A new method for the selective isolation of cysteine-containing peptides

Specific labelling of the thiol group with a hydrophobic chromophore

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A new method for the selective isolation of cysteine-containing peptides was designed. The method is based on the specific labelling of thiol groups with a hydrophobic chromophore followed by enzymic fragmentation of the labelled protein and reversed-phase high-pressure liquid-chromatographic separation of the peptide mixture. This new method has several distinct advantages: (1) the hydrophobic-chromophore-labelled cysteine-containing peptides are easily separated from non-cysteine-containing peptides by reversed-phase high-pressure liquid chromatography; (2) only cysteine-containing peptides are detected in the visible region with sensitivity at the low picomole level; this high sensitivity allows isolation of nanogram amounts of pure cysteine-containing peptide; (3) during sequence determination of the chromophore-labelled cysteine-containing peptides, the cysteine residues are released as coloured anilinothiazolinone derivatives and can be detected directly in the picomole range; (4) with proteins bearing several disulphide groups, each disulphide group may undergo a different degree of reduction, and therefore the recovery of individual cysteine-containing peptides may be used to deduce the disulphide linkages present in the native protein. Two thiol-specific reagents, 4-dimethylaminoazobenzene-4'-iodoacetamide and 4-dimethylaminoazobenzene-4'-*N*-maleimide, were synthesized and characterized. The method was successfully used to isolate five cysteine-containing peptides from a completely reduced monoclonal-antibody κ -light chain raised against the azobenzenearsonate determinant and six cysteine-containing peptides from a κ -light chain raised against streptococcal group A polysaccharide. The principle of this method is applicable to the isolation of any peptide containing amino acid residues that can be specifically labelled with a hydrophobic chromophore.

Thiol and disulphide groups play an important role in the biological activity and conformational stability of proteins. The determination of amino acid sequences around thiol and disulphide groups usually provides important information in understanding the structure–function relationships of proteins.

Isolation of cysteine-containing peptides can be achieved by diagonal paper electrophoresis (Perham, 1967; Hartley, 1970) or column chromatography (Hirs, 1967). Selective isolation of cysteine-

containing peptides can also be achieved by their covalent binding to (Egorov *et al.*, 1975; Svenson *et al.*, 1977) or affinity adsorption on (Wilchek & Miron, 1979) an insoluble matrix.

We have developed a new method for the one-step isolation of cysteine-containing peptides from non-cysteine-containing peptides as well as separation of cysteine-containing peptides from each other. Thiol groups of a polypeptide were covalently labelled with a hydrophobic chromophore. The labelled polypeptide was digested enzymically and the peptide mixture was separated by reversed-phase h.p.l.c. The selective labelling of thiol groups with a hydrophobic chromophore serves two major functions, as follows. (1) The hydrophobic labelling renders cysteine-containing peptides more hydrophobic than the non-cysteine-containing peptides. Thus cysteine-

Abbreviations used: h.p.l.c., high-pressure liquid chromatography; DABIA, 4-dimethylaminoazobenzene-4'-iodoacetamide; DABMA, 4-dimethylaminoazobenzene-4'-*N*-maleimide; DABCAM, 4-dimethylaminoazobenzene-4'-carboxyamidomethyl; PTH, phenylthiohydantoin.

containing peptides are eluted with longer retention times than are non-cysteine-containing peptides on the reversed-phase h.p.l.c. (whose separation mechanism is mainly based on the hydrophobic interaction). (2) The chromophoric labelling enables cysteine-containing peptides to be detected in the visible spectral region (with a sensitivity of 2–5 pmol), whereas non-cysteine-containing peptides are colourless.

We describe in the present paper (1) the synthesis of two thiol-specific reagents 4-dimethylaminoazobenzene-4'-iodoacetamide (DABIA) and 4-dimethylaminoazobenzene-4'-*N*-maleimide (DABMA), and (2) the application of this new technique for the isolation of cysteine-containing peptides from immunoglobulin light chains and allergen antigen E.

Materials and methods

Materials and h.p.l.c. instrumentation

The sources of immunoglobulin light chains 10K26-12A1 (Alkan *et al.*, 1983) and 7S34.1 (Herbst *et al.*, 1982; see also Chang *et al.*, 1983) are described elsewhere. Ragweed antigen E was supplied by the National Institutes of Health (Bethesda, MD, U.S.A.) (Research Resources Branch; cat. no. A601-902-185) and was a gift from Dr. H. Frost. All other chemicals and solvents used in the synthesis of both DABIA and DABMA and in h.p.l.c. analysis were commercial analytical grade from Sigma Chemical Co. (St. Louis, MO, U.S.A.),

Fluka (Buchs, Switzerland) and Merck (Darmstadt, West Germany). The details of the h.p.l.c. instrument have been described previously (Chang, 1981).

Synthesis of DABMA (Chang, 1977) (Fig. 1)

The starting material for synthesizing both DABMA and DABIA is 4-amino-4'-dimethylaminoazobenzene (*p*-dimethylaminophenylazo-aniline). The synthesis of 4-amino-4'-dimethylaminoazobenzene has been documented previously (Chang *et al.*, 1976).

4-Amino-4'-dimethylaminoazobenzene (0.565 g) was stirred with 0.25 g of maleic anhydride in 10 ml of anhydrous diethyl ether at room temperature overnight. The insoluble mixture was collected by filtration and washed with 40 ml of ethanol/water (1:1, v/v), and then dried in a vacuum desiccator overnight. The dried intermediate was stirred with 1.5 g of anhydrous sodium acetate and 7.5 ml of acetic anhydride in a boiling-water bath for 20 min (moisture was excluded from the reaction mixture). The mixture was then left for 1 h at room temperature and filtered. The crude product, in the precipitate, was washed twice with acetone/water (2:1, v/v), dried *in vacuo* and then recrystallized from boiling acetone. The final product was shown to be at least 98% pure by t.l.c. Found: C, 67.58; H, 5.11; N, 17.50; calc. for $C_{18}H_{16}N_4O_2$: C, 67.50; H, 5.00; N, 17.50. DABMA derivatives of cysteine and glutathione have molar absorption coefficients of $\epsilon = 4.0 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at 520 nm (in 2 M-HCl).

Fig. 1. Structure and synthesis of DABIA and DABMA

Synthesis of DABIA (Chang, 1977) (Fig. 1)

4-Amino-4'-dimethylaminobenzene (0.7 g) was stirred with iodoacetic acid (0.48 g) and dicyclohexylcarbodi-imide (0.52 g) in 15 ml of ethyl acetate at 5°C. The reaction mixture was left for 1 h and then filtered. The crude product, which was mainly in the precipitate, was washed with water and then dried *in vacuo*. The crude DABIA was recrystallized twice from hot ethanol. The final product has minimum purity of 98% as determined by h.p.l.c. and t.l.c. Found: C, 47.35; H, 4.27; N, 13.63; calc. for $C_{16}H_{17}IN_4O$: C, 47.06; H, 4.50; N, 13.50%. DABIA decomposed at 182–185°C. DABIA derivatives of cysteine and glutathione have molar absorption coefficients of $\epsilon = 2.9 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at 535 nm (in 2 M-HCl).

Synthesis of S-DABCAM-cysteine PTH derivative

Cysteine (500 nmol) was dissolved in 100 μl of water, and to it was added 50 nmol of DABIA in 100 μl of dimethylformamide. The mixture was incubated for 1 h at room temperature and then dried *in vacuo* (completeness of conversion of DABIA into S-DABCAM-cysteine can be assessed on polyamide t.l.c.). The dried sample was dissolved in 300 μl of aq. 50% (v/v) pyridine, and to it was added 25 μl (approx. 100 μmol) of phenyl isothiocyanate. After reaction for 30 min at 50°C, the mixture was again dried and the solid was redissolved in 150 μl of 50% (v/v) trifluoroacetic acid. The acid solution was heated for 40 min at 50°C and then dried *in vacuo*, and the solid was redissolved in 1 ml of 80% (v/v) ethanol for h.p.l.c. analysis (Fig. 7). Completeness of conversion of S-DABCAM-cysteine into the less-polar S-DABCAM-cysteine PTH derivative can also be assessed by polyamide t.l.c. The final product actually contains a mixture of derivatives. However, S-DABCAM-cysteine PTH derivative is the only coloured one.

Complete reduction of protein

Antibody light chains (10–15 nmol) were dissolved in 400 μl of 5 M-guanidinium chloride/0.5 M-Tris/HCl buffer, pH 8.4, and to the solution was added 3.5 mg of dithiothreitol. Reduction was performed for 6 h at 50°C. The reduced protein was separated from the excess reagent and salt by gel filtration (Sephadex G-25, superfine grade; eluted with 5% acetic acid; column size 1.4 cm internal diam. $\times$ 30 cm) and was freeze-dried immediately.

Labelling of protein with DABIA

Native protein or reduced protein was dissolved in 600 μl of 5 M-guanidinium chloride/0.5 M-Tris/HCl buffer, pH 8.4, and to this solution was added 600 μl of DABIA solution (0.5 mg/ml in dimethylformamide). Labelling of protein was conducted for 1 h at room temperature in the dark with magnetic stirring. The sample was acidified with 30 μl of

trifluoroacetic acid, and the excess reagent was removed by gel filtration (Sephadex G-25, superfine grade; eluted with 50% acetic acid; column size 1.4 cm internal diam. $\times$ 30 cm). DABIA-labelled protein was collected as a colour band with a volume of 8–9 ml. The solution was dried, redissolved by first adding 200 μl of 50% (v/v) formic acid and then 400–600 μl of water, transferred to a smaller tube suitable for enzymic digestion and dried again.

Enzymic digestion of DABIA-labelled protein

DABIA-labelled protein (10 nmol) was dissolved in 200–300 μl of 0.1 M- NH_4HCO_3 and digested with 20 μg of trypsin (Worthington Biochemical Corp., Freehold, NJ, U.S.A.) or chymotrypsin (Worthington) for 20 h at 37°C (a 6 mm magnetic stirring bar being used). The DABIA-labelled proteins were not always soluble in NH_4HCO_3 solution, but usually became soluble after enzymic digestion had taken place. After digestion, the sample solution can be injected directly into the h.p.l.c. apparatus or freeze-dried and redissolved in a suitable volume of aq. 20% (v/v) pyridine for h.p.l.c. analysis.

H.p.l.c. separation of DABIA-labelled cysteine-containing peptides

The strategy for the separation of DABIA-labelled cysteine-containing peptides is the same as that for separating DABTC-peptides (see the following paper, Chang *et al.*, 1983). (1) First, 10–30 pmol of the sample (approx. 0.5–1% of the total material) was used for each analytical run to determine the optimum conditions. This was usually achieved by selection of different gradient systems and different pH and salt concentrations of the solvent A. (2) Once the optimum conditions had been established, the rest of the material was then collected manually (Chang, 1981), preferably by one or at most two preparative runs, for sequence determination.

Sequence determination of cysteine-containing peptides

Automatic sequencing of cysteine-containing peptides is described in the following paper (Chang *et al.*, 1983).

Results

Specificities of DABMA and DABIA

Both DABMA and DABIA react specifically and quantitatively with thiol groups at pH 5 [0.2 M-acetate buffer (pH 5.0)/acetone, 1:1, v/v] and pH 8.4 [0.5 M-Tris buffer (pH 8.4)/dimethylformamide, 1:1, v/v] respectively (Fig. 2). Control experiments performed with lysine, histidine and methionine under identical conditions gave no detectable reaction products, as checked by t.l.c. (Chang, 1977).

Fig. 2. Recoveries of DABMA-cysteine (Δ), DABMA-glutathione ($\square$), DABCAM-cysteine ($\blacktriangle$) and DABCAM-glutathione ($\blacksquare$) as a function of concentrations of cysteine and reduced glutathione

The labelled cysteine and reduced glutathione were separated from excess of reagent by silica-gel t.l.c., extracted with 2M-HCl and quantitatively analysed at 520nm (DABMA derivatives) or at 535nm (DABCAM derivatives). The linearity indicates a quantitative and reproducible reaction of DABMA and DABIA with the thiol groups of cysteine and reduced glutathione.

Isolation of five cysteine-containing peptides from immunoglobulin 10K26-12A1 light chain

Mouse κ -light chain contains two intrapeptidic disulphide bonds linking residues 23 (Cys 1)–88 (Cys 2) and 134 (Cys 3)–194 (Cys 4), as well as one half-cystine residue at the C-terminal end (Cys 5) that bridges to the heavy chain (Kabat *et al.*, 1979). Five cysteine-containing peptides were isolated from the completely reduced immunoglobulin 10K26-12A1 light chain by using the DABIA method (Fig. 3). A 10nmol portion of the light chain was used as starting material and the recoveries of the five cysteine-containing peptides were as follows: Cys 1 peptide (peak 6, purity 98%, yield 3.6nmol); Cys 2 peptide (peak 2, purity 96%, yield 1.5nmol); Cys 3 peptide (peak 5, purity 95%, yield 1.4nmol); Cys 4 peptide (peak 3, purity 95%, yield 0.6nmol, plus peak 4, purity 83%, yield 0.6nmol); Cys 5 peptide (peak 1, purity 98%, yield 2.6nmol). Those peptides were sequenced by using an automatic sequencer (Table 1), and, except for Cys 2 peptide, were all sequenced to their C-terminal ends.

Minor peaks shown in Fig. 3, e.g. the one eluted between peaks 1 and 2, were also collected and subjected to the sequencing procedure; however, no conclusive result was obtained.

Amino acid sequences of the two cysteine-containing peptides derived from the variable region of immunoglobulin 10K26-12A1 light chain are homologous with those of the other four anti-azobenzene-

Fig. 3. Preparative isolation of cysteine-containing peptides derived from the tryptic cleavage of DABIA-labelled immunoglobulin 10K26-12A1 light chain (anti-azobenzene-arsenate monoclonal light chain)

Solvent A was 10mM-phosphate buffer, pH 7.0, containing 2% of dimethylformamide. Solvent B was acetonitrile. The flow rate was 1ml/min. The column was μ -Bondapak C-18 (Waters Associates). The column temperature was 22°C. The detector was set at 436 nm and at 1.0 AUFS (absorption unit, full scale).

Table 1. *Sequences of cysteine-containing peptides selectively isolated by the DABIA method*

Some tryptic peptides (f3 of Fig. 3 and f6 of Fig. 4) are non-specifically cleaved (chymotryptic) by-products. For other experimental details see the text. Residues are numbered according to the system of Kabat *et al.* (1979). The light chain of immunoglobulin 7S34.1 is five residues longer than the numbering system (see the following paper, Chang *et al.*, 1983). Cysteine-containing peptides are numbered according to their location starting from the *N*-terminus. The extra cysteine-containing peptide of immunoglobulin 7S34.1 light chain is termed Cys Extra. The one-letter notation for amino acid sequences (IUPAC-IUB Commission on Biochemical Nomenclature, 1969)

is used. Cysteine is identified as the coloured *S*-DABCAM-cysteine PTH derivative and is shown as $\overset{*}{C}$. Amino acid residues that were not identified are shown as ().

Origin	Fraction (peak no.)	Sequence	Location in native protein
10K26-12A1 (tryptic)	f1 (Fig. 3)	NE $\overset{*}{C}$	212-214 (Cys 5)
	f2 (Fig. 3)	FSGSGSGTDYSLTISNLEQE(IATYF $\overset{*}{C}$ QQGNTLP)	92-96 (Cys 2)
	f3 (Fig. 3)	T $\overset{*}{C}$ EATHK	193-199 (Cys 4)
	f4 (Fig. 3)	HNSYT $\overset{*}{C}$ EA(HK)	189-199 (Cys 4)
	f5 (Fig. 3)	ADAAPTVSIFPPSSEQLTSGGASVV $\overset{*}{C}$ FLNNFYPK	109-142 (Cys 3)
	f6 (Fig. 3)	VTIS $\overset{*}{C}$ R	19-24 (Cys 1)
7S34.1 (tryptic)	f1 (Fig. 4)	NE $\overset{*}{C}$	212-214 (Cys 5)
	f2 (Fig. 4)	VEAEDVG VYY $\overset{*}{C}$ MQQ()	78-92 (Cys 2)
	f3 (Fig. 4)	DIVMTQTADSALVTPG()SVSIS $\overset{*}{C}$ R	1-24 (Cys 1)
	f4 (Fig. 4)	T $\overset{*}{C}$ EAT(K)	193-199 (Cys 4)
	f5 (Fig. 4)	ADAAPTVSIFPPSSEQLTSGGASVV $\overset{*}{C}$ FLNNFY()	109-142 (Cys 3)
	f6 (Fig. 4)	$\overset{*}{C}$ MQQR	88-92 (Cys 2)
	f7 (Fig. 4)	SLLHSNG()TYLYWFLQ()PGQ $\overset{*}{C}$ ()QLLIY()	28-50 (Cys Extra)
7S34.1 (chymotryptic)	f1 (Fig. 5)	NRNE $\overset{*}{C}$	210-214 (Cys 5)
	f2 (Fig. 5)	T $\overset{*}{C}$ EATH	193-198 (Cys 4)
	f3 (Fig. 5)	$\overset{*}{C}$ MQQREY	88-94 (Cys 2)
	f4 (Fig. 5)	TSGGASVV $\overset{*}{C}$ F	126-135 (Cys 3)
	f5 (Fig. 5)	Y $\overset{*}{C}$ MQQREY	87-94 (Cys 2)
	f6 (Fig. 5)	QRPGQ $\overset{*}{C}$ P	38-44 (Cys Extra)
	f7 (Fig. 5)	IS $\overset{*}{C}$ RS()	21-26 (Cys 1)

arsonate monoclonal light chains (Siegelman & Capra, 1981) except for the third hypervariable region (residues 92 and 93) carried with Cys 2 peptide.

The sequences of three cysteine-containing peptides derived from the constant region completely agree with the sequence of immunoglobulin MOPC 21 light chain (Svasti & Milstein, 1972). It is

Fig. 4. Preparative isolation of cysteine-containing peptides derived from the tryptic cleavage of DABIA-labelled immunoglobulin 7S34.1 light chain [anti-(streptococcal group A polysaccharide) light chain]. Conditions for column chromatography were as described in the legend to Fig. 3 except for the use of a second μ -Bondapak C-18 column.

Fig. 5. Analytical separation of cysteine-containing peptides (30 pmol) derived from the chymotryptic digestion of DABIA-labelled immunoglobulin 7S34.1 light chain.

Solvent A was 10 mM-phosphate buffer, pH 7.0, containing 2% of dimethylformamide. Solvent B was acetonitrile containing 4% of dimethylformamide. The flow rate was 1 ml/min. The column was Lichrosorb RP-18 (Merck). The column temperature was 22°C. The detector was set at 436 nm and at 0.01 AUFS.

noteworthy that sequences at positions 127–129 (Ser-Gly-Gly) carried within the Cys 3 peptide are different from those of the immunoglobulin MOPC 41 and MOPC 70 light chains (Gly-Gly-Ser) (Gray *et al.*, 1967).

Isolation of six cysteine-containing peptides from immunoglobulin 7S34.1 light chain

The complete variable-region sequence of immunoglobulin 7S34.1 light chain has been determined (see Chang *et al.*, 1983). Amino acid analysis of performic acid-oxidized immunoglobulin 7S34.1 light chain gave 6.3 ± 0.2 residues of cysteic acid/

molecule of light chain or approx. 1.5 residues/molecule more than in immunoglobulin 10K26-12A1 light chain. Although one additional cysteic acid residue was found at residue 48 of the variable region (see Chang *et al.*, 1983), it was not clear whether this light chain contains one additional cysteine residue or one additional cystine residue. The DABIA method was used to clarify this ambiguity. Approx. 10 nmol portions of immunoglobulin 7S34.1 light chain, after complete reduction and labelling with DABIA, were digested separately with trypsin and chymotrypsin. The peptides were separated by h.p.l.c. (Figs. 4 and 5). The coloured

Fig. 6. Preparative isolation of one cysteine-containing peptide derived from the chymotryptic digestion of DABIA-labelled antigen E (native)

Solvent A was 10 mM-phosphate buffer, pH 7.0, containing 2% of dimethylformamide. Solvent B was acetonitrile. The flow rate was 1 ml/min. The column was μ -Bondapak C-18. The column temperature was 22°C. The detector was set at 436 nm and at 0.5 AUFS.

Fig. 7. H.p.l.c. analysis of the standard *S*-DABCAM-cysteine PTH derivative (bottom panel, 15 pmol) and the *S*-DABCAM-cysteine PTH derivative released at step 6 of the sequencing analysis of cysteine-containing peptide *f*6 from Fig. 5 (top panel)

Solvent A was 35 mM-acetate buffer, pH 5.0. Solvent B was acetonitrile. The flow rate was 1 ml/min. The column was Zorbax-ODS (Dupont). The column temperature was 22°C. The detector was set at 436 nm and at 0.01 AUFS.

cysteine-containing peptides were recovered with a yield ranging from 1 nmol to 3.4 nmol and were all sequenced by using an automatic sequencer (Table 1). In both preparations, only six cysteine-containing peptides were found, three at the variable region and three at the constant region.

Isolation of one cysteine-containing peptide from antigen E

Ragweed allergen antigen E (King *et al.*, 1964) contains one free thiol group (Paull *et al.*, 1980). Native antigen E was dialysed against 0.1% acetic

Fig. 8. Recoveries of *S*-DABCAM-cysteine PTH derivative at each degradation step from selected cysteine-containing peptides isolated by the DABIA method. *S*-DABCAM-cysteine PTH derivative was quantitatively analysed by h.p.l.c. (Fig. 7). Then 1–10% of each of the PTH derivatives was injected for assay of *S*-DABCAM-cysteine PTH derivative at 436 nm, and the yields were normalized to 100% injection.

acid, labelled with DABIA in 5M-guanidinium chloride and digested with chymotrypsin. Only one coloured cysteine-containing peptide was isolated by h.p.l.c. (Fig. 6). The yield was rather low (about 7.5%); 600pmol of the peptide was purified for sequencing and only the first four degradation steps gave identifiable results. The first step gave isoleucine PTH derivative (550pmol), the second degradation gave isoleucine PTH derivative (380pmol), the third degradation gave histidine PTH derivative (250pmol) and ϵ -phenylthiocarbamoyllysine PTH derivative (80pmol), and the fourth degradation gave the coloured *S*-DABCAM-cysteine PTH derivative.

Efficiency of DABIA labelling of the completely reduced protein

This can be readily determined by taking one portion of the labelled protein (after Sephadex G-25 separation from the excess reagent) for amino acid analysis and another portion for absorbance reading at 535nm after dilution with 2M-HCl (molar absorption coefficient of the DABCAM derivative $\epsilon = 29000 \text{ M}^{-1} \cdot \text{cm}^{-1}$). For immunoglobulin 10K26-12A1 light chain (calculated for five cysteine residues) and immunoglobulin 7S34.1 light chain (calculated for six cysteine residues) the labelling efficiencies were 87% and 92% respectively.

Identification of cysteine as *S*-DABCAM-cysteine PTH derivative

In sequencing DABIA-labelled cysteine-containing peptides by the Edman degradation, cysteine residues were released as the coloured *S*-DABCAM-cysteine PTH derivative, which can be quantitatively analysed by h.p.l.c. at the low picomole level (Figs. 7 and 8). As little as 100–200pmol of *S*-DABCAM-cysteine PTH derivative is directly visible in the conversion acid solution (100 μl of 30% trifluoroacetic acid).

Discussion

We have shown that thiol groups of polypeptides can be selectively labelled with a hydrophobic chromophore and that these chromophore-labelled cysteine-containing peptides can be isolated with high efficiency and analysed at the picomole level. Perhaps the most interesting feature of this new technique is the possibility of using the yield of each cysteine-containing peptide to pair the disulphide linkage present in the native protein. Immunoglobulin light chain has two well-characterized disulphide linkages, one in the variable region and the other in the constant region. If these two disulphide bonds undergo different degrees of reduction, the yield of the chromophore-labelled cysteine-containing peptides should be different and those derived from the same disulphide linkage should give approximately equal yields. For instance, the yields of cysteine-containing peptides derived from the constant region of light chain 7S34.1 (Fig. 5, peaks 2 and 4) are double those derived from the variable region (Fig. 5, peaks 3 and 7). However, successful application of this strategy relies upon three important prerequisites: (1) labelling of free thiol groups must be quantitative; (2) enzymic cleavage of the labelled protein must be quantitative; (3) recoveries of all cysteine-containing peptides from h.p.l.c. must be quantitative.

So far, the low recovery of cysteine-containing peptide (ranging from 10 to 35%) has been the major problem of this new technique. Although

material loss occurs during gel filtration as well as during dialysis, we have found that the major loss of peptide occurs at the step of reversed-phase h.p.l.c. separation. However, this low yield is not uncommon with other chromatographic methods, particularly when applied to the separation of low nanomole or picomole amounts of peptides.

The two thiol-specific reagents DABIA and DABMA were synthesized, but only DABIA was applied to the isolation of cysteine-containing peptides. DABMA is indeed more specific than DABIA, and DABMA derivatives also display higher molar absorption coefficients than the DABIA derivatives (40000 compared with $29000 \text{ M}^{-1} \cdot \text{cm}^{-1}$). However, we have also recognized the possibility of ring opening of maleimide under alkaline conditions, which would complicate the isolation of cysteine-containing peptides.

Thus we have developed a sensitive and efficient method for the selective isolation of cysteine-containing peptides based on the hydrophobic-chromatophoric labelling and reversed-phase h.p.l.c. The principle of this new method, like the diagonal electrophoresis method (Hartley, 1970), can in principle be applied to the isolation of any peptide containing residues of an amino acid that can be specifically labelled with hydrophobic chromophore, e.g. methionine-labelled or lysine-labelled peptides. Selective isolation and sequence determination of methionine-containing peptides (Tang & Hartley, 1967; Wilkinson, 1969; Degen & Kyte, 1978) and lysine-containing peptides (Butler *et al.*, 1967; Perham & Jones, 1967) can be used to investigate the overlap of CNBr-cleavage and tryptic peptides and should greatly facilitate the job of protein sequencing.

Part of the work described in this paper, the synthesis and characterization of DABIA and DABMA, is abstracted from Chang (1977). We acknowledge the report by Luedtke *et al.* (1981) on the use of DABIA in the biophysical study of immunoglobulin G. We thank Dr. H. Frost, Dr. S. Alkan, Dr. R. Ball, Dr. H. Herbst and Mr. R. Aebersold for providing proteins as demonstration samples. The excellent technical assistance of Mr.

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