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Induction of Antibody against Actin from Myxomycete Plasmodium and Its Properties†

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ABSTRACT: Plasmodium actin was highly purified by gel filtration of crude G-actin on Sephadex G-100 followed by ultracentrifugation after polymerization in the presence of 1 *M* urea and 1 m*M* ATP. Purified actin showed a single band in the sodium dodecyl sulfate gel electrophoretic pattern. Antibody against this purified actin was induced in rabbits. The antibody obtained was immunologically monospecific for plasmodium actin, judging from the following results. (1) The addition of the antibody to a plasmodium F-actin solution increased the turbidity of the mixed solution, showing the formation of the antibody–actin complex. (2) In immunodiffusion and immunoelectrophoresis, the antibody formed single precipitin lines with the purified actin preparation and with the crude actin extract from the acetone-dried powder of plasmodium. (3) The antibody inhibited polymerization of plasmodium G-actin. (4) Plasmodium F-actin filaments were decorated with antibody in electron micrographs. The antibody reacted not only with plasmodium F- and G-actin, but also reacted with sea urchin egg actin, but it did not react with actin from rabbit striated muscle.

Actin has been isolated from plasmodium of the myxomycete using its specific binding to muscle myosin (Hatano and Oosawa, 1966) and by column chromatography (Adelman and Taylor, 1969). This F-actin filament is decorated with heavy meromyosin (HMM)[1] from muscle to form the arrowhead like structures (Nachmias et al., 1970).

On the other hand, microfilaments, around 60 Å in diameter, have been observed in electron micrographs of living and glycerinated plasmodium of the same myxomycete (Wohlfarth-Bottermann, 1962; Rhea, 1966; Nagai and Kamiya, 1966). They existed as bundles of several hundreds of filaments in the gel layer of plasmodium. Nagai and Kamiya (1968) showed that when glycerinated plasmodium was treated with ATP in the presence of Mg^{2+}, the bundle was broken into small blocks in which filaments made dense aggregates similar to superprecipitated actomyosin of muscle. Using the method developed by Ishikawa et al. (1969), Alléra et al. (1971) ascertained that microfilaments in question were F-actin filaments. Namely, these filaments were decorated with HMM from muscle to form the arrowhead like structures. Similar actin filaments have been identified in many eukaryotic cells by this method (Pollard et al., 1970; Pollard and Korn, 1973a,b; Pollard and Weihing, 1974; Comly, 1973; Schroeder, 1973; Tilney et al., 1973; Williamson, 1974; Palevitz et al., 1974).

However, there are some technical problems in this method. For example, when actin exists in the soluble part of protoplasm, it or a part of it dissolves out during glycerination before the application of HMM. Some authors have pointed out the possibility that actin exists in the state of monomer or oligomer (Jockusch et al., 1971; Tilney et al., 1973) or in the different states of polymer from F-actin (Hatano et al., 1967; Hatano and Totsuka, 1972; Tilney, 1975). If actin exists in such states in vivo, it will be very difficult to show the existence of actin, even if they were decorated with HMM.

We have tried to produce the antibody to actin from plasmodium in order to examine the precise localization of actin in plasmodium immunohistochemically. We report in this paper the induction of antibody against highly purified actin from plasmodium and describe some properties of the antibody.

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[1] Abbreviations used are: HMM, heavy meromyosin; PBS, phosphate buffered saline.

Materials and Methods

Preparation and Purification of Plasmodium Actin. Plasmodium of a myxomycete, *Physarum polycephalum,* was cultured in 15-l. buckets by supplying oatmeal every day (Camp, 1936). The original G-actin fraction was obtained from plasmodia after acetone treatment by the method of Hatano and Oosawa (1966). This crude actin was further purified by gel filtration (Rees and Young, 1967; Adelman and Taylor, 1969) followed by ultracentrifugation. About 7 ml of the actin solution of concentration 4 mg/ml was applied to a column of Sephadex G-100 of 2 cm in diameter and 95 cm in height, and eluted with a 4 m*M* Tris-HCl (pH 8.2) solution containing 0.1 m*M* ATP. The eluted solution was collected every 4 ml in small test tubes and the absorbance at 280 nm of each solution was measured. The absorbance showed two peaks, which were termed FI and FII (Figure 1). Only FII had polymerizability. The FII (32 ml total) were collected and concentrated to about 8 ml (2 mg/ml) by Diaflo ultrafiltration (Amicon, Lexington, Mass.). G-Actin in the concentrated FII was polymerized to F-actin by the addition of 0.1 *M* KCl. F-Actin, of which the concentration was about 2 mg/ml, was incubated in 1 *M* urea in the presence of 0.1 *M* KCl, 2 m*M* $MgCl_2$, 1 m*M* ATP, and 10 m*M* Tris-maleate buffer (pH 7.0) for 4 hr, at 4°. Then it was centrifuged at 100,000*g* for 90 min at 5°. The precipitated F-actin was dissolved into a solvent containing 0.5 m*M* ATP and 3 m*M* cysteine (pH 8.2) and dialyzed against a solution containing 0.05 m*M* ATP and 3 m*M* cysteine (pH 8.2) for 2 to 3 days. Pure G-actin was finally obtained by ultracentrifugation of the solution at 100,000*g* for 30 min. About 4 mg of purified actin was obtained from 8 g of acetone-dried powder of plasmodium or 100 g of fresh plasmodia.

In the latter half of the experiment the original actin fraction was prepared from plasmodium myosin B directly, without acetone treatment. Crude actin was separated from myosin by heating myosin B at 55° for 15 min in the presence of 0.1 *M* KCl and 5 m*M* ATP at pH 7.0. This method, which is very simple and useful for obtaining actin in high yield, will be reported in another paper.

Production of Antibody. Adult female white rabbits were used in this experiment. One-half milliliter of the purified plasmodium F-actin solution which contained 1 mg of actin was emulsified with an equal volume of Freund's complete adjuvant and then injected intradermally, subcutaneously, intramuscularly, and intraperitoneally at multiple sites of rabbits. The injections were carried out four times once a week and repeated further four or five times at intervals of 2 to 3 weeks. As an adjuvant, bacterial endotoxin was also injected. Namely, 1 mg of alum-precipitated plasmodium F-actin was suspended in 1 ml of phosphate buffered saline (PBS) containing 20 μg of *Salmonella* endotoxin and the suspension was injected intravenously 3 times at an interval of 2 days during the first 10 days of the course of immunization. Ten days after the last injection the rabbits were bled. Blood was allowed to clot at room temperature and was kept overnight at 4°. Antiserum was obtained by centrifugation of blood at 1500*g* for 15 min and further clarified at 10,000*g* for 30 min.

Antibody in the serum was fractionated into the γ-globulin fraction by salting out of serum with half-saturated ammonium sulfate. The salting out was repeated twice. The precipitated γ-globulin was then suspended in PBS and dialyzed against the same solution for 3 days and stored at −20° at a protein concentration of 35 mg/ml. This γ-globulin fraction was used as an "antibody fraction" in the following experiments. For electron microscopic observation the antibody fraction was further purified by DEAE-cellulose column chromatography (Sober and Peterson, 1958) and gel filtration on Sephadex G-200.

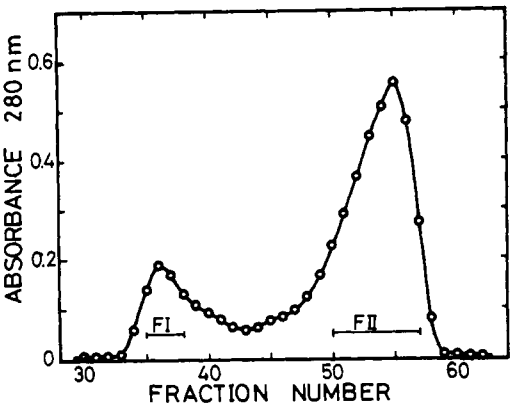

FIGURE 1: Gel filtration of original plasmodium actin on Sephadex G-100. Actin (28 mg) was applied to a 2 × 90 cm column and was eluted with 4 m*M* Tris-HCl buffer (pH 8.2) containing 0.1 m*M* ATP. Fractions of 4 ml were collected at a flow rate of 12 ml/hr. FI and FII were pooled for the further purification.

Preparation of Muscle Myosin, Muscle Actin, and Sea Urchin Egg Actin. Muscle myosin was prepared from rabbit striated muscle by the method described by Perry (1955) and muscle G-actin was prepared by almost the same method as that of Straub (1943) except that tropomyosin and troponin were carefully removed before the acetone treatment of myosin-extracted minced muscle (Ebashi and Ebashi, 1964). Sea urchin egg actin was prepared by the method of Hatano et al. (1969) from eggs of *Hemicentrotus pulcherrimus.*

Determination of Protein Concentration. Protein concentration was measured by the biuret method (Gornall et al., 1949) using absorbances of 0.068 at 540 nm for plasmodium actin at 1 mg/ml, 0.070 for muscle actin, and 0.066 for muscle myosin, respectively.

Viscosity was measured by Ostwald type capillary viscometers, of which the flow times were around 30 sec for the buffer solution.

Sodium dodecyl sulfate gel electrophoresis was carried out by the method of Weber and Osborn (1969) with a slight modification. The 7.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate were used. Samples were dissolved into 0.05 *M* phosphate buffer of pH 7.2 which contained 1% sodium dodecyl sulfate and 1% mercaptoethanol and then were boiled for 3 min before the electrophoresis.

Immunodiffusion and Immunoelectrophoresis. These were carried out by the methods described by Clausen (1969). Agar plates for immunodiffusion were prepared with 1.3% agar, 0.02% sodium azide, and 0.02 *M* Tris-HCl buffer of pH 8.2, and those for immunoelectrophoresis were prepared with 1.3% agar, 0.02% sodium azide, and 0.022 *M* Veronal buffer of pH 8.6.

Electron Microscopy. A drop of the plasmodium F-actin solution of about 0.07 mg/ml was placed on a Formvar-coated grid stabilized with a carbon film. After 1 or 2 min the solution was sucked up with a small piece of filter paper and a drop of the antibody solution of 2 mg/ml was transferred on the grid to let antibody react with F-actin on the surface of the grid for about 3 min. The complex of F-actin

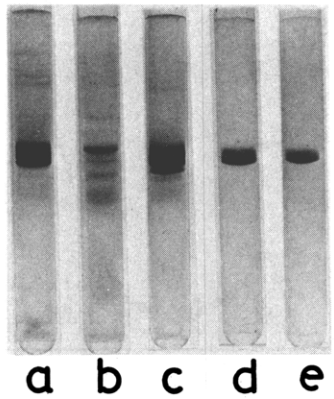

FIGURE 2: Purification of plasmodium actin as monitored by sodium dodecyl sulfate gel electrophoresis: (a) original plasmodium actin; (b) FI; (c) FII; (d) purified plasmodium actin (40 μg); (e) the same as d (20 μg).

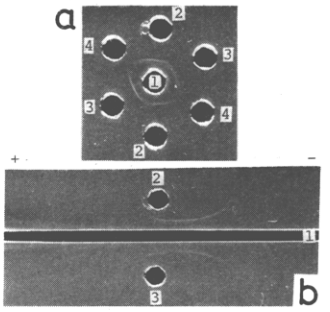

FIGURE 3: Comparison of precipitation patterns produced in immunodiffusion (a) and immunoelectrophoresis (b): (1) antibody fraction to plasmodium actin; (2) purified plasmodium actin; (3) water extract from acetone-dried powder of plasmodium; (4) muscle actin. Each well contained approximately the same amount of actin. Single precipitin line appeared in every case except in the case of muscle actin.

and antibody was observed with a Hitachi electron microscope at a magnification of 21,000 to 30,000 after negatively staining with 1% uranyl acetate (Huxley, 1963).

Results

Purity of Antigen. When the original actin fraction which was prepared by the ordinary method of Hatano and Oosawa (1966) was used as the antigen, the immunoelectrophoretic pattern of antigen and antiserum from immunized rabbits showed a few bands, suggesting that antibodies against proteins other than actin were also induced. Actually, the sodium dodecyl sulfate gel electrophoresis revealed that the original actin preparation contained small amounts of a few impurities (Figure 2a). Therefore, further purification of the original G-actin was carried out by gel filtration on Sephadex G-100. The elution pattern of the original G-actin had two peaks, FI and FII, as described previously (Figure 1). Polymerization activity localized only in the second fraction, FII. However, FII still contained small amounts of the other protein components (Figure 2c). One component, the molecular weight of which is a little smaller than that of actin, remarkably increased in FII after chromatography. This is not due to the concentration of this component in FII, but seems to be a result of autolysis of G-actin during chromatography, although the reason for this is not known now.

To remove these components various procedures have been tried. Ultracentrifugation of F-actin at a low salt concentration (0.03 *M* KCl, pH 7.0; Laki et al., 1962; Martonosi, 1962), or at a high salt concentration (0.6 *M* KCl, pH 7.0; Spudich and Watt, 1971) in the presence or absence of 10 m*M* EDTA was not very effective. Finally, it was found that ultracentrifugation of F-actin in the presence of 1 *M* urea at low temperature was most useful for removing the impurities. As shown in Figure 2d and e, pure actin was obtained in high yield. As Szent-Györgyi and Joseph (1951) reported in the case of muscle F-actin, plasmodium F-actin did not show any denaturation by such a treatment. G-Actin thus purified polymerized to F-actin on the addition of 0.1 *M* KCl or 2 m*M* $MgCl_2$. The reduced viscosity (η_{sp}/C) of F-actin was 10 to 11 dl/g which was much higher than that (4 to 7 dl/g) of F-actin from the original plasmodium actin and was similar to that of F-actin from purified muscle actin.

Specificity of Antibody; Titers. Antisera from immunized rabbits showed the precipitin reaction in the interfacial ring test. Titers which were expressed by the reciprocal of dilution were between 2^5 and 2^6.

Immunodiffusion and Immunoelectrophoresis. As illustrated in the immunodiffusion pattern of Figure 3a, anti-actin antiserum formed single precipitin lines with purified actin and with water extract from the acetone-dried powder of plasmodium, but did not precipitate with muscle actin. Figure 3b shows an immunoelectrophoretic pattern of purified plasmodium actin and that of water extract from the acetone-dried powder of plasmodium. Both samples formed single precipitin arc lines against anti-actin antiserum. These experiments show that antiserum contains antibody which reacts with plasmodium actin and does not contain any other antibodies which react with the other proteins from plasmodium. Namely, antibody obtained here was immunologically of a single component specific to plasmodium actin.

Turbidimetry. One-fifth volume of the antibody fraction of concentration 35 mg/ml was added to F-actin solution of 1 mg/ml and the change of the turbidity of the mixed solution was measured at 500 nm at 36.8°. The turbidity increased gradually and reached a constant level after 90 min as shown in Figure 4. The turbidity of the mixed solution of plasmodium F-actin and a normal γ-globulin fraction which was prepared from a nonimmunized rabbit did not increase. Moreover, the turbidity of the mixed solution of muscle F-actin and the antibody fraction did not show any increase. However, when F-actin from sea urchin egg was mixed with the antibody fraction, the turbidity of the solution increased in a similar way as in the case of the mixture of plasmodium F-actin and the antibody fraction. Thus, the antibody to plasmodium actin does not interact with F-actin from rabbit striated muscle, but interacts with F-actin from sea urchin eggs.

Inhibition of Polymerization of G-Actin by Antibody. Plasmodium G-actin of 1 mg/ml was preincubated with the antibody fraction of 7 mg/ml in 3 m*M* cysteine, 0.05 m*M* ATP, and 10 m*M* Tris-maleate of pH 7.0 at 0° for 5 min. Then it was polymerized by the addition of 0.1 *M* KCl at 21.9°. After about 45 min, the viscosity of the mixed solution was measured. The viscosity was only about 30% of that of F-actin polymerized without antibody. Polymerization of plasmodium G-actin was not inhibited by the normal γ-globulin fraction from a nonimmunized rabbit. Thus, the antibody interacted with plasmodium G-actin to inhibit its polymerization. On the other hand, polymerization of muscle G-actin was not inhibited by the antibody fraction.

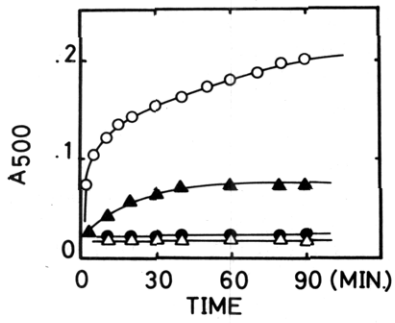

FIGURE 4: Turbidity change of mixture of actin and antibody fraction. Antibody was added to each actin solution at 0 min and turbidity of each solution at 500 nm was measured at 36.8°: (○) plasmodium actin and antibody; (▲) sea urchin egg actin and antibody; (●) plasmodium actin and normal γ-globulin fraction; (△) muscle actin and antibody.

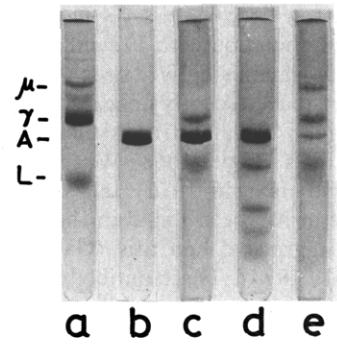

FIGURE 5: Sodium dodecyl sulfate gel electrophoresis of actin–antibody complex: (a) antibody fraction: (b) purified plasmodium actin; (c) precipitate of plasmodium actin with antibody; (d) sea urchin egg actin; (e) precipitate of egg actin with antibody; (γ) γ chain; (μ) μ chain; (A) actin.

Sodium Dodecyl Sulfate Gel Electrophoresis of the Antigen–Antibody Complex. Protein components of the antigen–antibody precipitates were analyzed by sodium dodecyl sulfate gel electrophoresis. After plasmodium F-actin and sea urchin egg F-actin were incubated with the antibody fraction in the weight ratio of 1 mg of F-actin to 7 mg of the antibody fraction at 36.8° for 60 min, the solutions were kept at 4° for 2 days. The precipitates formed were collected by low-speed centrifugation and washed with 20 vol of PBS twice. Then sodium dodecyl sulfate gel electrophoresis of the precipitates was carried out. In the case of the precipitate of plasmodium F-actin the band of actin as well as the bands of γ-chain (heavy chain) and light chain of IgG were clearly seen in the electrophoretic pattern (Figure 5c). The band of actin was stronger than those of the subunits of the antibody, because only the definite ratio of the antibody fraction was added to the F-actin solution as mentioned above. It has been shown by turbidimetric examination that such a ratio of the antibody fraction was enough to precipitate F-actin to the maximum level in the solution. In the case of the precipitate of egg F-actin the band of actin as well as those of the μ chain of IgM, the γ chain of IgG (heavy chains), and the light chain were also observed as main bands (Figure 5e). It can be said that the precipitates formed by antigen–antibody reaction were actin and the anti-actin complex. When plasmodium G-actin was incubated with the antibody fraction in the same way as mentioned above, the electrophoretic pattern of the resultant precipitate was nearly the same as that of the precipitate of plasmodium F-actin and antibody fraction. These results also show that the antibody reacts not only with plasmodium F-actin, but also with plasmodium G-actin.

Electron Microscopic Observation. When the antibody fraction was added to F-actin on a grid for electron microscopy, the F-actin filaments were decorated with antibody, so that their surface became rough. Antibody molecules were observable on some parts of the electron micrograph (Figure 6b). The antibody strongly promoted the aggregation of actin filaments into parallel arrays of two or more filaments. These appearances are very similar to those of actin filaments treated with *Acanthamoeba* myosin (Pollard and Korn, 1973a,b) of which the molecular weight (about 180,000) and the shape are very similar to γ-globulin. On the contrary, the surface of plasmodium F-actin remained smooth when normal γ-globulin was added to F-actin. Similarly, no change of the appearance of F-actin filaments was observed when the antibody fraction was added to muscle F-actin.

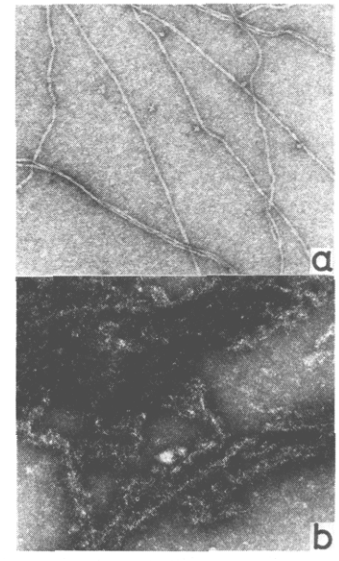

FIGURE 6: Electron micrographs of plasmodium F-actin treated with antibody: (a) plasmodium F-actin, ×42,000; (b) plasmodium F-actin treated with antibody, ×42,000.

Discussion

Actin has been isolated from various nonmuscle cells (Hatano and Oosawa, 1966; Hatano et al., 1969; Adelman and Taylor, 1969; Tatsumi et al., 1973; Weihing and Korn, 1971; Zucker-Franklin and Grusky, 1972; Yang and Perdue, 1972). It has been reported that physicochemical properties of these actins are similar to those of actin from rabbit striated muscle. For example, the molecular weight of plasmodium actin determined by the sodium dodecyl sulfate gel electrophoresis was the same as that of actin from rabbit striated muscle, and the amino acid composition of plasmodium actin appeared to be very similar to that of muscle actin (Hatano and Oosawa, 1966). Therefore, it is considered that plasmodium actin has only weak antigenicity to rabbit. In the case of a weak immunogen an appropriate dosage of the antigen is necessary to elicit antibody, because higher doses of the antigen produce high zone tolerance and lower doses of the antigen produce low zone tolerance. Here, 1 mg of plasmodium actin was chosen as an appropriate dose for single administration. Adjuvants are known to be useful in eliciting the antibody having a high titer. F-Actin may have many identical antigenic determinants and is resistant to hydrolytic enzymes such as trypsin. In such a case endotoxin is effective as an adjuvant to stim-

ulate bone marrow derived cells. *Salmonella* endotoxin was used here in combination with Freund's complete adjuvant which stimulates thymus-derived cells.

A few reports have recently appeared on the induction of antibodies to actins isolated from skeletal muscle of several species of animals (Pepe, 1968; Wilson and Finck, 1971; Hirabayashi and Hayashi, 1972). In a special case, an autoantibody to actin has been found in the serum of a patient with chronic aggressive hepatitis (Gabbiani et al., 1973; Trenchev et al., 1974). These antibody preparations, however, contained more or less antibodies which interacted with proteins other than actin. At the beginning of this study, actin prepared by the ordinary method of Hatano and Oosawa (1966) was used as the antigen. Then the immunoelectrophoretic pattern showed a few bands suggesting that antibodies against proteins other than actin in the original preparation were also induced as mentioned above.

Lazarides and Weber (1974) purified actin from mouse fibroblasts by gel electrophoresis in the presence of sodium dodecyl sulfate. Then they obtained antibody by using this actin as the antigen. We have tried to induce the antibody against plasmodium actin by a similar method. After the sodium dodecyl sulfate gel electrophoresis of plasmodium actin, actin was extracted from a band containing actin with an 8 *M* urea solution (pH 8.2). After dialysis against PBS to remove urea and sodium dodecyl sulfate, it was used as the antigen. The elicited antibody clearly showed passive hemagglutination, when it was mixed with rabbit red blood cells which had been coated with plasmodium actin. But this antibody did not precipitate with plasmodium actin showing that titer of the antibody was very low.

In this study the further purification of plasmodium actin was essential to successfully obtain pure antibody specific to this actin. That is, the antibody fraction (γ-globulin fraction) from rabbits which were immunized with this purified actin reacted with pure plasmodium actin to form the precipitate of actin and antibody. Moreover, according to the immunoelectrophoresis, the antibody fraction was immunologically monospecific. Therefore, this antibody will be useful for the immunological histochemistry of actin in plasmodium. Considering the fact that the antibody interacts with sea urchin egg actin, it will also be useful in various nonmuscle cells or tissues.

Acknowledgments

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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.