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The Application of 0.1 *M* Quadrol to the Microsequence of Proteins and the Sequence of Tryptic Peptides†

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ABSTRACT: In an effort to extend automated Edman degradation to nanomole quantities of protein, the method of sequenator analysis described by Edman and Begg (Edman, P., and Begg, G. (1967), *Eur. J. Biochem. 1,* 80) was modified to permit long degradations in the absence of carrier proteins. By using an aqueous 0.1 *M* Quadrol program with limited, combined benzene–ethyl acetate solvent extractions, as well as a change in the delivery system for heptafluorobutyric acid, it was possible to recover and identify the first 30 amino acid residues from a sequenator run on 7 nmol of myoglobin. For 3 nmol of myoglobin, 20 steps could be identified. PTH-amino acids were identified by gas–liquid chromatography and thin-layer chromatography on polyamide sheets. Without using a carrier protein in the cup to prevent mechanical losses (Niall, H. D., Jacobs, J. W., Van Rietschoten, J., and Tregear, G. W. (1974), *FEBS Lett. 41,* 62), the repetitive yield using this program was 93–96%. The same program has been applied successfully to peptides of 14 or more residues with or without modification by Braunitzer's reagent and to a number of larger peptides and proteins including a 216 residue segment of rabbit antibody heavy chain in which a sequence of 35 steps was accomplished on 25 nmol.

The availability in only trace amounts of many proteins of biological interest and recent developments in the detection of subnanomole quantities of phenylthiohydantoin[1] amino acid derivatives (Summers et al., 1973; Niall et al., 1974b) make it desirable to have simple automated techniques for extended Edman degradation on nanomole quantities of proteins. When proteins in amounts less than 50 nmol are subjected to automated Edman degradation in the protein sequenator, the repetitive yields are poor because of increasing mechanical losses during solvent washes. Niall et al. (1974a) have demonstrated that such losses may be decreased by using synthetic, long polar polypeptide carriers containing amino acids that would not interfere with identification of the unknown sequence. We report here a program for automated sequence analysis that permits extended degradations on less than 10 nmol of protein by using conventional identification systems (Summers et al., 1973; Pisano and Bronzert, 1969) without the use of a carrier. This program was originally developed for use in the automated sequencing of short tryptic peptides and, with minor modifications, was found to facilitate microsequence technique on larger peptides and proteins. These modifications are directed toward the problems of losses caused by solubility of the remaining peptide in extracting solvents and the mechanical losses occurring with nanomole quantities of protein. They include the use of aqueous 0.1 *M* Quadrol[2] buffer and a change in the delivery scheme for heptafluorobutyric acid in a single cleavage program.

Experimental Procedure

Reagents and Solvents. Phenyl isothiocyanate, heptafluorobutyric acid, benzene, *n*-chlorobutane, and ethyl acetate were obtained from Beckman Instruments, Inc. Hexane, Quadrol, butanedithiol, and 1-propanol are Sequanal grade purchased from Pierce Chemical Co.

Reagent 1 is phenyl isothiocyanate, 2.5% v/v in hexane.

Reagent 2 is 0.1 *M* Quadrol in 1-propanol–distilled water (3:4, v/v) adjusted to pH 9.0 with trifluoroacetic acid.

Reagents 4 and 5 are heptafluorobutyric acid and N_2, respectively.

Solvent 1 is benzene–ethyl acetate, 1:1 v/v.

Solvent 3 is *n*-chlorobutane containing 10^{-5}% v/v butanedithiol.

Sperm whale apomyoglobin and fully reduced and alkylated porcine insulin A and B chains were obtained from Schwarz/Mann.

Modification of Lysine-Containing Peptides. A 20-fold molar excess of SO_3PhNCS[3] (Pierce Chemical Co.) (Braunitzer et al., 1970) was added to the peptide and was dissolved in 0.2 ml of Me_2allylamine buffer (Beckman Instru-

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[1] Abbreviations used are: PTH, phenylthiohydantoin; SO_3PhNCS, 4-sulfophenyl isothiocyanate (Braunitzer's reagent); Me_2allylamine, dimethylallylamine; F_7butyric acid, heptafluorobutyric acid.

[2] Quadrol, *N,N,N',N'*-tetrakis(2-hydroxypropyl)ethylenediamine.

[3] Recently we have found an eightfold molar excess of Braunitzer's reagent to be sufficient for coupling; a larger excess results in a greater decrease in yields in the first several sequence steps because of residual reagent in the cup.

Table I: Sequenator Programs for Use with Microsequence of Proteins and Short Tryptic Peptides.[a]

Function	Program Step Time (sec)		Cup Speed (rpm)
	Protein Microsequence	Peptides	
Reagent 1 delivery (phenyl isothiocyanate)	4	4	1800
Restricted vacuum	60	60	1800
N_2 dry	30	30	1800
Reagent 2 delivery (0.1 *M* Quadrol)	14[b]	14	1800
Coupling reaction—high	300	300	1800
Coupling reaction—low	900	900	1200
Restricted vacuum	400	800	1200
Rough vacuum	200	200	1200
Fine vacuum + N_2	300	500	1200
Solvent 1 precipitation (benzene–ethyl acetate)	120	120	1800
Solvent 1 extraction	600	300–500[c]	1800
Restricted vacuum	300	300	1800
Rough vacuum	100	100	1800
Fine vacuum + N_2	600	600	1800
Reagents 4 and 5 vent	30	30	1200
Reagents 4 and 5 pressurize	30	30	1200
Reagent 4 delivery	3	3	1200
Reagent 5 delivery	6	6	1200
Cleavage reaction	180	180	1200
Rough vacuum	60	40	1200
Fine vacuum + N_2	30	30	1200
Solvent 3 precipitation (*n*-chlorobutane)	120	120	1800
Solvent 3 extraction	200 (~8 ml)	150 (~6 ml)	1800
Restricted vacuum	200	200	1800
Rough vacuum	100	100	1800
Fine vacuum	600	600	1800

[a] Some of the venting and pressurizing steps are not shown. The total cycle time is approximately 95 min for the microsequence program and 100–105 min for the peptide program. [b] Fourteen seconds in our machine delivers buffer up to the undercut (18 mm) in the cup. [c] In general, we suggest a 300-sec solvent 1 extraction for short (less than 15 residues) peptides and 500 sec for longer peptides. It is recognized that choice of the shorter extraction time would also depend on the relative hydrophobicity of the residual peptide.

ments Inc.). Coupling was performed at 50° for 30 min, followed by the addition of 20 μl of triethylamine (Pierce Chemical Co.) with further incubation for 10 min. The reaction mixture was then extracted twice with 0.4 ml of benzene and lyophilized for 30 min. The dried SO_3-PheNCS-coupled peptide was then transferred to the sequenator cup and the automated program was begun at the cleavage step with F_7butyric acid.

Sequenator. The sequenator used in these experiments was a Beckman sequencer, Model 890B, with nitrogen blowdown and an undercut cup adaptation (Beckman Kit 336481). One notable design change was effected: the coupled function involving reagent 3 (F_7butyric acid) and solvent 3 (*n*-chlorobutane), which are delivered through a common delivery valve in the typical Beckman instrument, was altered so as to maintain F_7butyric acid in an isolated delivery scheme (Waterfield et al., 1970). By switching F_7butyric acid from reagent 3 to reagent 4 and using nitrogen pressure from the reagent 5 reservoir, a brief 150-λ pulse of F_7butyric acid can be delivered to the cup. Reagents 4 and 5 vessels share a common delivery valve in the Beckman sequencer. The reagent 5 container is used solely for nitrogen to deliver and clear F_7butyric acid from delivery line 4. In our machine, a 3-sec delivery of F_7butyric acid and a 6-sec delivery of nitrogen are sufficient to completely clear this amount of F_7butyric acid from the line into the cup.

Programs. The generalized programs for use in microsequence of proteins and with peptides are shown in Table I. The only major differences between the two programs are the more abbreviated solvent extractions and the lengthened drying times for peptides. Both programs employ a decreased concentration of quadrol in the buffer, which permits abbreviated solvent extractions (Niall, 1973). The samples were dissolved in 0.5 ml of distilled, deionized water containing 2% triethylamine, applied directly to the cup at high speed, and dried by application first of restricted vacuum and then high vacuum. Coupling was initiated at a cup speed of 1800 rpm; the speed was reduced to 1200 rpm to lower the protein film. Coupling for 20 min at 55° was found to be sufficient.

Identifications of PTH-Amino Acids. The phenylthiazolinone amino acids in the chlorobutane extracts were completely dried and maintained overnight in the fraction collector at 3°. PTH-norleucine was added to each tube as an internal standard. Samples were converted to the PTH by the addition of 0.2 ml of 1 *N* HCl and heated at 80° for 10 min (Edman and Begg, 1967). Two 1-ml extractions with ethyl acetate were sufficient to extract the PTH.

PTH derivatives of alanine, dehydroserine, glycine, valine, dehydrothreonine, proline, leucine, isoleucine, methionine, and phenylalanine were identified by gas–liquid chromatography using 10% SP 400 on acid-washed Supelcon (Supelco, Inc.). Analyses were performed with a Varian series 2800 gas chromatograph equipped with a multilinear temperature programmer. The temperature increased from 190 to 290° during an 11-min period. All of the Varian columns were 2 mm i.d. × 3 ft of silanized glass. With a well-

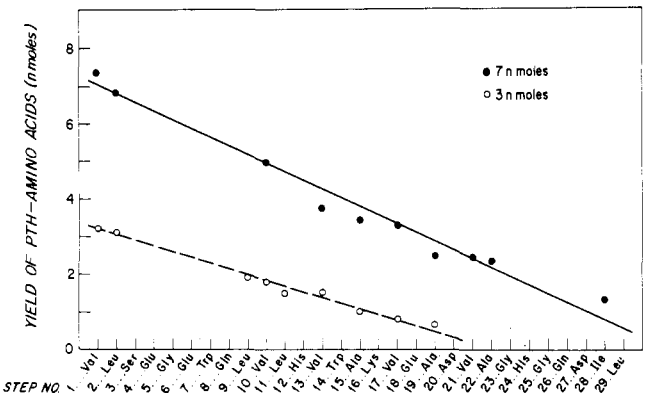

FIGURE 1: Yields of stable PTH-amino acids found on gas-liquid chromatography from sequenator run using 0.1 *M* Quadrol microsequence program on 7 (●) and 3 (○) nmol of sperm whale apomyoglobin.

conditioned column, 0.1–0.5 nmol of PTH could be identified consistently.

The PTH derivatives of asparagine, glutamine, lysine, tyrosine, and tryptophan were identified by using 3% OV-25 on finely deactivated Supelcon. Acid-washed Supelcon was further acid washed (Pisano et al., 1972) for 24 hr in concentrated HCl, then washed with distilled and deionized water, defined, and silanized twice with 5% dimethylchlorosilane (Eastman Organic Chemicals) in toluene. A Varian 1520 series gas chromatograph was used with a linear temperature program ranging from 240 to 310° during a 12-min period. It was possible with this system to identify 0.1–0.5 nmol of PTH-tyrosine and PTH-tryptophan and 2.0 nmol of ϵ-PTH-lysine; 1–2 nmol of PTH-asparagine and PTH-glutamine could be identified. Sensitivity with respect to PTH derivatives of amides varied depending on the accumulation of nonvolatile salts in the front of the column.

Direct on-column silylation with *N,O*-bis(trimethylsilyl)acetamide on 10% DC-560 was used primarily to identify the PTH derivatives of aspartic and glutamic acids and to distinguish leucine from isoleucine. Consistent identification of silylated PTH derivatives of aspartic and glutamic acids proved difficult from samples less than 2 nmol. Separation of leucine from isoleucine was effected by silylation at levels above 0.2 nmol. Isoleucine gives a split peak on DC-560 when silylated because of the formation of some alloisoleucine (Niall, 1973).

All gas chromatographic analyses were supplemented by two-dimensional thin-layer chromatography on polyamide

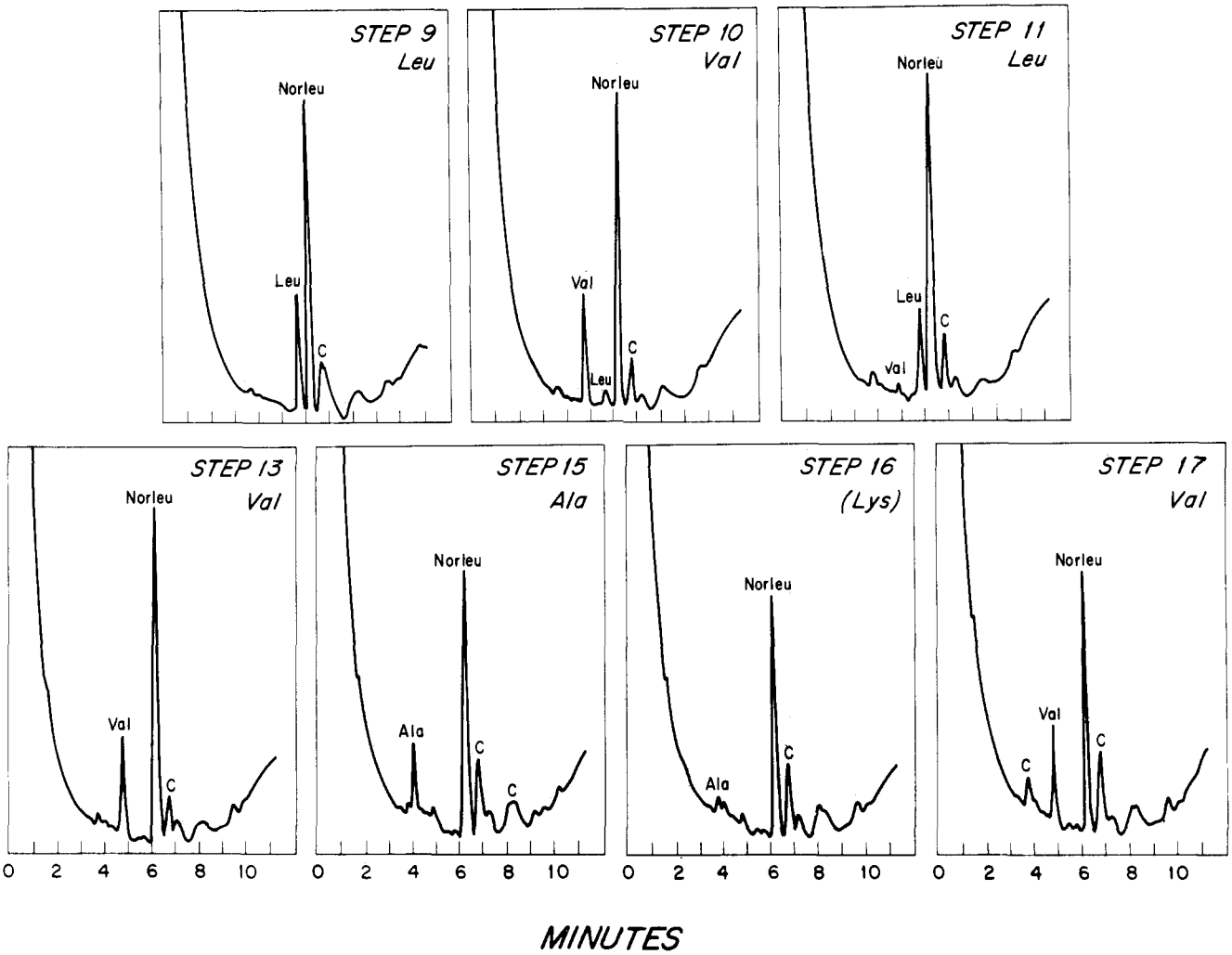

FIGURE 2: Representative gas-liquid chromatographic tracings of PTH-amino acid derivatives on SP-400, from sequenator analysis of 3 nmol of sperm whale apomyoglobin. Norleucine was added to each sample prior to conversion; 5 nmol was used for steps 1–13, and 3 nmol was used thereafter. The degree of overlap at steps 10, 11, and 16 may be appreciated by noting the relative areas of leucine, valine, and alanine from steps 9, 10, and 15, respectively. Lysine at step 16 is not detected on SP-400, but was detected on OV-25. "C" indicates contaminant peak. Ordinate is mass response of the gas chromatographic detector in arbitrary units.

Table II: Large Peptides Sequenced with the 0.1 *M* Quadrol Microsequence Program.

Peptide	Amount (nmol)	Approximate Length in Residues	Successive Steps Identified	Average Repetitive Yield (%)
CNBr peptide C_1 from H chain of rabbit anti-S8[a] 3322A	25	216	35[b]	95
CNBr peptide C_1 from H chain of rabbit anti-S3[a] 3381	40	253[c]	36	95
Tryptic V_H peptide from rabbit anti-S3[a] 3381	[d]	123	27	93
Rabbit anti-S3[a] 3381 L chain	80	215	47[e]	97
Rabbit anti-S3[a] 3374 L chain	150	215	58[f]	95
Glucose-6-phosphate dehydrogenase[g]	35	490	25[h]	95
Sperm whale apomyoglobin[i]	7	153	30	96
Sperm whale apomyoglobin[i]	3	153	20	94

[a] S3, pneumococcal polysaccharide Type III; S8, pneumococcal polysaccharide Type VIII. [b] Residue 15, arginine by homology, was not identified; residue 29 was not identified. [c] This peptide was CNBr cleaved at methionine-34. A disulfide bridge between the pyrrolidonecarboxylic acid blocked amino terminal fragment and the other fragment was not reduced. [d] The first step yield was 36 nmol of leucine. The amount of peptide analyzed was not determined in this instance. [e] The identification at step 44 is uncertain; leucine/isoleucine found at steps 46 and 47 were not resolved by silylation. [f] A proline at step 45 was not unequivocally identified. Leucine/isoleucine found at steps 48 and 49 were not resolved by silylation. Arginine known to be at step 51 was not identified. [g] A gift of Dr. D. Fraenkel. [h] Step 22 was either histidine or arginine. [i] Present study.

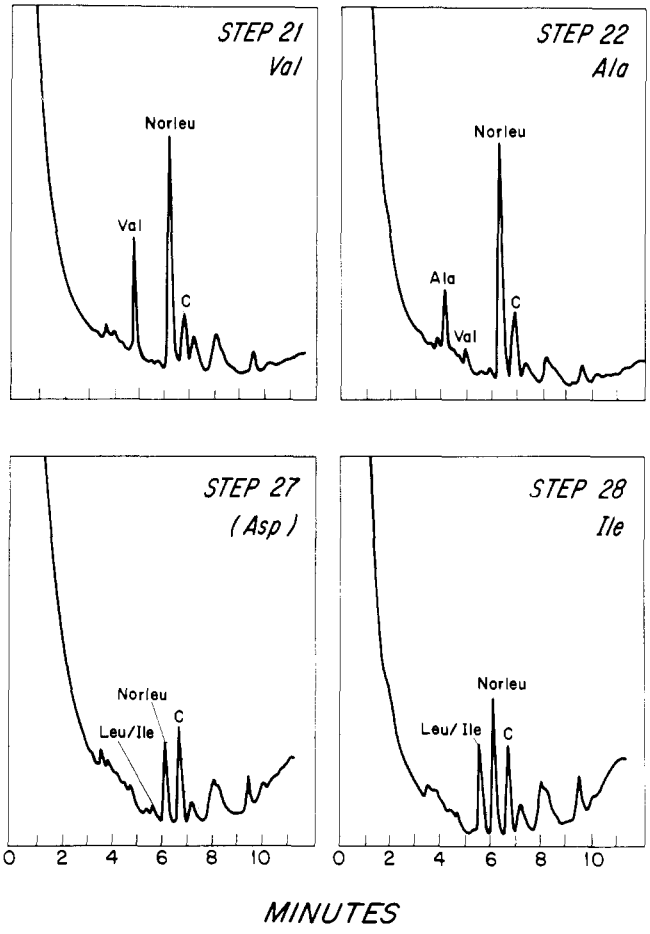

FIGURE 3: Gas-liquid chromatographic tracings on SP-400 of PTH-amino acids from the sequenator analysis on 7 nmol of sperm whale apomyoglobin. Norleucine (5 nmol) was used as internal standard in steps 21 and 22; 2 nmol of norleucine was used in steps 27 and 28. The attenuation of the detector mass response in steps 27 and 28 was half of that in steps 21 and 22. The degree of overlap and background may be appreciated by comparison of valine at step 21 and 22, and leucine/isoleucine at steps 27 and 28. Aspartic acid is not detected on SP-400, but was detected on polyamide sheets. "C" indicates contaminant peaks.

sheets by the method of Summers et al. (1973) or, for larger samples, by thin-layer chromatography on silica gel plates (Analtech, Inc.) according to Edman (1970). For the protein microsequence work, 10–40% of the total sample was used for gas chromatography and 20–40% for thin-layer chromatography on polyamide sheets. The latter method proved invaluable in the identification of less than 0.1 nmol of the PTH derivatives of aspartic and glutamic acids, asparagine and glutamine, and lysine derivatives that often proved difficult to detect at these levels by gas chromatography. The identification of PTH-histidine and PTH-arginine in the aqueous phase became a problem when the working sequence was less than 4–5 nmol based on the presence of easily quantified residues.

Results

The yields of PTH derivatives from two separate sequenator runs using the 0.1 *M* Quadrol protein microsequence program on 7 and 3 nmol of sperm whale apomyoglobin are shown in Figure 1. The repetitive yields were calculated from gas chromatographic analysis by using the more stable PTH derivatives of valine and leucine. All repetitive yields found for these nanomole amounts of protein were regularly between 93 and 96%. These calculations are based only on the in step residues without adding the overlap from the succeeding step. The quantitations were corrected for detector response differences directly against norleucine, which was used as a standard, and adjustments were made for successive overlap and cumulative background. The degradations were not protected by the addition of a synthetic carrier such as poly(norleucylarginine) as described by Niall et al. (1974a).

Figure 2 shows representative gas chromatographic tracings from the degradation of 3 nmol of myoglobin. All of the residues except histidine-12 were identified through step 20. Identifications for the 7-nmol run were completed through step 30 except for histidine-24. Several scattered residues beyond leucine-30 could be detected on polyamide sheets. This compares favorably with the results of Niall et al. (1974a) in which the sequence on 6 nmol in the presence

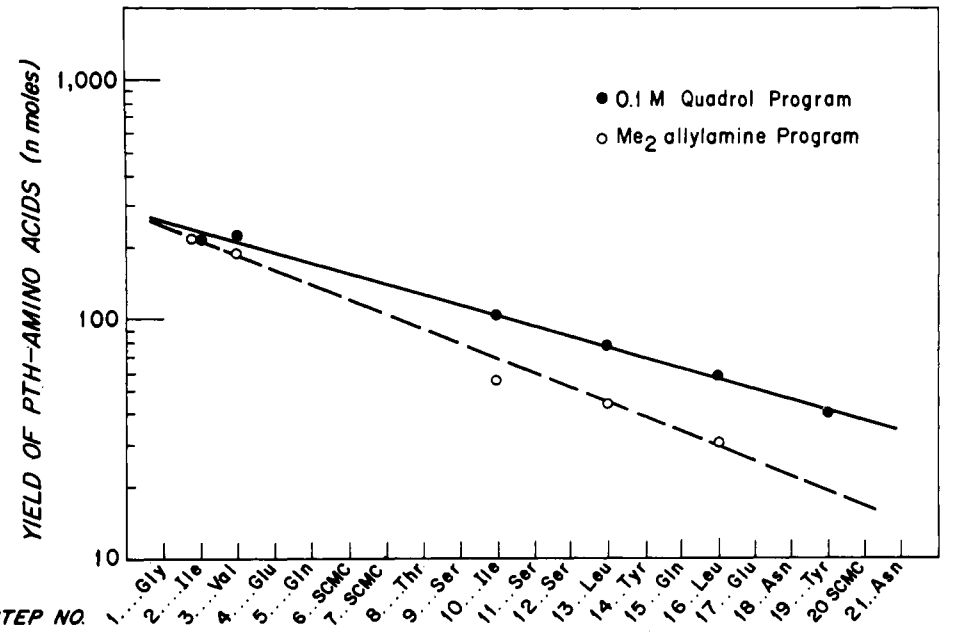

FIGURE 4: Yields of stable PTH-amino acids by gas-liquid chromatography from sequenator analysis on 0.3 μmol of porcine insulin A chain using the 0.1 *M* Quadrol program (●) and the Me_2allylamine program (○). At step 16 (leucine) the yield on the 0.1 *M* Quadrol program was 68 nmol, and the yield using the Me_2allylamine program was 32 nmol.

of carrier extended 21 steps, while the sequence on 10 nmol in the absence of carrier extended 11 steps.

As shown in Figure 3, the successive overlap at step 22 for the valine that was identified at the previous step is approximately 18%. This corresponds to less than 1% out-of-step degradations per cycle. The successive overlap based on averaging leucine-3, tryptophan-8, leucine-20, valine-11, valine-14, valine-22,[4] and alanine-23[4] for the several degradations was between 0.7 and 0.9% per cycle. In these myoglobin degradations, the background never became a significant factor. In Figure 3 it may be seen that the leucine–isoleucine background at step 27 is approximately 14%. These results are comparable (in terms of background and overlap) to prior runs with the 1 *M* Quadrol program using ten times the amount of protein (Beckman Program 050972).

Table II lists several large peptides and proteins examined with the microsequence program. It should be noted that even a chain of approximately 490 residues can be sequenced for 25 steps before the background hinders identification.

In Figure 4 the yields are shown for the completed sequence on 0.3 μmol of porcine insulin A chain determined with the 0.1 *M* aqueous Quadrol peptide program reported here, and by a single cleavage aqueous Me_2allylamine program using the same F_7butyric acid delivery scheme. Although a complete sequence was obtained in both instances, the repetitive yield using the Me_2allylamine program was lower in this experiment (85%) in comparison to the 0.1 *M* Quadrol program (91%). In addition, greater successive overlap per cycle (approximately 4%) occurred when using Me_2allylamine, whereas less than 2% per cycle was found for 0.1 *M* Quadrol.

Figure 5 contrasts the gas chromatography yields in the sequencing of native and modified porcine insulin B chain when using the 0.1 *M* Quadrol program. With 0.35 μmol of insulin B, the sequence was complete through phenylalanine-25. However, a rapid decline in yield occurred after the cleavage of arginine-22, presumably because of the increased solubility of the remaining peptide in the extracting solvents. The repetitive yield was 93–94% through step 18. This run was repeated on 0.28 μmol of insulin B chain after coupling with SO_3PhNCS (see Experimental Procedure). This permitted a completed sequence to be obtained through proline-28 without the falloff in yield that is seen in the absence of Braunitzer's reagent. The repetitive yield was 96–97% beyond step 11. Because of the known suppression of extractive yields in the first several steps in the presence of residual SO_3PhNCS, a repetitive yield calculation made by using the early steps would have been falsely high.

Another example of the application of the 0.1 *M* Quadrol peptide program is shown in Figure 6. This is a tryptic peptide from the constant region of a homogeneous rabbit antipneumococcal antibody light chain 3315 (M. N. Margolies, et al., 1975, manuscript in preparation). This peptide is 14 residues in length and ends in arginine. A complete sequence was obtained from 0.25 μmol of peptide, the last step being identified in the amino acid analyzer.

Discussion

The use of an aqueous 0.1 *M* Quadrol program has potentially broad utility. A major application is in the sequencing of proteins that are available only in nanomole quantities. It also shows considerable promise in degradations on tryptic peptides, especially when the carboxyl terminal lysine has been reacted with SO_3PhNCS. Quadrol was chosen as the coupling buffer primarily for the consistently superior repetitive yields afforded in comparison to Me_2allylamine. When Quadrol is used in low concentration, it can be removed efficiently in abbreviated solvent extractions, and does not form an impenetrable film under a strong vacuum (Niall, 1973). We have found that a combined extraction using equal volumes of ethyl acetate and benzene does not result in excessive extractive losses when compared to the Me_2allylamine program. The mixture appears to penetrate the protein or peptide film satisfactorily and no apparent accumulation of side products occurs. In

[4] Only in the 7 nmol run.

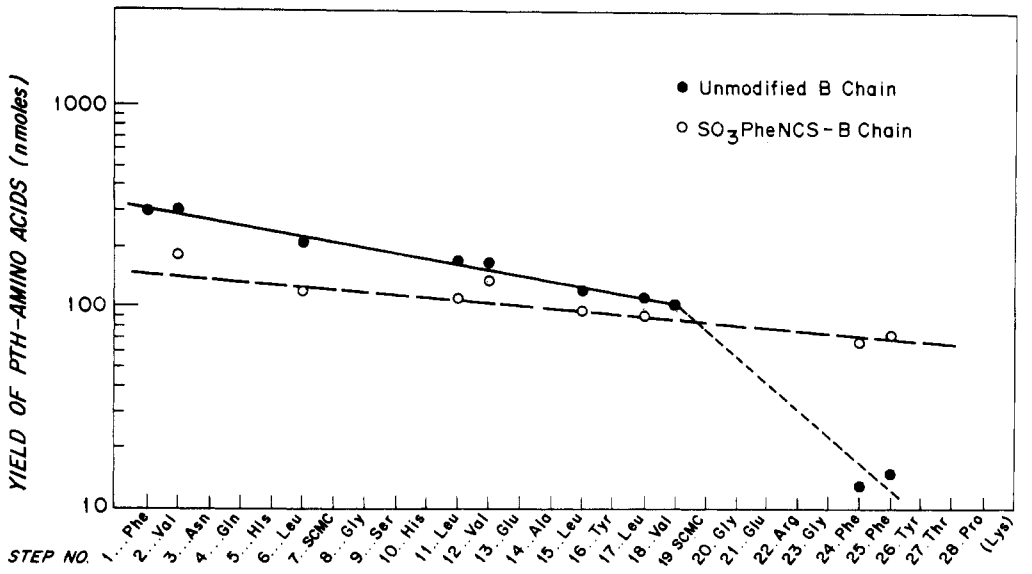

FIGURE 5: Yields of stable PTH-amino acids by gas–liquid chromatography from sequenator analysis using the 0.1 *M* Quadrol peptide program on 0.35 μmol of unmodified porcine insulin B chain (●), and 0.28 μmol of porcine insulin B chain modified with SO_3PhNCS(○).

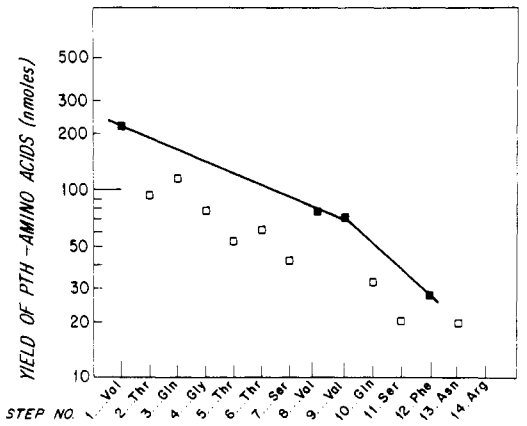

FIGURE 6: Yields of PTH-amino acids from sequenator analysis of 0.25 μmol of a 14 residue peptide from the constant region of b_4 rabbit antipneumococcal antibody light chain (3315). The line is plotted on the basis of yields of the stable PTH-amino acids (■); those PTH-amino acids known to be less stable are indicated by (□).

direct comparison with Me_2allylamine as a coupling buffer, the low concentration Quadrol program had fewer spurious contaminating peaks in the gas chromatography tracings and a lack of extraneous spots on the sensitive thin-layer polyamide sheets.

We have modified our sequenator so as to separate F_7butyric acid delivery from chlorobutane delivery (see Experimental Procedure). Because a common line is not used, contamination with acid during extraction of the thiazolinone is prevented. In addition, the F_7butyric acid delivery line is empty of acid during the vacuum stage after cleavage by virtue of the nitrogen delivery scheme. It is possible that residual F_7butyric acid was the major cause of "mechanical extraction" because of partial solution of the sample, which causes rapid fall in yields when degradation of nanomole amounts of protein is attempted (Niall et al., 1974a). When using the delivery scheme reported here, the fluoro acid is completely removed, and consequently we have not found it necessary to use carrier proteins to prevent losses of material.

It was expected that the absence of residual acid might cause some extraction problems, especially with polar amino acids. In over 100 sequenator experiments performed by using this delivery scheme, a slight improvement in the recovery of polar amino acids has been seen. Smithies et al. (1971) have reported improved recoveries of histidine and arginine by raising the cup temperatures to decrease residual fluoro acid. The 2-min preincubation in *n*-chlorobutane that immediately precedes extraction has been sufficient to penetrate the film for adequate extraction of the thiazolinone.

In our experience, single cleavage of the phenylthiocarbamyl derivative with F_7butyric acid has proven to be adequate, as reported previously by Hermodson et al. (1972). With extended sequences on proteins, the additional F_7butyric acid in a second cleavage appears to increase the background by nonspecific cleavage along the protein chain. The degree of overlap did not become a significant problem during 58 cycles of degradation on 150 nmol of intact light chain from a homogeneous rabbit antipneumococcal antibody 3374 (see Table II) (M. N. Margolies, et al., 1975, manuscript in preparation) using this single cleavage 0.1 *M* Quadrol program. Recovery of the more labile PTH derivatives of serine, threonine, and tryptophan late in the sequence is also improved in comparison with the double cleavage program. The indole ring of tryptophan is attacked by F_7butyric acid and is progressively destroyed during long degradations. Niall (1973) has reported that tryptophan, at position 35 in a protein, could not be identified by using a double cleavage program. We have successfully identified tryptophan at steps 36–38 in four different intact rabbit light chains (E. Cannon et al., 1975, manuscript in preparation; F. Chem, unpublished data; M. N. Margolies et al., 1975, manuscript in preparation) and at step 40 on rabbit b_4 C region (M. N. Margolies et al., unpublished data).

The lack of appreciable cumulative background and low successive overlap per cycle are perhaps the most attractive features of the method reported because additional identifications can be made with highly sensitive two-dimensional thin-layer chromatography. With long proteins during ordinary automated degradation, the utility of polyamide may become limited after approximately 25 cycles because of increasing background. However, with the 0.1 *M* Quadrol microsequence program, we have been able to make identifica-

tions using polyamide sheets through step 35 during a degradation on 40 nmol of a 250 residue fragment from rabbit heavy chain 3381 (Table II) (M. Rosemblatt et al., 1975, manuscript in preparation). Selected residues may be detected in long runs depending on their background level. For example, a lysine at step 41 from degradation of 90 nmol of a citraconylated tryptic peptide from rabbit light chain, 150 residues in length, could easily be identified on polyamide sheets (M. N. Margolies, unpublished data).

Obtaining lengthy sequences on small amounts of material is an obvious advantage in the structural studies of proteins. These results reported here have permitted the regular application of the 0.1 *M* Quadrol program to sequence analyses employing 10–30% of the material previously required.

Acknowledgments

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Incorporation of Fluorotryptophans into Proteins of *Escherichia coli*†

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ABSTRACT: A tryptophan-requiring strain of *Escherichia coli* can go through two doublings of optical density after L-tryptophan is replaced in the medium by 4-fluorotryptophan, during which the fluoro analog displaces ~75% of the L-tryptophan in cell protein. One doubling occurs in the presence of 5- or 6-fluorotryptophan, with 50–60% replacement of L-tryptophan by analog. When β-galactosidase is induced at the time of addition of analog, it reaches 60% of the control specific activity in the presence of 4-fluorotryptophan, 10% of normal in the presence of 5- or 6-fluorotryptophan. Lactose permease activity is 35% of the control in the presence of 4- and 6-fluorotryptophan, less than 10% in the presence of 5-fluorotryptophan. D-Lactate dehydrogenase shows a specific activity twice that of the control in the presence of 4-fluorotryptophan, one-half with 5- or 6-fluorotryptophan. Thus fluorotryptophan can be incorporated into proteins and affect their activities, although the nature and magnitude of the effect cannot be predicted for any given enzyme. Such substituted proteins should be useful for the study of protein structure and function by ^{19}F nuclear magnetic resonance and other techniques.

In order to understand structure–function relationships in proteins, one needs to correlate the dynamics of protein–ligand interactions and subunit–subunit interactions at the atomic level with the structural information provided by X-ray diffraction studies. An ideal approach to investigate such problems is to introduce some kind of "probe" selectively into the system which will not significantly perturb the host and which can provide spectroscopic signals that are comparatively free from interference. A number of spectroscopic probes (such as the attachment of spin-labels and/or ^{13}C or ^{19}F nuclei and/or fluorescent dyes to selected amino acid residues on the protein and/or the ligands) have been used to gain insight into this important problem (for recent reviews, see Yguerabide, 1972; Dwek, 1973). Ideally, such studies should provide detailed and specific information about the conformational transitions and the molecular motion of the active sites, regulatory sites, etc., as well as about quaternary transitions in some cases. This type of study has contributed significantly to our understanding of the molecular mechanism of a number of enzyme systems. However, it requires the attachment of a spectroscopic

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