiency again was 98–100% throughout. Deblocking of the first four amino acids was normal. The fifth residue, BOC-tryptophan, was very difficult to deblock, but was finally accomplished by pretreating the resin with 1.5 *M* urea in DMF, washing with DMF and dioxane, and deblocking for 2 hr with reagent C. Mercaptoethanol was present in all solutions. The next two residues, BOC-arginine and BOC-phenylalanine, deblocked normally. However, the eighth residue, BOC-histidine, required three trials using standard reagents. Five additional residues coupled and deblocked normally.

Peptides I–IV illustrate the following points: (1) when glycine-resin-ester (III) was used in peptide synthesis, the most difficult residues to deblock were no. 6 and 9, residues less difficult were no. 7 and 10; (2) when valine-resin-ester (IV), was used in peptide synthesis, residue no. 5 was most difficult to deblock, residue no. 8 less difficult; (3) all other residues in all peptides deblocked normally; (4) the BOC-glutamic and BOC-methionine which were poorly deblocked in II were normal in IV, alternatively the BOC-tryptophan and BOC-histidine which were difficult to deblock in IV were normally deblocked in II. These findings indicate that the position of the amino acid in the peptide rather than the nature of the amino acid determined the difficulty in deblocking.

A plausible explanation for the difficulty in deblocking various BOC-amino acids is that the terminal amino group is sterically hindered. Since the reactions are carried out in organic solvents, one may visualize around this amino group a cage of hydro-

(1) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. L. Cook, *Anal. Biochem.*, 34, 595 (1970).
(2) B. Gutte and R. B. Merrifield, *J. Amer. Chem. Soc.*, 91, 501 (1969).

phobic amino acid side chains and protecting groups. Most of the improved deblocking procedures involve the use of more polar solutions. Unfortunately these solvents shrink the resin and tend to prevent reagent penetration into the resin. Therefore we have compromised to include CH_2Cl_2 in most reagents to minimize resin shrinkage. Our aim is to find a single deblocking reagent which succeeds on every step of the synthesis.

Since reporting on the difficulty in deblocking certain residues in I and III,[3] we have been informed of similar results in the synthesis of other peptides.[4] Our results illustrate the importance of measuring the efficiency of deblocking as well as coupling in solid phase peptide synthesis.

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(3) F. C.-H. Chou, R. K. Chawla, R. F. Kibler, and R. Shapira, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970.

(4) R. N. Piasio, Manager, Organic R&D, Schwarz/Mann, Orangeburg, N. Y., peronal communication.

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On Potential Model Systems for the Nitrogenase Enzyme

Sir:

Nitrogenase as isolated from *Azotobacter vinelandii*,[1] *Clostridium pasteurianum*,[2] and subsequently from other sources, consists of two proteins, one containing both molybdenum and iron (in the ratio of ~1:15) and the second containing iron only. When combined, these two proteins are capable of catalyzing the reduction of N_2 and a variety of other substrates, *e.g.*, azide,[3] cyanide,[4] acetylene,[5] nitrous oxide,[6] and methylisonitrile.[7] In the absence of added substrate, and to a lesser extent in its presence, hydrogen gas is evolved.[8] All reactions involve the concomitant hydrolysis of ATP.

A recent communication,[9] in which the reduction of some of these same substrates has been demonstrated, has led to the conclusion that the essential chemical processes catalyzed by the N_2-reducing enzymes occur at a molybdenum-containing binding site. However, some of our results, reported herein, from attempts to produce model systems based on iron for the nitrogenase enzyme, emphasize that sufficient evidence is *not* yet available to distinguish between molybdenum, iron, or a multimetal center as the active site of the enzyme.

The reaction mixture usually employed in the present work consisted of 0.25 mmol of iron(II) complex and 1–2.5 mmol of both substrate and reductant (sodium borohydride or sodium dithionite) in 10 ml of solvent (ethanol, water, or buffer) in a 35-ml bottle fitted with a serum cap. For reactions involving gaseous substrates (acetylene, ethylene, etc.), the reaction bottle was purged with the particular gas for 4–5 min, while the solid (potassium cyanide, sodium azide) or liquid (acetonitrile) substrates were introduced under an argon atmosphere before the addition of solvent which initiated the reaction. Parallel series of reactions were used; one series was followed by gas–liquid chromatography[10] of samples of the gas phase and the other (not for the hydrocarbon reduction) by continuously flushing with a slow stream of argon and trapping evolved ammonia in 1 *M* hydrochloric acid. Ammonia was determined on aliquots spectrophotometrically using Nessler's reagent after isothermal distillation.[8] These reactions have lead to the reduction of azide, cyanide, acetonitrile, acetylene, methylacetylene, and ethylene and low levels of reduction of dinitrogen also. Control reactions were conducted in all cases.

True catalytic activity was found in these iron systems, *i.e.*, more than 1 mol of substrate/mol of iron complex is reduced, a phenomenon not yet demonstrated in the molybdenum–thiol system.[9] For example, even using the known compound dichlorotetrapyridinoiron(II) as catalyst, sodium borohydride as reductant, and sodium azide as the substrate in ethanol, 4.5 mol of ammonia/mol of catalyst was obtained at 25° in 5 hr, at which time the reaction was stopped. It was also found that CN^- was reduced to methane plus a trace of ethane and ammonia (0.4 mol/mol of catalyst), and acetonitrile was reduced to ethane and ammonia under similar conditions by, for example, dichlorobis(*N*-phenyl-*S*-methyl-2-aminoethanethiol)-iron(II).[11] The rate of the latter reaction was slower by a factor of 10 than that for cyanide. Slower reductions of acetonitrile were also observed with the enzyme,[7] but here the difference was 500-fold and methane was the product. Acetylene and methylacetylene were slowly reduced to ethylene and propylene, respectively, with 5 and 3% of the initial gas converted to products in 4 hr at 25°, respectively. Ethylene was also utilized as a substrate (this hydrocarbon is not reduced enzymatically). Using a system consisting of 0.25 mmol of the same iron complex and 2.5 mmol of sodium borohydride in 10 ml of ethanol, ethylene (1 mmol) was completely reduced to ethane in 2 hr at 25°. When smaller amounts (~0.7 mmol) of $NaBH_4$ were used, only 40% of the ethylene was reduced in this time, but addition of a further quantity of reductant completed the reduction. If the system was then purged with ethylene, reduction would begin again at a similar rate to that observed initially. With a mixture of acetylene and ethylene, it was observed

(1) W. A. Bulen and J. R. LeComte, *Proc. Nat. Acad. Sci. U. S.*, **56**, 979 (1966).
(2) L. E. Mortenson, J. A. Morris, and D. Y. Jeng, *Biochim. Biophys. Acta*, **141**, 516 (1967).
(3) R. Schöllhorn and R. H. Burris, *Proc. Nat. Acad. Sci. U. S.*, **57**, 1317 (1967).
(4) R. W. F. Hardy and E. Knight, Jr., *Biochim. Biophys. Acta*, **139**, 69 (1967).
(5) M. J. Dilworth, *ibid.*, **127**, 285 (1966).
(6) R. W. F. Hardy and E. Knight, Jr., *Biochem. Biophys. Res. Commun.*, **23**, 409 (1966).
(7) M. Kelly, J. R. Postgate, and R. L. Richards, *Biochem. J.*, **102**, 1C (1967).
(8) W. A. Bulen, R. C. Burns, and J. R. LeComte, *Proc. Nat. Acad. Sci. U. S.*, **53**, 532 (1965).
(9) G. N. Schrauzer and G. Schlesinger, *J. Amer. Chem. Soc.*, **92**, 1808 (1970).
(10) An F and M 700 chromatograph with a 10 ft × 0.25 in. 80–100 mesh alumina column at 144° and a flame ionization detector was used.
(11) This and other new compounds used in this work have satisfactory elemental analyses.