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Selection and Characterization of Phage Miniantibodies to Actins of Different Origin

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Abstract—Single-chain miniantibodies (scFv's) to actin were obtained by the phage display method. A naive combinatorial phage display library of murine scFv's (containing $2 \cdot 10^8$ independent recombinant clones) was used to select miniantibodies. After three rounds of selection two clones producing miniantibodies to chicken smooth muscle actin with affinity constants of $1.4 \cdot 10^7$ and $1.2 \cdot 10^6$ M^{-1} were chosen. The isolated miniantibodies could specifically detect various plant and animal actins.

Key words: actin, phage display of miniantibodies, affinity selection, single-chain miniantibodies, colloidal gold, dot-blot assay

Actin is one of the main eukaryotic structural proteins. Its intracellular concentration may reach 100 mM. Actin plays a crucial role in muscle contraction by forming (together with myosin) components of the actomyosin motility system. The monomeric form of this protein can readily undergo polymerization with formation of filaments of 6 nm in diameter. The polymeric form of actin is present not only in muscle cells but also in many other cells of eukaryotic organisms. In contrast to the majority of cellular proteins it can undergo reversible polymerization/depolymerization, and this phenomenon is crucial for numerous processes in living cells such as directed movement of cytoplasm and organelles, mitosis, meiosis, plasma membrane receptor signal transduction, etc. [1]. This has stimulated much interest in studies of biochemical and physiological properties of both actin and actin-binding proteins. Actin-binding proteins control the specific polymerization/depolymerization cycle, cross-linking, and tight package of actin filaments into fibers and networks [2, 3]. These sequential polymerization/depolymerization cycles yield actin-rich preparations *in vitro*.

Actin is one of the most conservative proteins in the living world. Amino acid sequences of amoeba and human actins revealed their 95% identity [4]; yeast and soybean actins share 85% identity with mammalian actins [5, 6]. However, the number of genes encoding actins and their localization in the genome vary in different taxonomic groups. Recently actins have also been found in plant cells, prokaryotes, and in viruses [7-9]. In lower algae, actin is encoded by a single gene. Genes encoding actins in higher eukaryotes usually exist in several copies [10]. Similarity and differences of amino acid sequences of eukaryotic, prokaryotic, and viral actins underline both biochemical properties and morphological parameters of filaments recognized by electron microscopy [8].

An immunochemical method plays an important role in detailed structural–functional studies of various actins including actins of plant cytoskeleton [11, 12]. However, high evolutionary conservativeness of actins prevents elaboration of highly specific and high affinity antibodies to such antigen in animal organisms. High identity degree of amino acid sequences (and consequently low immunogenicity) seriously complicates elaboration of antibodies to actin. In report [13], only one of 15 rabbits gave positive immune response to chicken smooth muscle actin with antibody titer of 1 : 250 in

Abbreviations: CFU) colony forming unit; DTT) dithiothreitol; ELISA) enzyme-linked immunosorbent assay; scFv) single chain miniantibodies; IPTG) isopropyl-β-D-thiogalactopyranoside; PMSF) phenylmethylsulfonyl fluoride; PEG) polyethylene glycol; TLCK) 1-chloro-3-tosylamido-7-amino-2-heptanone.

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blood serum. Nevertheless, immunocytochemical and histochemical analyses of actins in plant cytoskeleton require preparative quantities of stable affinity antibodies to actin. So the major goal of this study was to develop the method of preparation of such antibodies using a phage display library followed by subsequent heterologous expression of antigen-binding immunoglobulin fragments, known as miniantibodies, using an *in vitro* system.

The method of phage display implies selection of clones producing recombinant miniantibodies of required specificity using representative combinatorial libraries of miniantibodies exposed in one of the capsid proteins of the bacteriophage surface [14]. Miniantibodies (in our case scFv-fragments) represent expression products of V_H and V_L chains of immunoglobulins randomly linked by a flexible hydrophilic peptide $(G_4S)_{3-4}$, providing spatial approaching of domains and normal functioning of the resultant molecule. For elaboration of these miniantibodies, a combinatorial phagemid library of murine scFv has been employed [15]. Each bacteriophage (as well as B-lymphocyte) exposes specific antibody. With a relatively large size of such library a "repertoire" of variable sites is comparable with the antibody repertoire in animal organisms. Bacteriophages carrying antigen-binding antibody fragments of required specificity can be chosen by selection on an immobilized antigen.

Use of the method of phage display, representing one of the perspective directions of biotechnology, can provide miniantibodies of required specificity; these can be used for both diagnostics and therapeutics. Use of the method of phage display in relation to antibodies to actin may overcome problems of low antigen immunogenicity. In the present study, we have selected miniantibodies specific to chicken smooth muscle actin. Using a naive combinatorial phage display library of murine scFv, we obtained a preparation of specific miniantibodies to actin by heterologous expression in *E. coli* cells, and characterized antibody affinity to antigen. We have also demonstrated the possibility of application of these antibodies for detection of various actins.

MATERIALS AND METHODS

Bacterial strains *Escherichia coli* XL1-Blue and BL21 (DE3) pLys S were used in the study. Yeast extract and tryptone were from Difco (USA); acrylamide and phenylmethylsulfonyl fluoride (PMSF) were from Serva (Germany); *bis*-acrylamide, EDTA, dithiothreitol (DTT), and Tween-20 were from Fluka (Switzerland); 2-mercaptoethanol and polyethylene glycol (PEG)-6000 were from Ferak (Germany); Tris, leupeptin, pepstatin A, 1-chloro-3-tosylamido-7-amino-2-heptanone (TLCK), and isopropyl-β-D-thiogalactopyranoside (IPTG) were from Sigma (USA); ATP was purchased from Boehringer (Germany). Other chemicals produced by Russian suppliers had "chemically pure" and "specially chemically pure" grades.

Isolation of actin preparations. Animal actins were isolated from rabbit back muscles and chicken stomachs. Rabbit actin was isolated by the classical method of polymerization/depolymerization as described [16, 17]. Chicken actin was purified from stomach acetone powder [18].

Leaves of *Vicia faba* L. (Black Russian) were used as the source of plant actin, which was isolated by the method of polymerization/depolymerization [19]. Leaves frozen at −70°C, were ground in a mortar and mixed (1 : 4 w/v) with extraction buffer (0.4 M Tris-HCl, pH 7.5, 5 mM DTT, 0.5 M KCl, 0.1 mM PMSF, 0.01 mM pepstatin A, 0.5 μM leupeptin, 0.5 μM TLCK). This homogenate preincubated at 4°C for 1.5 h was then homogenized using a blender at 3000 rpm for 3 min. The resultant homogenate was centrifuged under refrigeration (4°C) for 30 min at 5000*g* and the supernatant was filtered through two layers of coarse calico and subjected to ammonium sulfate fractionation. The protein fraction sedimented at 30-60% of ammonium sulfate saturation was dialyzed against depolymerizing buffer (5.5 mM Tris-HCl, pH 7.7, 0.5 mM ATP, 0.2 mM $CaCl_2$, 0.1 mM PMSF, 0.01 mM pepstatin A, 0.5 μM leupeptin, 0.5 μM TLCK) at 4°C for two days. The dialyzate was further centrifuged using a Beckman L7-55 centrifuge (USA) at 80,000*g* for 30 min. After addition of KCl and $MgCl_2$ (final concentrations 100 and 2 mM, respectively) the resultant supernatant was incubated for 2 h at room temperature and then KCl concentration was increased to 0.5 M. The preparation was left for overnight incubation at 4°C to get better actin polymerization. The polymeric actin was sedimented by ultracentrifugation at 100,000*g* for 1.5 h. The sediment was suspended in the extracting buffer.

Purity of actin preparations was monitored by electrophoresis in 12% polyacrylamide gel under denaturing conditions [20]. Electrophoretic purity of animal and plant actins was 99 and 90%, respectively.

Antibody elaboration by immunization of animals. Rabbits were immunized with homogenous chicken smooth muscle actin using colloidal gold as an antigen carrier. Actin conjugate with colloidal gold (average particle size of 15 nm in diameter) was prepared as described [21]. Conjugation was carried out by simple mixing of gold sol and actin without use of cross-linking agents; stabilizing concentration of actin determined by a "golden number" was 10 μg/ml of sol. Conjugate stability was evaluated by lack of aggregation (change of color from red to blue or gray) after addition of 10% aqueous solution of NaCl to the final concentration 1%.

For the first immunization, we used a mixture of conjugate with complete Freund's adjuvant; this mixture was subcutaneously injected into 10 points along the spine (0.5 μg of the antigen per point). Mixing of the conjugate with complete Freund's adjuvant did not result in

aggregation. The next immunization was carried out two weeks later by injecting the conjugate with incomplete Freund's adjuvant. The third immunization was carried out using the colloidal gold conjugate with actin without adjuvant [22, 23].

Blood was collected 10 days after the last immunization; serum titer was determined using ELISA. Immunoglobulin fractions were obtained by ammonium sulfate fractionation [24] followed by subsequent FPLC anion-exchange chromatography (Pharmacia, Sweden) using a Mono-Q column.

Affinity selection of miniantibodies from the phage library. For selection of phages carrying antibodies to actin, a nitrocellulose membrane (Millipore, USA) was used as a solid phase for antigen fixation. The membrane (1 × 1 cm) was incubated overnight in 1 μM actin solution at 4°C. Unbound antigen was washed out with TBS-T buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20). The washed membrane was incubated in 1% horse serum albumin for 30 min and washed five times with TBS-T. The membrane with immobilized actin was placed into 1 ml TBS-T containing a library of phagemid particles (single chain miniantibodies) exposing miniantibodies at the N-terminus of gIIIp protein (concentration 10^{12} CFU/ml). The system was incubated overnight at 4°C. After the incubation, the membrane was washed five times with TBS-T. For elution of bound phagemid particles, the membrane was incubated in 1 ml of 1 μM actin at room temperature for 2 h. Eluted phagemid particles were used for infection of *E. coli* cells (strain XL1). The *E. coli* cells infected with the selected phages were cultivated overnight at 37°C in 10 ml of liquid 2YT medium (16 g tryptone, 10 g yeast extract, 5 g NaCl in 1 liter of distilled water) containing 100 μg/ml of ampicillin and 1% glucose. Ten milliliters of the same medium was inoculated with 1% of the resulting culture, and the cells were cultivated with intensive aeration up to cell concentration A_{600} = 0.3. At this stage of cell cultivation, we added a helper phage M13 K07 (10 phage particles per bacterial cell), and the system was incubated for 1 h at 37°C. After the adsorption of phage particles cells were sedimented by centrifugation at 2000*g* for 10 min. The cell pellet was resuspended in 50 ml of the 2YT medium containing 100 μg/ml ampicillin and 50 μg/ml kanamycin, and the cells were grown overnight at 37°C under intensive aeration. The overnight cell culture was centrifuged at 3000*g* for 40 min. The supernatant containing phage particles was mixed with a solution containing 20% PEG-6000 and 2.5 M NaCl (in the proportion 5 : 1 v/v), and this mixture was incubated in ice for 1.5 h. Phage particles were sedimented by centrifugation at 8000*g* for 10 min, and the sediment was resuspended in the TE buffer containing 10 mM Tris-HCl, pH 7.5; 1 mM EDTA, pH 8.0 (in 1/10 part of the initial volume of culture medium). The resultant preparation was centrifuged under the same conditions and phage particles were sedimented again by adding of PEG/NaCl solution followed by centrifugation; the proportion of supernatant to the PEG/NaCl solution was 5 : 1. The pellet was dissolved in 1 ml of TE buffer. The concentration of phage particles was determined spectrophotometrically using the ratio A_{269} 30 ~ $2 \cdot 10^{14}$ phage particles per ml.

The same method of preparation of phage particles was used for two subsequent rounds of selection, which were carried out under the same conditions but using shorter incubation time and less number of phage particles at the stage of their interaction with the immobilized antigen (1.5 and 1 h incubation at room temperature and 10^{11} and 10^{10} phage particles for the second and the third rounds, respectively).

Immunoscreening of the selected library. This was carried out after the third round of selection. The isolated phage particles were used for infection of *E. coli* cells (strain XL1), which were plated on the agarose 2YT medium containing 100 μg/ml ampicillin and 1 mM IPTG (500-600 colonies per Petri dish of 86 mm in diameter). Dishes were incubated at 30°C up to colony size of 0.2 mm in diameter and the nitrocelloluse disk of the same diameter as the Petri dish (86 mm) was then applied on the agar surface with grown colonies. Before application this disk was sequentially incubated for 1 h in 1 μM actin solution at room temperature and for 30 min in 1% horse serum albumin, washed in TBS-T, and dried. After overnight incubation at 25°C, the filter was carefully taken from the agar surface and washed five times with TBS-T buffer to remove *E. coli* cells. The filter was then incubated with 9E10 antibody solution (Dako, Denmark; working dilution 1 : 3000 in TBS-T) to C-*myc* peptide located at the C-terminus of miniantibodies. The bound 9E10 antibodies were detected by peroxidase labeled antibodies to murine immunoglobulins (Amersham, England; working dilution 1 : 3000 in TBS-T) using the chemiluminescence system ECL (Amersham).

Isolation of recombinant pHEN 1 DNA and transformation of *E. coli* cells (strain BL21). Isolation of recombinant pHEN 1 DNA from the selected clones was carried out using the conventional method [25]; this recombinant pHEN 1 contains an insert encoding a single chain antibody of required specificity.

For preparation of competent cells *E. coli* cells (strain BL21) were cultivated in 2YT medium at 37°C and intensive aeration up to cell concentration A_{600} = 0.5. The resultant culture was cooled in ice for 30 min and the cells were sedimented by centrifugation at 3000*g* for 15 min at 4°C. The sediment was suspended in cooled 10% glycerol (v/v) prepared using Milli-Q water; the volume of glycerol solution corresponded to the initial volume of culture medium. This procedure was repeated twice; after that cells were resuspended in 10% glycerol (in 0.001 volume of the initial culture liquid). Cell suspension was aliquotted into tubes (50 μl), frozen in liquid nitrogen, and kept at −70°C.

Electroporation was carried out in 0.2 cm cuvettes using 50 μl of the competent cells and 1 ng DNA. Right after electric impulse (2500 V) 1 ml of 2YT medium containing 1% glucose and 10 mM $MgCl_2$, cells were carefully suspended, transferred into sterile tubes, and incubated at 37°C for 1 h. One hundredth part was plated on Petri dishes containing agar 2YT medium containing 1% glucose and 100 μg/ml ampicillin. After addition of glycerol to final concentration of 10% the remaining cells were frozen in liquid nitrogen and kept at −70°C.

Isolation of periplasm preparation from *E. coli* cells. Fifty milliliters of 2YT medium containing 1 mM IPTG and 100 μg/ml ampicillin were inoculated with one colony of *E. coli* cells (strain BL21) transformed with the recombinant plasmid pHEN 1. After overnight cultivation at 29°C and intensive aeration the cell culture was cooled in ice to 4°C and centrifuged at 4000*g* for 10 min. Cells were resuspended in cold 200 mM borate buffer, pH 8.0, and incubated in ice for 30 min. The cell suspension was centrifuged again. The sediment was discarded and the supernatant was centrifuged at 20,000*g* for 30 min at 4°C. The resultant periplasm preparation containing scFv was used for immunochemical tests.

Determination of scFv affinity constants. Affinity constants K_{aff} were determined as described in [26] using indirect ELISA. Periplasm extract containing secreted miniantibodies was pipetted into wells of an immunoplate (Lenmedpolymer, Russia) in the following concentrations: 10, 5, and 2.5 μg total protein per ml. Electrophoretic analysis revealed that miniantibodies represented about 20% of total protein content of the periplasm extract. Free binding sites in the plate wells were then blocked by adding 1% horse serum albumin and biotinylated chicken smooth muscle actin was added in sequential twofold dilution series. (Initial concentration of the biotinylated protein was 50 μg/ml in TBS-T.) The content of bound actin was quantified after interaction with a streptavidin–peroxidase conjugate (Amersham; working dilution of 1 : 3000 in TBS-T) using *o*-phenylenediamine (Sigma) as peroxidase substrate for photometric detection of the colored oxidation product. Affinity constants were calculated using titration plots of the dependence of absorbance value at 492 nm on $-\log_2$ (actin dilutions). The K_{aff} was calculated using the following formula:

$$K_{aff} = 1/(2[Ag'] - [Ag]),$$

where [Ag] and [Ag′] are the antigen concentrations corresponding to the bend points at C_0 and $C_0/2$ antibody concentrations, respectively.

Actin biotinylation. Actin was biotinylated as described in [27]. Solution of purified smooth muscle actin (1 mg/ml) in 0.1 M sodium carbonate/bicarbonate buffer, pH 9.5, was mixed with 70 mM biotin N-hydroxysuccinimide ether in dimethyl formamide (protein/biotin ether ratio of 1 : 3). The mixture was incubated at room temperature for 2 h. The reaction was stopped by adding 1 M NH_4Cl (20 μl NH_4Cl per mg protein) and the reaction mixture was dialyzed against 1000 volumes of TBS-T. Antibodies to biotin labeled with colloidal gold were used as a developing agent [22].

Dot-analysis. Specificity of single chain miniantibodies and rabbit antibodies to plant and animal actins was evaluated by the method of dot-analysis. Antibodies labeled with horseradish peroxidase and colloidal gold were used as the detecting agents [23]. One microliter of sequential twofold dilution series of antigen solution (initial concentration of 300 μg/ml) was applied to centers of framed quadrants (5 × 5 mm) on a nitrocellulose membrane. Sites of nonspecific binding were blocked by adding 1% horse serum albumin for 30 min. This pretreated membrane was incubated in the periplasm extract. Monoclonal antibodies 9E10 were used as secondary antibodies (working dilution 1 : 3000 in TBS-T). After washing, biotinylated rabbit antibodies to murine immunoglobulins (working dilution 1 : 300 in TBS-T) were added. After these treatments, the membrane was incubated with the marker, antibodies to biotin labeled with colloidal gold (average diameter of particles of 15 nm, absorbance $A_{520} = 0.5$). We described the synthesis of this marker previously [21]. The result of reaction is developed as characteristic red staining in places of specific interaction between the marker and antibodies.

Sensitivity of rabbit polyclonal antibodies to actin was evaluated by the method of dot-analysis similar to that described above. A membrane with adsorbed antigens was incubated in solution of rabbit anti-actin IgG (working dilution of 1 : 300 in TBS-T). Bound antibodies to actin were detected by sheep antibodies to rabbit IgG labeled with horseradish peroxidase (Gamaleya Institute of Microbiology and Epidemiology, Russian Academy of Medical Sciences, Russia; working dilution 1 : 200) using diaminobenzidine (Sigma).

RESULTS AND DISCUSSION

For selection of miniantibodies, we have used a murine combinatorial phage library constructed in Pushchino Branch of Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry [15]. The repertoire of this library includes $2 \cdot 10^8$ independent recombinant clones. Electrophoretically homogenous chicken smooth muscle actin was used as the antigen for miniantibody selection.

For selection of the most specific and high affinity miniantibodies we used three rounds. During this selection, we decreased the concentration of phage particles from round to round from 10^{12} to 10^{10} ml^{-1}. We also reduced time interval of actin incubation with the phage library: in the first round there was overnight incubation at 4°C, during the second and the third rounds incuba-

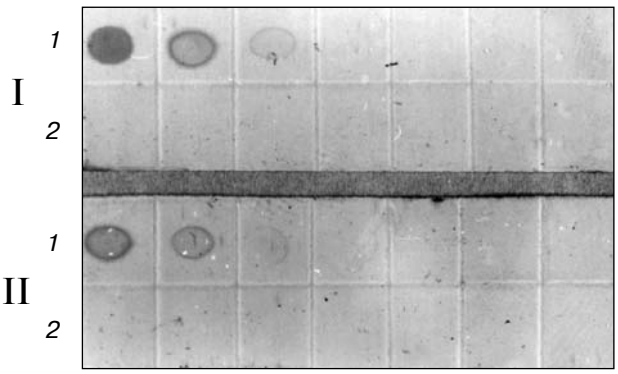

Fig. 1. ELISA dot-analysis of sequential twofold dilution series of chicken smooth muscle actin (initial concentration 300 μg/ml) using miniantibodies produced by clones I and II (*1*); ovalbumin (initial concentration 1 mg/ml) was used as control (*2*).

tions were carried out at room temperature for 1.5 and 1 h.

The enriched library was plated on agarose medium (500-600 colonies per Petri dish). After three rounds of affinity selection, colonies were subjected to immunoscreening using the ECL chemiluminescence system. The grown colonies (~80%) gave positive signal indicating high effectiveness of the procedure of affinity selection. For subsequent work, we selected 13 clones exhibiting the most pronounced signal (evaluated by spot brightness) and isolated pHEN 1 recombinant phagemid DNA from them. This DNA was then used for transformation of *E. coli* cells of non-suppressor strain BL21, which expressed the secreted form of single chain miniantibodies.

Subsequent analysis revealed two clones producing a secreted form of scFv; they were characterized by high viability during long-term cultivation under conditions of induction of scFv secretion. The other 11 clones did not revealed positive signal in the dot-analysis. Figure 1 shows the results of dot-analysis of chicken actin with miniantibodies produced by the two selected clones.

For the miniantibodies produced by the two selected clones, we determined affinity constants by means of the method described in [26]. Calculation of K_{aff} by this method requires precise information about only one reaction component. In contrast to full sized immunoglobulins, the miniantibodies have only one antigen binding site; consequently it is not important which of two components will be used in precise concentration. Since miniantibodies have been obtained as a periplasm preparation representing a mixture of proteins and antigen (actin) preparation as a homogenous protein, the latter has been used as the component with firmly detected concentration. Figure 2 shows the dependence of actin binding by scFv of one clone on actin concentration. This was used for K_{aff} calculation. The K_{aff} values for scFv were $1.4 \cdot 10^7$ and $1.2 \cdot 10^6$ M^{-1} for the first and the second clone, respectively. Results of K_{aff} calculation demonstrate that use of the naive combinatorial phage library of scFv fragments and several rounds of selection with sequential reduction of antigen concentration and incubation time with the phage library resulted in selection of one clone producing soluble miniantibodies with average moderate affinity to chicken smooth muscle actin. These antibodies are suitable for immunohistochemical actin detection; employment of antibodies with higher K_{aff} values often gives false positive results [28].

ELISA assay revealed that titer of the polyclonal anti-actin antiserum obtained by rabbit immunization by the antigen adsorbed on gold nanoparticles was 1 : 1024. Since antiserum contains a mixture of antibodies to actin with various affinities to antigen, K_{aff} was not determined.

We compared sensitivity and specificity of anti-actin polyclonal antiserum and *E. coli* periplasm preparation containing expressed scFv of the highest affinity (K_{aff} of $1.4 \cdot 10^7$ M^{-1}) in the immunodot analysis of various actins. The actin preparations were obtained from smooth and striated animal muscles (rabbit, chicken) and plant leaves (*Vicia faba* L.). Figure 3 shows that miniantibodies expressed by clone I detected all three actins. The sensitivity of immunodot analysis in detection of the three various actins differed insignificantly. The detection limit was 12, 4, and 15 ng for chicken, rabbit, and plant actin, respectively. Broad specificity with respect to various

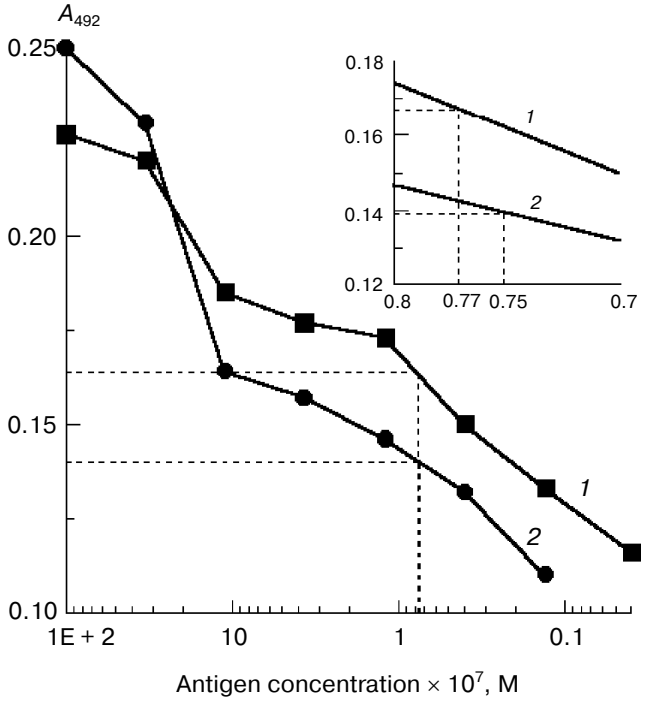

Fig. 2. Determination of affinity constant of clone I antibodies by the method of indirect ELISA. Antigen concentrations: [Ag′] = $0.77 \cdot 10^7$ M^{-1} (*1*), [Ag] = $0.75 \cdot 10^7$ M^{-1} (*2*). The insert shows enlarged scale for more accurate K_{aff} determination.

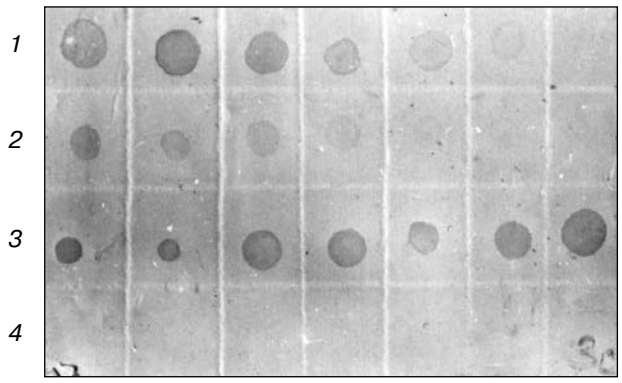

Fig. 3. Dot analysis of various actins using miniantibodies produced by clone I. Sequential twofold dilutions: *1*) chicken smooth muscle actin (initial concentration 0.4 mg/ml); *2*) plant actin from *V. faba* leaves (0.25 mg/ml); *3*) rabbit actin (0.25 mg/ml); *4*) ovalbumin (control). The method is described in the "Materials and Methods" section.

actins and similar values of the detection limits suggest that the single chain miniantibodies of clone I bind to the antigenic determinant, which is highly conservative for all actins used in the experiment.

In contrast to miniantibodies expressed by clone I, the rabbit anti-actin polyclonal antibodies for the immunodot analysis detected only chicken and rabbit actins with the same detection limit of 25 ng. This antibody did not detect the plant actin in the dot-analysis. Thus, rabbit antibodies obtained against chicken smooth muscle actin by immunization of animals with antigen conjugate with colloidal gold exhibited narrower specificity to various actins than did single chain miniantibodies selected from the phage library and expressed in *E. coli* cells.

Rather high sensitivity of secreted single chain miniantibodies in detection of various actins can be subsequently used for the development of methods for actin detection in biological samples by various methods of immune analysis including immunohistochemistry. The ability of the selected clone I for scFv secretion to periplasm of *E. coli* provides for elaboration of preparative quantities of these miniantibodies. These miniantibodies can be further conjugated with the detecting molecules (e.g. horseradish peroxidase or colloidal gold) including methods of gene engineering followed by heterologous expression.

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REFERENCES

1. Williamson, R. E. (1993) *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **44**, 181-202.
2. Esters, J. E., and Higgins, P. J. (eds.) (1994) *Actin: Biophysics*, *Biochemistry*, *and Cell Biology*, Kluwer Academic/Plenum Publishers, Hardbound.
3. Dos Remedios, C. G., and Thomas, D. D. (eds.) (2001) *Molecular Interactions of Actin: Actin Structure and Actin-Binding Proteins*, Springer Verlag, Berlin.
4. Vandekerckhove, J., and Weber, K. (1978) *J. Mol. Biol.*, **126**, 783-788.
5. Ng, R., and Albelson, J. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3912-3917.
6. Shah, D. M., Hightower, R. C., and Meagher, R. B. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 1022-1026.
7. Mathur, J., and Hulskamp, M. (2001) *Curr. Biol.*, **12**, 669-676.
8. Van den Ent, F., Amos, L. A., and Lowe, J. (2001) *Nature*, **413**, 39-44.
9. Nermut, M. V., Wallengren, K., and Pagert, J. (1999) *Virology*, **260**, 23-34.
10. Baskin, T. I. (2000) in *Biochemistry and Molecular Biology of Plants* (Buchanan, B., Gruissem, W., and Jones, R., eds.) American Society for Plant Physiology, Rockville, pp. 202-258.
11. Zak, E. A., Sokolov, O. I., Greengauz, O. K., Bocharova, M. A., and Klyachko, N. L. (1997) *J. Exp. Botany*, **48**, 1019-1026.
12. Greengauz, O. K., Negretskii, V. A., and Sokolov, O. I. (1998) *Cell Biol. Int.*, **21**, 870-872.
13. Craig, S. W., Sanders, S. K., and Pardo, J. V. (1986) in *Methods in Enzymology* (Colowick, S. P., and Kaplan, N. O., eds.) Vol. 134, Academic Press, Orlando, pp. 460-467.
14. McCafferty, J., Griffiths, A. D., Winter, G., and Chiswell, D. (1990) *Nature*, **348**, 552-554.
15. Laman, A. G., Shepelyakovskaya, A. O., Ulitin, A. B., Markova, E. V., Mareeva, T. Yu., Bystrov, N. S., Brovko, F. A., and Nesmeyanov, V. A. (2002) *Bioorg. Khim.*, **28**, 126-134.
16. Pardee, J. D., and Spudich, J. A. (1982) in *Methods in Cell Biology* (Wilson, L., ed.) Vol. 24, Academic Press, New York, pp. 271-289.
17. Spudich, J. A., and Watt, S. (1971) *J. Biol. Chem.*, **246**, 4866-4871.
18. Ebashi, S. (1985) *J. Biochem.*, **97**, 693-695.
19. Sokolov, O. I., Greengauz, O. K., and Richter, T. Ya. (1998) *Fiziol. Rast.*, **45**, 227-234.
20. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
21. Roth, J. (1983) in *Techniques in Immunocytochemistry* (Bullock, G. R., and Petrusz, P., eds.) Vol. 2, Academic Press, London, pp. 217-284.
22. Dykman, L. A., Matora, L. Yu., and Bogatyrev, V. A. (1996) *J. Microbiol. Meth.*, **24**, 247-248.
23. Dykman, L. A., Bogatyrev, V. A., Zaitseva, I. S., Sokolova, M. K., Ivanov, V. V., and Sokolov, O. I. (2002) *Biofizika*, **47**, 632-640.
24. Catty, D., and Raikundalia, Ch. (1991) in *Antibodies. Methods* (Catty, D., ed.) [Russian translation], Mir, Moscow, pp. 33-115.
25. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1984) *Methods of Gene Engineering. A Laboratory Manual*, *Molecular Cloning* [Russian translation], Mir, Moscow.
26. Beatty, J. D., Beatty, B. G., and Vlahos, W. G. (1987) *J. Immunol. Meth.*, **100**, 173-179.
27. Bayer, E. A., and Wilchek, M. (1980) *Meth. Biochem. Anal.*, **26**, 1-45.
28. Burton, D. R. (1995) *Immunotechnology*, **1**, 87-94.