PROTEIN EXPRESSION AND PURIFICATION 3, 50–56 (1992)

Purification of Recombinant Antigenic Epitopes of the Human 68-kDa (U1) Ribonucleoprotein Antigen Using the Expression System pH6EX3 Followed by Metal Chelating Affinity Chromatography

Heike Berthold, Mirco Scanarini, Charles C. Abney, Brigitte Frorath, and Wolfgang Northemann[1]
Department of Molecular Biology, ELIAS Entwicklungslabor, Obere Hardtstrasse 18, D-7800 Freiburg, Germany

Received July 15, 1991, and in revised form October 5, 1991

A novel plasmid expression vector (pH6EX3) that directs the synthesis of a fusion protein with a histidine hexapeptide at its N-terminus and a foreign protein at its C-terminus was constructed. The fusion gene is controlled by a strong *tac* promoter, leading to high-level expression of recombinant protein in several bacterial strains; the protein is deposited mainly as an insoluble mass in inclusion bodies. The fusion protein can be purified from the insoluble cell fraction by one-step affinity chromatography based on the selective interaction between the histidine hexapeptide and a metal chelating matrix charged with Ni^{2+} ions. The principle of this new system was tested by expressing and purifying antigenic epitopes of the human 68-kDa (U1) ribonucleoprotein autoantigen. With the use of column chromatography and pH gradient elution, about 25 μg recombinant protein/ml of bacterial culture was obtained.

Many eukaryotic proteins that are protentially useful for basic research or for medical applications such as tools for suitable diagnostic assay systems are often unavailable because of their low natural abundance. Expression of heterologous genes at very high levels in various cell cultures is therefore of considerable importance in providing more abundant sources of these proteins. The cloning of foreign genes in *Escherichia coli* plasmids and their expression after transformation of cells are common procedures in recombinant DNA-based biotechnological methods. Even though a large variety of different host/vector systems are used routinely for the overproduction of proteins, the purification of expressed proteins to homogeneity remains a limiting problem in the practical use of this recombinant technology (1).

Recently, several bacterial vectors have been constructed to facilitate the purification of recombinant proteins from lysates of bacterial cells by specific affinity chromatography. The construction of fusion genes encoding a bifunctional fusion protein is accomplished by joining the protein-coding sequence of the protein of interest to a well-characterized ligand-binding moiety. Such fusion proteins can be purified efficiently by taking advantage of the specific binding of the ligand moiety to an immobilized matrix, often providing the simplicity of a one-step purification protocol. The choice of gene fusion system depends on the properties of the fusion protein and the final use for the gene product. A major consideration of purification is whether the fusion protein is produced in soluble form or is, instead, sequestered by the cells in insoluble inclusion bodies (2). Many of the current systems are useful only for purification of soluble expressed recombinant proteins such as proteins fused to β-galactosidase, protein A, protein G, maltose-binding protein, glutathione *S*-transferase, chloramphenicol acetyltransferase, streptavidin, phosphate-binding protein, or the so-called flag peptide (for review see Ref. (3)). The solubility of a fusion protein product is determined by empirical factors; often two normally soluble proteins are rendered insoluble on fusion (4). Successful purification depends entirely on the natural conformation of the synthesized fusion proteins or on the normal binding affinity to the immobilized matrix after refolding (5).

In general, most of the expressed proteins accumulate intracellularly as insoluble inclusion bodies (2). The inclusion bodies are soluble in detergents, in strong chao-

[1] To whom correspondence should be addressed.

1046-5928/92 $3.00

tropic agents such as guanidinium-hydrochloride, and in urea, all of which mainly affect hydrogen bonding. These agents destroy the natural conformation of the recombinant proteins (5). Only a partial refolding to the natural conformation is observed, often with a concomitant loss of the ligand-binding affinity. Thus, an efficient and quantitative purification of expressed insoluble proteins using affinity chromatography and the fusion gene systems listed above is still not possible.

Here, we describe a genetic approach that provides a method for expressing recombinant proteins and for purifying them to homogeneity from insoluble inclusion bodies. The cDNA coding for two autoantigenic epitopes of the human 68 kDa (U1) ribonucleoprotein antigen was cloned in the new vector pH6EX3 and highly expressed as a fusion protein with a histidine hexapeptide at its N-terminus and the recombinant epitopes at its C-terminus under the control of the *tac* promoter in *E. coli*. The recombinant fusion protein was solubilized from the inclusion bodies and purified by affinity chromatography using a chelating matrix charged with Ni^{2+} ions. The Ni^{2+} column selects proteins containing neighboring histidine residues (6,7). We show that this new vector system is useful for high-level expression of recombinant proteins with respect to their purification from inclusion bodies with a single-step affinity chromatography. The purified recombinant proteins provide usable sources of antigens for the development of corresponding immunoassay systems for diagnosis of autoantibodies in the sera of patients with autoimmune diseases such as mixed connective tissue disease (MCTD).[2]

MATERIAL AND METHODS

Construction of Expression Vector pH6EX3

A 76-bp linker was synthesized (Gene Assembler Plus, Pharmacia) and cloned into the *Eco*NI site (position 269) and the *Eco*RI site (position 941) of the vector pGEX-2T (8). The DNA fragment inserted contains sequences coding for a histidine hexapeptide (H_1–H_6), a hexapeptide representing the recognition sequence for the site-specific proteinase thrombin (LVPRGS), and sequences of a multicloning site containing eight restriction sites (Fig. 1). The downstream *Eco*RI site was inactivated by mutation. The new vector, pH6EX3, is 4856 bp long. The correct insertion of the linker was proved by sequence analysis from both directions using sequencing primers ONPGEX.5 and ONPGEX.3. The 5′-primer, ONPGEX.5 (5′-GAGCTGTTGACAAATTAATCATCGG-3′), and the reverse 3′-primer, ONPGEX.3 (5′-GCTGCATGTGTCAGAGGTTTTCACCG-3′), are respectively 24 and 26 long and identical or complementary to the sequence between positions 177 and 200 and between positions 389 and 414 of pH6EX3. Both sequencing primers are also useful for proving correct insertion of a cloned cDNA into the multicloning site of pH6EX3 from both directions. A 506-bp fragment containing the sequence for the strong *rrnB* ribosomal RNA transcription terminator (9) was isolated from the vector pKK223-3 ((10); Pharmacia) and inserted downstream of the translational stop signals of pH6EX3.

Expression of the His–P68(A′-B′) Fusion Protein

Transformed *E. coli* strains were cultured overnight in LB medium containing 150 μg/ml ampicillin, diluted 10-fold with fresh prewarmed LB medium without ampicillin, and incubated for 90 min at 37°C prior to induction with 1.0 mM IPTG. Aliquots of 100 μl were sedimented. The cell pellets obtained were resuspended in 10 μl SDS sample buffer and resolved electrophoretically in a 12.5% SDS–polyacrylamide gel under reducing conditions. As a control, *E. coli* strain LE392 was transformed with either pGEX-2T (8) or pP68.2 (11). The pGEX-2T and pP68.2 directed the synthesis of the 26-kDa glutathione *S*-transferase and the 43-kDa glutathione *S*-transferase–P68(A′-B′) fusion protein, respectively (Fig. 2, lanes 1, 2). Protein markers (Bio-Rad) were used as reference.

Purification of His–P68(A′-B′) Fusion Protein by Metal Chelate Chromatography

For the purification of recombinant His–P68(A′-B′), 50 ml *E. coli* culture was induced with 1 mM IPTG for 8 h at 37°C. The cells were sedimented and resuspended in 10 ml PBS. For lysis, the cells were incubated in the presence of 1.0 mg/ml lysozyme (Sigma) and 1 mM phenylmethylsulfonyl fluoride (Sigma) for 20 min at 0°C and then in the presence of 1% Triton X-100 (Sigma) for a further 10 min at 0°C, followed by sonication with two pulses for 20 s each at about 100 W at 0°C. The soluble and insoluble cell fractions were separated by centrifugation of the cell homogenate at 8000*g* and 4°C for 5 min. The pellet containing the insoluble recombinant His–P68(A′-B′) was dissolved in 6 ml of 6 M urea in Tris–acetate buffer (50 mM Tris–acetate, pH 7.2, 0.5 M NaCl) and incubated for 60 min at 0°C prior to sonication with two pulses of 2 s each for the final dissolving of the proteins. To remove remaining insoluble material the solution was centrifuged at 17,000*g* and 4°C for 10 min. The supernatant was directly applied to a 2-ml chelating Sepharose FF column (Pharmacia), which had been charged with nickel ions according to the man-

[2] Abbreviations used: GST, glutathione *S*-transferase; His, histidine; His–P68(A′-B′), fusion protein of histidine hexapeptide and the antigenic epitopes A′ and B′ of P68; IPTG, isopropyl-β-D-thiogalactoside; MCTD, mixed connective tissue disease; P68, 68-kDa (U1) ribonucleoprotein antigen; P68(A′-B′), antigenic epitopes A′ and B′ of P68; PBS, phosphate-buffered saline.

ufacturer's standard protocol (Pharmacia) and equilibrated with loading buffer. Before chromatography, the column was washed with Tris–acetate buffer at pH 4.0 and reequilibrated with sample buffer. The column was developed with a pH-step gradient in 6 M urea and Tris–acetate buffer. The solubilized proteins were loaded onto the column at pH 7.2 and washed sequentially with buffers at pH 6.5, pH 6.0, and pH 5.5. The recombinant protein was eluted with pH 5.0 buffer, dialyzed against water, and lyophilized for storage.

All fractions derived from the purification steps were adjusted to total amounts of proteins of a 100-μl aliquot of the cell culture and analyzed by 12.5% SDS–polyacrylamide gel electrophoresis. The yield of purified recombinant protein was measured with the bicinchoninic acid protein assay kit (Sigma).

Western Blotting Analysis of the Recombinant His–P68(A'-B') Protein

The total cell lysate was resuspended in sample buffer, separated by 12.5% SDS–polyacrylamide gel electrophoresis under reducing conditions, and transferred electrophoretically onto a nitrocellulose filter (Amersham) using a trans-blot semi-dry electrophoretic transfer cell (Bio-Rad). Unoccupied protein-binding sites on the filter were blocked with 5% nonfat dried milk in TBST buffer (10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) for about 16 h. The immobilized proteins were incubated for 60 min with a 1000-fold dilution of autoimmune sera from MCTD patients. The diluted sera had been preabsorbed with 0.2 mg/ml *E. coli* crude cell lysate for 16 h at 4°C prior to incubation with antigen. The bound antibodies were visualized with anti-human immunoglobulins conjugated with alkaline phosphatase (Promega) after incubation for 45 min followed by a color reaction using nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate as substrates (Promega).

Autoantisera from MCTD Patients

The autoimmune sera from MCTD patients studied here were kindly made available by Professor H. J. Lakomek, University of Minden, Germany, and positively identified and characterized by ELISA techniques using human ribonucleoprotein antigens (12).

RESULTS

Construction of the Expression Vector pH6EX3

The initial vector pGEX-2T (8) was modified by replacement of the GST gene with a 76-bp linker containing the coding sequences for a histidine hexapeptide and the recognition site for the proteinase thrombin followed by the multicloning site with eight restriction sites. The thrombin-cleavage site provides the option of cleaving the histidine hexapeptide of the purified fusion protein, if necessary. The expression vector constructed, pH6EX3, is 4856 bp long (Fig. 1) and carries both the ampicillin-resistant and the lacI^q repressor genes. The restriction sites of the multicloning site allow the insertion of cDNA in all three open reading frames in order to obtain a correct fusion protein with the histidine hexapeptide. In addition, the locations of the *Eco*RI and *Xho*I sites in the multicloning site were designed to accept cDNA inserts directly from λ-ZAPII clones positively identified by immunoscreening (13). The fusion genes constructed are strongly controlled by the trp–lac (*tac*) promoter (14). The protein synthesis directed by the *tac* promoter is normally repressed by the lac repressor mediated by the lacI^q repressor gene of pH6EX3 until induction with IPTG. This allows cloning and expression using the pH6EX3 vector regardless of the lacI status of the *E. coli* host used. The strong *rrnB* ribosomal RNA termination sequence located after the multicloning site and the translational stop signal stabilizes the host–vector system and prevents possible overexpression of downstream sequences within the vector (data not shown).

Construction and Expression of pP68.201

A 432-bp cDNA fragment coding for the antigenic epitopes A' and B' of the human 68-kDa (U1) ribonucleoprotein antigen was isolated and cloned as described previously (11). For expression the corresponding cDNA was inserted into the vector pH6EX3, yielding a fusion gene with an open protein reading frame coding for the fusion protein His–P68 with a histidine hexapeptide and a thrombin-cleavage site (2.4 kDa) at its N-terminus and the recombinant P68 antigenic epitope (17.5 kDa) at its C-terminus.

Five *E. coli* strains, LE392, CAG440, CAG456, GE196, and K5254, were transformed with pP68.201 and tested for optimal yield of the expressed recombinant His–P68 fusion protein. As controls, the same *E. coli* strains were transformed with pH6EX3. Strains CAG440, CAG456, GE196, and K5254 are deficient in the *lon* proteinase, which may allow increased stability of foreign proteins (15). After induction with 1 mM IPTG for 8 h, bacterial lysates were separated by SDS–polyacrylamide gel electrophoresis and analyzed either by staining (Fig. 2A) or by Western blotting using a characterized autoantiserum from a patient with MCTD (Fig. 2B). The expression of the His–P68 fusion protein with apparent molecular weight of 20 kDa was observed in all *E. coli* strains transformed with pP68.201 (Fig. 2, lanes 4, 6, 8, 10, 12). The highest yield of recombinant protein was obtained with *E. coli* strain K5254 (Fig. 2, lane 12). The recombinant His–P68 fusion protein gradually accumulated in the K5254 cells over 8 h (Fig. 3). Longer incubation periods did not increase the yield of recombinant

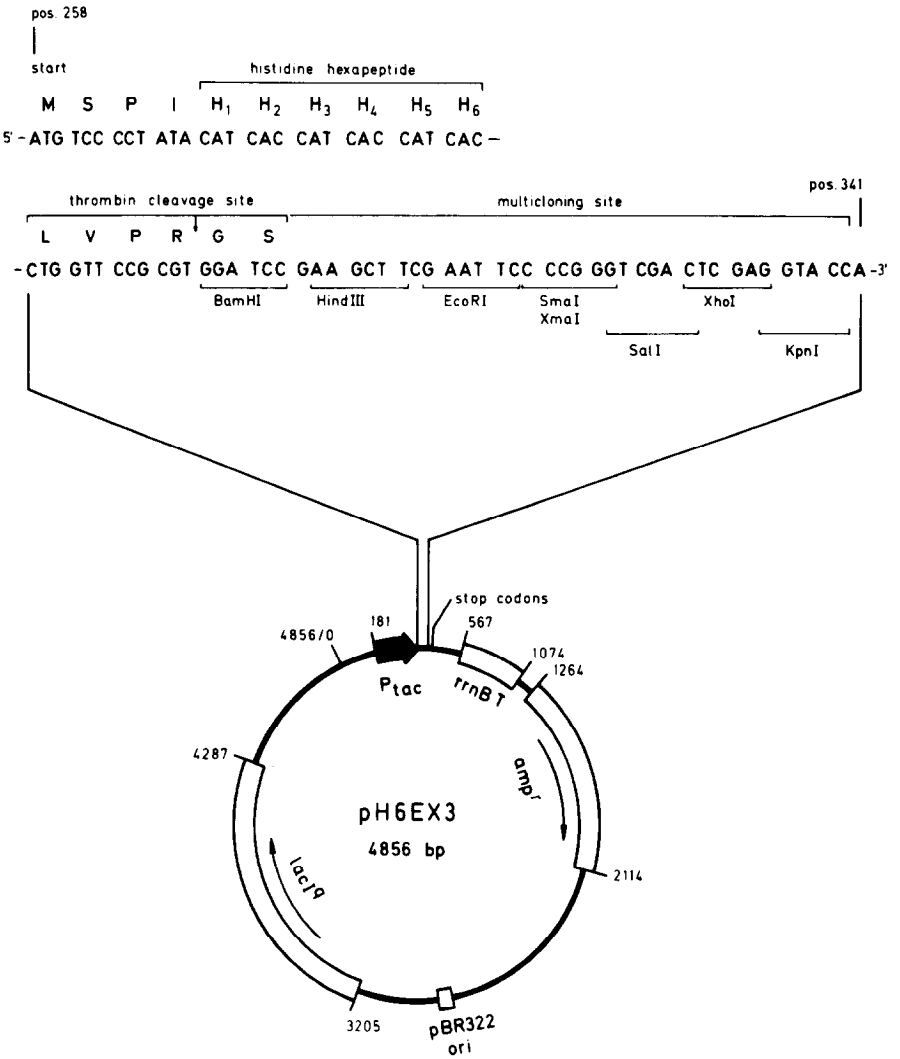

FIG. 1. Structure of the expression vector pH6EX3. The 4856-bp pH6EX3 possesses the ampicillin-resistant gene (amp^r), the modified *E. coli* lac repressor gene ($lacI^q$), and the transcriptional termination sequence (*rrnB*T). The *tac* promoter (P_{tac}) controls the expression of the fusion protein with a histidine hexapeptide (H_1–H_6) at its N-terminus. A multicloning site between positions 300 and 340 containing eight different restriction sites and a sequence coding for a site-specific recognition for thrombin are located adjacent to the histidine hexapeptide followed by three stop codons in all three reading frames.

protein (data not shown). Under optimal conditions the recombinant His–P68 fusion protein constituted up to 20% of total cellular proteins. As a control, *E. coli* strain LE392 was transformed with plasmids pGEX-2T (8) and pP68.2 (11), which direct the synthesis of GST (26 kDa) and GST–P68(A'-B') fusion protein (43 kDa), respectively (Fig. 2, lanes 1, 2).

Purification of the Recombinant His–P68 Fusion Protein

The *E. coli* K5254 cell culture transformed with pP68.201 was induced with 1 mM IPTG for 8 h and harvested. After lysis of cells by treatment with lysozyme and sonication, the cell homogenate was separated into soluble and insoluble cell fractions (Fig. 4, lanes 1, 2, 3). As shown in Fig. 4 (lane 3) the His–P68 fusion protein remained entirely in the insoluble cell fraction representing the inclusion bodies. The insoluble proteins were dissolved in 6 M urea, loaded onto a chelating Sepharose column charged with Ni^{2+} ions, and washed with a pH-step gradient from pH 7.2 to 5.5 (Fig. 4, lanes 4, 5, 6, 7). The His–P68 fusion protein was specifically eluted at pH 5.0 in a highly purified form (Fig. 4, lane 8). About 1.25 mg purified His–P68 fusion protein was isolated from a 50-ml bacteria culture. The yield was strongly dependent on the synthesis in inclusion bodies and on the efficiency of the solubilization of the inclusion bodies containing the recombinant protein.

The purity of the His–P68 fusion protein was studied by Western blotting analysis using two selected autoim-

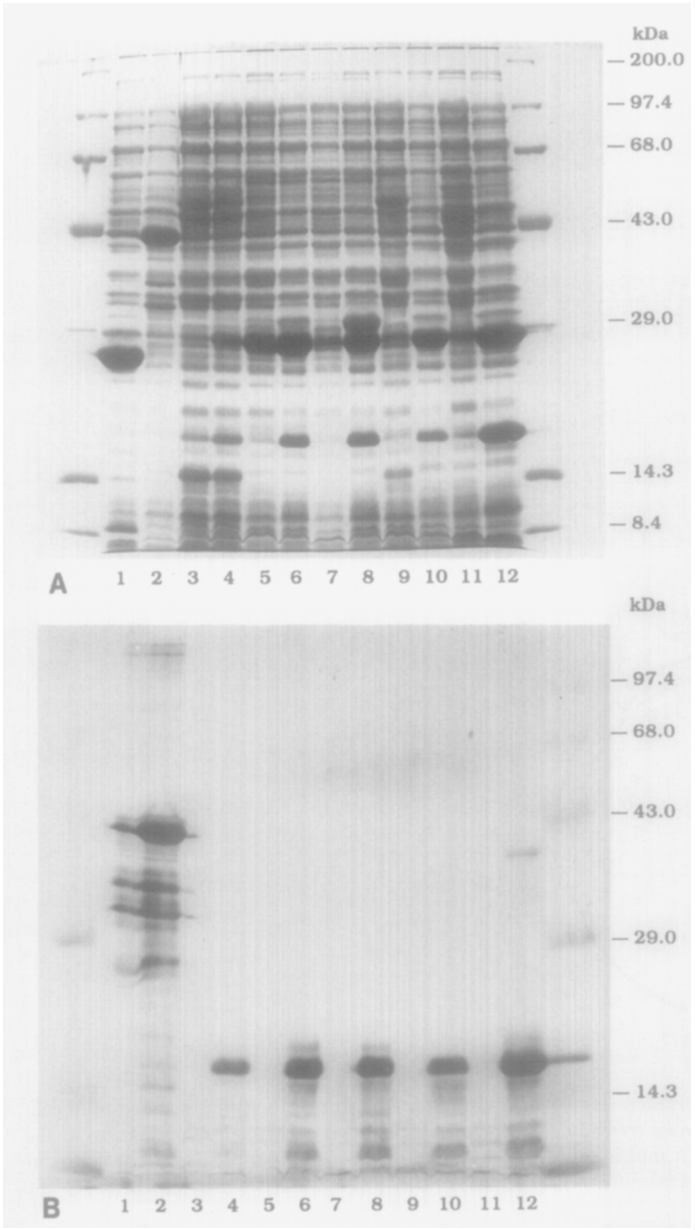

FIG. 2. The expression of recombinant His–P68(A′-B′) in various *E. coli* strains. Five different *E. coli* strains (LE392, CAG440, CAG456, GE196, and K5254) were transformed with pH6EX3 or pP68.202. Aliquots of 100 μl of bacterial culture induced with 1 mM IPTG for 8 h were sedimented, resuspended in sample buffer, and applied to a 12.5% SDS–polyacrylamide gel. After separation the proteins were stained with Coomassie brilliant blue (A) or analyzed by Western blotting (B). LE392 transformed with pH6EX3 (lane 3) or pP68.202 (lane 4). CAG440 transformed with pH6EX3 (lane 5) or pP68.202 (lane 6). CAG456 transformed with pH6EX3 (lane 7) or pP68.202 (lane 8). GE196 transformed with pH6EX3 (lane 9) or pP68.202 (lane 10). K5254 transformed with pH6EX3 (lane 11) or pP68.202 (lane 12). As a control, LE392 was transformed with pGEX-2T (lane 1) or pP68.2 (lane 2).

mune sera from MCTD patients (Figs. 5A and 5B). The main immune signal corresponded to the eluted protein band representing the His–P68 fusion protein. Stained protein bands with lower apparent molecular weights that are visible in the Western blot analysis but not after staining with Coomassie brilliant blue either represent protein chains that were not completed during the synthesis or are due to partial degradation of the fusion protein.

DISCUSSION

The use of modern recombinant technology enables the transfer and expression of heterologous genes in bacterial cell systems such as *E. coli*, providing a facile source of purified proteins and polypeptides for research and diagnostic systems. Many vector/host systems have been developed for high-level production of the foreign proteins. Proteins expressed directly or as fusion proteins constitute up to 25% of total cell proteins. High-level production of recombinant proteins usually leads to an intracellular accumulation of proteins in the form of insoluble inclusion bodies (1,2). This was also observed with proteins that are normally produced in soluble form. The prediction and the cause of formation of inclusion bodies are not yet understood. Formation is believed to be independent of amino acid sequence, although some investigators have hinted that certain sequences can trigger inclusion body formation (4). Proteins sequestered in inclusion bodies are protected from proteolytic degradation and do not disturb the osmotic balance in the cell after accumulation. Insoluble proteins cannot be purified using normal chromatographic approaches to separate the recombinant pro-

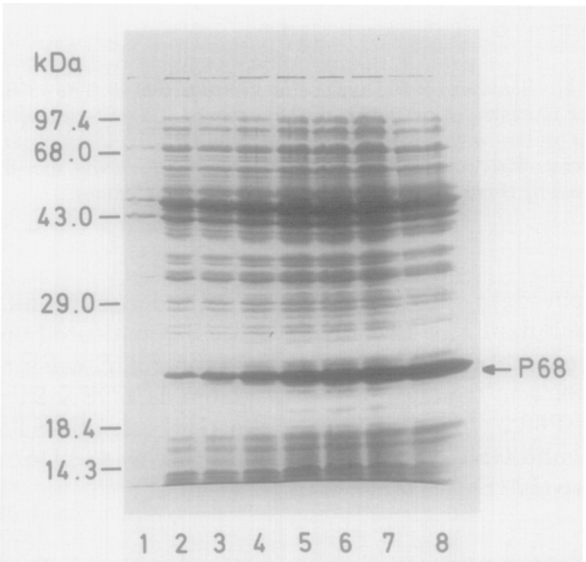

FIG. 3. Expression kinetics of recombinant His–P68 (A′-B′). Cultures of *E. coli* K5254 transformed with pP68.202 were induced with 1 mM IPTG for 0 h (lane 1), 2 h (lane 2), 4 h (lane 3), 6 h (lane 4), 8 h (lane 5), 10 h (lane 6), 12 h (lane 7), and 24 h (lane 8). Aliquots of resuspended total bacterial proteins representing 100-μl cultures were separated by 12.5% SDS–polyacrylamide gel electrophoresis and stained.

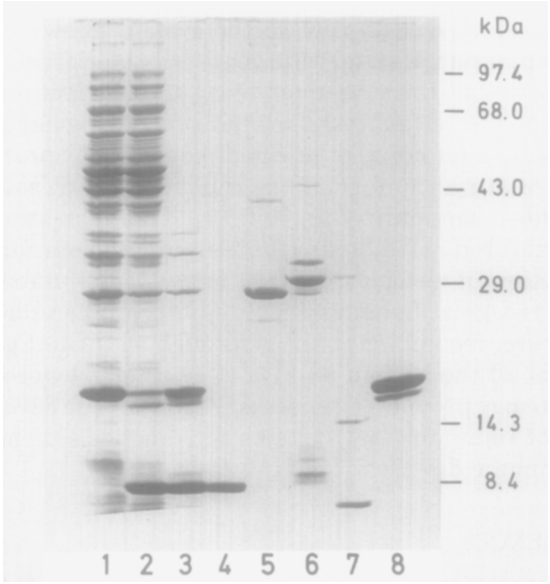

FIG. 4. Purification of recombinant His–P68(A'-B'). *E. coli* strain K5254 transformed with pP68.202 was cultured, induced with 1 mM IPTG for 8 h, and fractionated to purify the recombinant His–P68(A'-B') protein. Aliquots containing equal amounts of protein were taken from different cell fractions and from several steps of the purification procedure and analyzed by 12.5% SDS–polyacrylamide gel electrophoresis. The insoluble cell fraction was dissolved in 6 M urea and purified by affinity chromatography using chelating Sepharose loaded with Ni^{2+}. All samples analyzed were adjusted to represent 100-μl aliquots of the original cell culture. *E. coli* cell lysate (lane 1), soluble cell fraction (lane 2), insoluble cell fraction containing the His–P68(A'-B') protein (lane 3), column flowthrough (lane 4), fraction eluted at pH 6.5 (lane 5), fraction eluted at pH 6.0 (lane 6), fraction eluted at pH 5.5 (lane 7), fraction eluted at pH 5.0 containing the purified His–P68(A'-B') protein (lane 8).

teins from the bacterial cell lysate. Several vectors have been constructed for the expression of fusion proteins composed of the recombinant protein and a ligand-binding moiety which is used for specific affinity chromatography (for review see Ref. (3)). Most of these techniques were developed and are only useful for the purification of soluble recombinant proteins that have been restored their natural conformation and their normal binding affinity to an immobilized matrix. Few of the methods previously described allow the purification of insoluble proteins from bacterial cell lysates to homogeneity with acceptable yields using specific affinity chromatography.

Normally, an excessive accumulation of foreign proteins in bacterial cells is prevented by proteolytic degradation. The proteinases serve as a cellular sanitation system that is selectively regulated. The problem in expressing recombinant proteins in a bacterial host is realized when this host security system is antagonistic to expression of the desired proteins. There are three main approaches can be used to avoid unnecessary degradation of intracellularly expressed recombinant proteins in bacterial cells.

1. The expressed proteins must share some common conformational or sequential homologies with host housekeeping or metabolically active proteins, preventing their rapid turnover. The fusion gene strategy allows the expression of bifunctional fusion proteins with the recombinant protein of interest and a fusion partner that mimicks the bacterial degradation system protecting the fusion protein. In addition, the use of fusion genes facilitates the purification of fusion proteins by specific affinity chromatography. Depending on the properties and structure of its fusion partner, the recombinant protein may lose its biological activity or/and conformational integrity. For kinetic studies of active proteins and for refolding to native conformation, the fusion gene approach often requires the removal of the recombinant protein from the fusion protein and its subsequent purification.

2. *E. coli* strains deficient in the *lon* proteinase (15), such as strains CAG440, CAG456, GE196, and K5254 used in this study (Fig. 2), can be constructed. The *lon* proteinase is one of several proteinases responsible for the generally low stability of foreign or otherwise abnormal proteins in *E. coli* (15).

3. Strong bacterial promoters allow overexpression of recombinant proteins, which often accumulate as an insoluble protein mass shown as inclusion bodies. Recombinant proteins sequestered in these inclusion bodies are also protected from the proteinase system of the host cell, but are difficult to purify by traditional methods.

It was necessary to obtain a bacterial expression system that not only provides high-level expression of re-

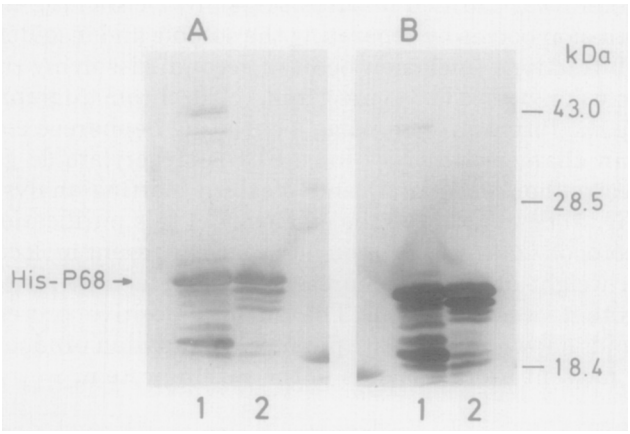

FIG. 5. Western blotting analysis of purified recombinant His–P68(A'-B'). Aliquots of 10 μl of resuspended total bacterial proteins from a 100-μl culture of K5254 transformed with pP68.201 after induction with 1 mM IPTG for 8 h (lane 1) and 5 μg of purified recombinant His–P68(A'-B') (lane 2) were separated by 12.5% SDS–polyacrylamide gel electrophoresis. The proteins were electrophoretically transferred onto nitrocellulose filters and analyzed with two selected autoantisera from MCTD patients by Western blotting (A and B).

combinant protein without fusion to complicated and sensitive ligand moieties but also simplifies the protocol for purification of recombinant proteins even from inclusion bodies. Here we describe the construction of a new expression plasmid vector designated pH6EX3 (Fig. 1) which is based on the expression of a fusion protein with a histidine hexapeptide at its N-terminus and the recombinant protein at its C-terminus. This vector system allows facile purification of recombinant proteins stored in bacterial inclusion bodies and is compatible with cloning systems commonly used today. The synthesis of the fusion protein is controlled by the *tac* promoter (14), which is inducible by IPTG. The powerful *tac* promoter mediates high-level synthesis of recombinant proteins, which are mainly deposited as insoluble masses in inclusion bodies, as shown by experiments with six cDNAs coding for different eukaryotic structural and serum proteins (data not shown). A multicloning site containing eight restriction sites allows the insertion of cDNA sequences in all three possible protein reading frames fused to the sequence coding for the histidine hexapeptide. The histidine-hexapeptide moiety allows easy purification of insoluble proteins made soluble in either 8 M urea or 6 M guanidinium-hydrochloride by retention on a Ni^{2+}-Sepharose column and elution over a pH gradient.

To test the designed expression vector, pH6EX3, we inserted a 432-bp cDNA fragment coding for the antigenic epitopes A′ and B′ of the human 68-kDa (U1) ribonucleoprotein autoantigen (11,12,16) in this vector and transformed several *E. coli* strains. The highest yield of the fusion protein composed of the histidine hexapeptide and the recombinant autoepitopes was observed in *E. coli* strain K5254 (Fig. 2). K5254 cells are deficient in the *lon* proteinase. The recombinant His–P68 fusion protein was purified in three steps: first, enriching the inclusion bodies by separating the soluble and insoluble cell fractions (inclusion bodies); second, dissolving the inclusion bodies in 6 M urea; and, third, chromatographing the fusion protein using a chelating Sepharose column charged with Ni^{2+} ions. SDS–polyacrylamide gel electrophoresis (Fig. 4) and Western blotting analysis (Fig. 5) demonstrated the efficiency of this purification protocol. One main protein band with apparent molecular weight of 20 kDa, representing the His–P68 fusion protein, was observed. The smaller positive protein bands shown in Fig. 5 are possible degradation products or nascent protein chains of the fusion protein.

In conclusion, this new vector system allows high-level expression of eukaryotic genes in bacterial cell systems as well as the subsequent easy and efficient purification of the recombinant proteins to homogeneity. If necessary, a refolding of purified recombinant proteins to their natural conformations is possible using several techniques summarized in Ref. (5). The expression of the denatured recombinant proteins is sufficient for the production of antigens because many of the autoantibodies recognize linear antigenic epitopes. Therefore, in the future, recombinant antigens such as the antigenic epitopes of the human 68-kDa (U1) ribonucleoprotein antigen may provide the necessary tools for the development of immunoassay systems for diagnosis of many autoimmune diseases.

REFERENCES

1. Marston, F. A. O. (1987) *in* "DNA Cloning: A Practical Approach" (Glover, D. M., Ed.), Vol. 3, pp. 59–88, IRL Press, Oxford Washington.
2. Williams, D. C., Van Frank, R. M., Muth, R. M., and Burnett, J. P. (1982) *Science* **215,** 687–689.
3. Uhlén, M., and Moks, T. (1990) *in* "Methods in Enzymology" (Goeddel, D. V., Ed.) Vol. 185, pp. 129–143, Academic Press, San Diego.
4. Tonouchi, N., Oochi, N., Kashima, N., Kawai, M., Nagase, K., Okano, A., Matsui, H., Yamada, K., Hirano, T., and Kishimoto, T. (1988) *J. Biochem.* **104,** 30–34.
5. Kelley, R. F., and Winkler, M. E. (1990) *Genet. Eng.* **12,** 1–19.
6. Hochuli, E., Doeblei, H., and Schacher, A. (1987) *J. Chromatogr.* **411,** 177–184.
7. Hochuli, E., Bannewarth, W., Doebli, H., Gentz, R., and Stueber, D. (1988) *Biotechnology* **6,** 1321–1326.
8. Smith, D. B., and Johnson, K. S. (1988) *Gene* **67,** 31–40.
9. Brosius, J. (1984) *Gene* **27,** 161–172.
10. Brosius, J., and Holy, A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6929–6933.
11. Frorath, B., Scanarini, M., Netter, H. J., Abney, C. C., Liedvogel, B., Lakomek, H. J., and Northemann, W. (1991) *Biotechniques* **11,** 364–371.
12. Netter, H. J., Guldner, H. H., Szostecki, C., Lakomek, H. J., and Will, H. (1988) *Arthritis Rheum.* **31,** 616–622.
13. Short, J. M., Fernandez, J. M., Sorge, J. A., and Huse, W. D. (1988) *Nucleic Acids Res.* **16,** 7583–7600.
14. Aman, E., Brosius, J., and Ptashne, M. (1983) *Gene* **25,** 167–178.
15. Charnay, P., Gervais, M., Louise, A., Galibert, F., and Tiollais, P. (1980) *Nature* **286,** 893–895.
16. Theissen, H., Etzerodt, M., Reuter, R., Schneider, C., Lottspeich, F., Argos, P., Lührmann, R., and Philipson, L. (1986) *EMBO J.* **5,** 3209–3217.