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Expression immunoassay based on antibodies labeled with a deoxyribonucleic acid fragment encoding the α-peptide of β-galactosidase

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An immunoassay is reported which uses, as a label, an expressible DNA fragment encoding the α-peptide of β-galactosidase. This inactive peptide consists of 97 amino acid residues containing an amino-terminal portion of the enzyme. Antigen (an anti-thyrotropin immunoglobulin) immobilized in microtiter wells is allowed to react with specific antibodies which are then linked to the DNA label *via* biotin–streptavidin interaction. After completion of the immunoreaction, the solid phase bound DNA is subjected to a cell-free, one-step transcription/translation reaction to produce the α-peptide. The α-peptide is allowed to react (complementation reaction) with the remaining part of the β-galactosidase (M15 protein, also inactive) to give fully active enzyme molecules. 4-Methylumbelliferyl galactoside is used as a substrate. The fluorescence is linearly related to the amount of antigen in the well. As little as 3 fmol of antigen can be detected. The RSDs (within-run) obtained for 8 and 20 fmol of antigen were 10.7 and 9.3%, respectively ($n = 4$). The present work illustrates the utility of expressing a non-detectable peptide capable of triggering a signal generating system.

Keywords: *Expression immunoassay; transcription/translation; α-peptide of β-galactosidase*

Immunoassay is a highly sensitive analytical technique used broadly in research and clinical laboratories.[1] Recent advances in the development of reagents and detection systems used with immunoassays have improved their sensitivity. There is a current trend away from the use of radioactive labels which has led to the production of a number of fluorescent, chemiluminescent and enzymatic antibody labels.[1–3] The enzyme is perhaps the most widely used reporter molecule because it introduces signal amplification through turnover of an appropriate substrate to detectable products. Further improvements in the sensitivity of the enzyme immunoassay have come from the replacement of chromogenic substrates with substrates that result in the generation of fluorescent or chemiluminescent products.[4] Recently, we introduced a new analytical system, named expression immunoassay, which uses an expressible, enzyme-coding DNA fragment as a reporter molecule.[5] In this system the antibody was labeled with a DNA fragment encoding luciferase. After completion of the immunoreaction, the DNA label (DNA template) was subjected to a cell-free (*in vitro*) coupled transcription/translation reaction that produced several luciferase molecules in solution. It was found that the enzymatic activity was linearly related to the amount of antigen (analyte) present in the sample. The expression immunoassay offers significant advantages over current immunoassays. To date, the efforts towards enhancing the sensitivity of enzyme immunoassays have been focused on the design of improved substrates. Expression immunoassay entails an increase in the number of enzyme molecules, thus introducing an additional level of amplification. Furthermore, by using an enzyme-coding DNA fragment as a label (instead of the enzyme itself) the problem of enzyme inactivation on conjugation to antibodies is eliminated and the enzyme remains free in solution.

In this paper we report a novel expression immunoassay which uses, as a label, a DNA fragment that encodes a relatively small polypeptide with no inherent enzymatic activity. The peptide, however, is able to trigger a sensitive enzymatic reaction as follows. After transcription/translation of the DNA label, the generated peptide interacts with an excess of a large protein molecule (also inactive) in a 'complementation reaction' to form fully active enzyme molecules. A well known intracistronic complementation reaction is the α-complementation of fragments of β-galactosidase to produce a molecule with enzymatic activity.[6–10] This system has been used extensively in molecular cloning[11,12] and protein expression studies.[13] β-Galactosidase consists of four identical sub-units. A small peptide (α-peptide) at the amino-terminal end of each sub-unit is necessary for the enzymatic activity. Protein molecules that lack the α-peptide (*e.g.*, the M15 protein from lacZΔM15 bacterial strains) have no activity. The M15 protein exists in solution as an inactive dimer of identical sub-units.[14] In the presence of the α-peptide an active enzyme is formed. This occurs through the formation of a pseudo-tetrameric complex consisting of four M15 proteins held together with four α-peptides through non-covalent interactions.[9] The α-peptide allows the formation of an activating interface between the subunits which, in turn, leads to the formation of a complete active site.[6] The complemented enzyme has full β-galactosidase activity and differs from the native enzyme only by a slightly lower stability at increased temperatures or high urea concentrations.

Experimental

Instrumentation

An imaging densitometer (Model GS-670, Bio-Rad Laboratories, Mississauga, Ontario, Canada), together with the Molecular Analyst PC software version 1.2 was used for the quantification of DNA fragments after agarose gel electrophoresis. A miniature horizontal gel system, MLB-06, from Tyler Research (Edmonton, Alberta, Canada) was used for electrophoresis. High-performance liquid chromatography (HPLC) was performed using a Shimadzu system (Shimadzu, Kyoto, Japan) with absorbance monitoring. A G24 environmental incubation shaker from New Brunswick Scientific (Edison, NJ, USA) was employed for culturing bacteria. A Fluoroskan II fluorimeter from Labsystems (Ingram and Bell Scientific, Don Mills, Ontario, Canada) was used for fluorescence measurements in microtiter wells.

Materials

The cloning vector pGEM 13Zf(+), and *E. coli* JM109 cells {genotype: recA1, endA1, gyrA96, thi, hsdR17, supE44, relA1, λ^-, Δ(lac–proAB), [F′ traD36, proAB, lacIqZΔM15]},[15] were

purchased from Promega (Madison, WI, USA). The Wizard maxipreps DNA purification system was also from Promega. The restriction enzymes Afl III and Ngo MI were obtained from New England Biolabs (Beverly, MA, USA). The Klenow fragment of DNA polymerase I (exonuclease-free) was from United States Biochemical (Cleveland, OH, USA). Ultrapure 2′-deoxyribonucleoside 5′-triphosphates were from Pharmacia Biotech. (Montreal, Quebec, Canada). Linear DNA markers (lambda-DNA digested with Eco RI and Hind III) containing fragments from 0.12 to 21.2 kbp, supercoiled DNA markers (sizes 2.07–16.2 kbp), streptavidin, β-galactosidase (isolated from *E. coli*), isopropyl thiogalactopyranoside (IPTG) and 4-methylumbelliferyl galactoside (4-MUG) were purchased from Sigma (St. Louis, MO, USA). Adenosine triphosphate (ATP), bovine serum albumin and the 'blocking reagent' (Cat. No. 1096 176) were obtained from Boehringer (Laval, Quebec, Canada). Biotin-14-dCTP (biotin attached at the N^4 position of cytidine through a 14-atom linker) and U-bottomed polystyrene microtiter wells (Nunc, Maxisorp) were obtained from Life Technologies (Burlington, Ontario, Canada). White, flat-bottomed polystyrene wells, Microlite-2, were from Dynatech Laboratories (Chantilly, VA, USA). Monoclonal anti-thyrotropin antibody was from Jackson Immunoresearch Laboratories (BioCan, Mississauga, Ontario, Canada). Microcon-30 microconcentrators were obtained from Amicon (Beverly, MA, USA).

The blocking solution contained 1% blocking reagent in 0.1 mol l^{-1} maleate, 0.15 mol l^{-1} NaCl, pH 7.5. The wash solution consisted of 50 mmol l^{-1} Tris, pH 7.4, 0.15 mol l^{-1} NaCl and 0.1% v/v Tween-20. The Tris–EDTA (TE) buffer consisted of 10 mmol l^{-1} Tris and 1 mmol l^{-1} EDTA, pH 8.0. The transcription/translation reagent mixture (*E. coli* S-30 Extract System for Linear Templates from Promega) was prepared according to the manufacturer's instructions. The complete mixture contained the S-30 extract (with ribosomes, translation factors, *etc*.), the S-30 premix (with NTPs, tRNAs, an ATP generating system, IPTG, and a buffer system) and amino acids. M-9 plus thiamine-HCl agar plates were made from an autoclaved solution of 42.3 mmol l^{-1} Na_2HPO_4, 22 mmol l^{-1} KH_2PO_4, 8.5 mmol l^{-1} NaCl, 18.6 mmol l^{-1} NH_4Cl, and 1.5% agar to which were added 2 ml of 1 mol l^{-1} $MgSO_4$, 0.1 ml of 1 mol l^{-1} $CaCl_2$, 10 ml of 20% glucose, and 1 ml of 1 mol l^{-1} thiamine-HCl. LB broth contained 10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 0.17 mol l^{-1} NaCl and 2 mmol l^{-1} NaOH.

Preparation of the M15 extract

The extract was prepared using a procedure based on the work of Lin and Zabin.[16] Briefly, *E. coli* JM109 cells were initially grown on M-9/thiamine-HCl agar plates in order to select for the presence of the F′-episome which contains the sequence that codes for the M15 protein. A colony of the JM109 cells was used to inoculate LB broth containing 0.5 mmol l^{-1} IPTG, to stimulate production of the M15 protein. The resulting 6 l culture was harvested, during the exponential growth phase at 37 °C, by centrifugation and was resuspended in 20 ml of 0.1 mol l^{-1} NaH_2PO_4, pH 7.2. The cells were lysed using a French pressure cell and the cellular debris was removed by ultracentrifugation at 49 000*g* for 40 min. The supernatant, containing 17 g l^{-1} of protein (determined using the Bio-Rad Protein Assay), was divided into 1 ml aliquots and stored at −20 °C until use.

Preparation and purification of the expressible DNA template encoding the α-peptide

For growth of *E. coli* JM109 cells, preparation of competent cells and transformation with the plasmid DNA, standard procedures were followed.[17] Bacteria transformed with the plasmid pGEM 13Zf(+) were grown overnight in LB broth containing 0.1 g l^{-1} ampicillin. The plasmid DNA was purified from a 1 l bacterial culture using the Wizard maxipreps DNA purification system according to the manufacturer's instructions. The size of the plasmid was confirmed by agarose (1.2%) gel electrophoresis and ethidium bromide staining, using the supercoiled markers. The plasmid concentration was determined from its absorbance at 260 nm.

For preparation of the DNA template, 200 μg of plasmid DNA were first digested overnight at 37 °C with 100 U of Ngo MI in 80 μl of digestion buffer (50 mmol l^{-1} potassium acetate, 20 mmol l^{-1} Tris acetate, 10 mmol l^{-1} magnesium acetate, 1 mmol l^{-1} dithiothreitol, pH 7.9). Subsequently a fill-in reaction was initiated by adding 22 μl of a solution containing 43.6 μmol l^{-1} each of biotin-14-dCTP and dGTP as well as 80 U of the Klenow fragment of the DNA polymerase I in the digestion buffer. After incubation at 37 °C for 1.5 h, the reaction was terminated by heating the mixture at 65 °C for 10 min. A second digestion was then carried out by adding 22 μl of a solution containing 100 U of Afl III, to the digestion buffer, and incubating at 37 °C overnight. After digestion, the DNA fragments were separated by agarose (1.2%) gel electrophoresis and stained with ethidium bromide. The band corresponding to 737 bp was excised and isolated by centrifugation of the agarose slices according to the method of Wu and Welsh.[18] Then, the DNA was purified by ethanol precipitation according to standard procedures.[17] In order to quantify the purified DNA template, another agarose (1.2%) gel electrophoresis was performed and the DNA was stained, as above. A lane containing the linear DNA markers was included for the construction of a calibration graph. The gel was photographed under UV excitation using a Polaroid 665 film and the negatives were scanned by an imaging densitometer.

Preparation and purification of streptavidin–DNA template complex

The streptavidin–DNA template complex was prepared in a final volume of 70 μl containing 2.2 μg (4.5 pmol) of purified, biotinylated DNA template and 13 μg (21 nmol) of streptavidin in 2.2 mmol l^{-1} Tris and 0.22 mmol l^{-1} EDTA, pH 8.0. The mixture was incubated for 30 min at room temperature. The complex was then purified by HPLC using a size-exclusion column (Bio-Sil Sec 400-5, 300 × 7.8 mm, from Bio-Rad Laboratories) isocratically. The mobile phase was 0.5 mol l^{-1} NaH_2PO_4–0.5 mol l^{-1} Na_2HPO_4–0.15 mol l^{-1} NaCl, pH 6.8. The flow rate was 0.5 ml^{-1} min. Absorbance was monitored at 260 nm. A fraction, with an approximate volume of 2 ml, corresponding to a peak centered at 10.4 min was collected. Blocking solution (200 μl) was added, as a carrier, and the mixture was concentrated using a Microcon-30 microconcentrator. Then, 6 μl of 10 mmol l^{-1} EDTA were added to the concentrated DNA complex solution. The final volume was 66 μl. A 5 μl aliquot of this preparation was subjected to electrophoresis and the DNA concentration was determined by scanning densitometry of the negative, as described above.

Fluorimetric assay of β-galactosidase

A 60 mmol l^{-1} stock solution of 4-MUG in dimethyl sulfoxide was prepared. A 0.2 mmol l^{-1} substrate solution for the β-galactosidase assay was made by diluting the stock in reaction buffer containing 10 mmol l^{-1} NaH_2PO_4, 0.1 mol l^{-1} NaCl, 1 mmol l^{-1} $MgCl_2$, 0.1% m/v sodium azide and 0.1% m/v bovine serum albumin, pH 7.0. For the β-galactosidase assay, 50 μl of the sample were added to 50 μl of the substrate solution in a microcentrifuge tube and incubated at 37 °C for 1 h. The reaction was terminated by adding 50 μl of 1 mol l^{-1} glycine, pH 10.3. A 130 μl aliquot was removed and placed in a flat-

bottomed microtiter well and the fluorescence was measured using the Fluoroskan II instrument, with excitation and emission wavelengths set at 355 and 460 nm, respectively.

Quantification of immobilized antigen by expression immunoassay

A monoclonal anti-thyrotropin immunoglobulin was used as the antigen (analyte) throughout this work. The antigen was detected by reaction with a goat anti-mouse immunoglobulin. Solutions with various analyte concentrations were prepared by dilution in coating buffer (0.1 mol l^{-1} carbonate, pH 9.6). The analyte was immobilized by pipetting 25 μl of solution into U-bottomed polystyrene microtiter wells and incubating overnight at 4 °C. The coating solution was then aspirated and the remaining binding sites were blocked with blocking solution for 90 min at room temperature. The wells were washed once with wash solution and 25 μl of a solution of biotinylated goat anti-mouse antibody (5 μl ml^{-1}), diluted in blocking solution, were pipetted into each well. After incubation for 30 min with shaking at room temperature, the wells were washed four times in order to remove the excess of biotinylated antibody. Then, 25 μl of the DNA–streptavidin complex (3.0 ng μl^{-1} with respect to DNA), diluted in blocking solution, were added to each well. The wells were incubated at room temperature for 15 min, to allow binding of the complex to the biotinylated antibody. Excess of DNA complex was removed by washing five times with wash solution and three times with TE buffer. Then, 25 μl per well, of the transcription/translation reagent mixture were added and the wells were incubated at 37 °C for 90 min to allow expression of the DNA template bound to the immunocomplexes. Subsequently, 20 μl were removed from each well, pipetted into separate microcentrifuge tubes and incubated at 70 °C for 30 min to destroy any endogenous β-galactosidase activity in the *E. coli* S-30 extract. After cooling, 20 μl of the M15 extract were added to each tube and the complementation reaction was allowed to proceed for 1 h at 37 °C. The mixture was brought to 50 μl with galactosidase reaction buffer and assayed for β-galactosidase activity, as described above.

Results and discussion

The principle of the proposed immunoassay is illustrated in Fig. 1. The DNA template that was used as a label in this work was a linear DNA fragment isolated from plasmid pGEM 13Zf(+), which contains the sequence coding for the α-peptide of β-galactosidase downstream from the Lac operator and promoter sequences (Fig. 2) Ampicillin resistance is conferred by the β-lactamase gene [amp(r) region]. The expressible DNA fragment containing the α-peptide sequence was prepared by first digesting the plasmid with Ngo MI to produce linearized plasmid with recessed 3′ ends. The recessed ends were filled-in with the Klenow fragment of DNA polymerase in the presence of biotin-14-dCTP and dGTP, thus labeling both ends of the linearized plasmid with biotin. A second restriction digestion was carried out on the labeled DNA using Afl III to produce two fragments (Fig. 2); a 2444 bp fragment and a 737 bp fragment. The latter had a single biotin moiety at the 3′ terminus and contained the required sequences for synthesis of the α-peptide (Fig. 2). The two DNA fragments were separated by agarose gel electrophoresis and the 737 bp fragment was excised, purified, and used as a label (template) in this study. After electrophoresis, the concentration of the DNA template was determined using scanning densitometry. Expression of the 737 bp DNA by coupled (one step) cell-free transcription/translation produces the α-peptide. The transcription by *E. coli* RNA polymerase is directed from the Lac promoter sequence. The synthesized RNA contains a ribosome binding site (RBS) for translation in a prokaryotic system. By itself the α-peptide is inactive. However, after complementation with M15 protein, an active enzyme is formed. The M15 protein was expressed in JM109 cells and the cell extracts were used directly for complementation.

The initial experiments indicated that the *E. coli* S-30 extract used in the transcription/translation mixture contained endogenous β-galactosidase activity. Heat inactivation was attempted in order to remove this activity. Aliquots of the transcription/translation mixture were heated to temperatures ranging from 65 to 75 °C for 15 or 30 min. It was found that incubation for 30 min at 70 °C was sufficient to eliminate the endogenous galactosidase activity without affecting the α-peptide produced from expression of the DNA template. Further, it was found that the M15 extract contained no detectable β-galactosidase activity and required no treatment before use.

The time dependence of the complementation reaction was studied by subjecting 8.6 ng of DNA template to transcription/translation, followed by heat inactivation of the endogenous galactosidase. The α-peptide produced was allowed to react with the M15 protein for various time periods at 37 °C and the activity of the generated galactosidase was measured. The results are summarized in Fig. 3. It was estimated that, under these conditions, approximately 90% of the complementation reaction was completed after 1 h.

Further optimization of the complementation reaction was performed by studying the amount of M15 added to the reaction

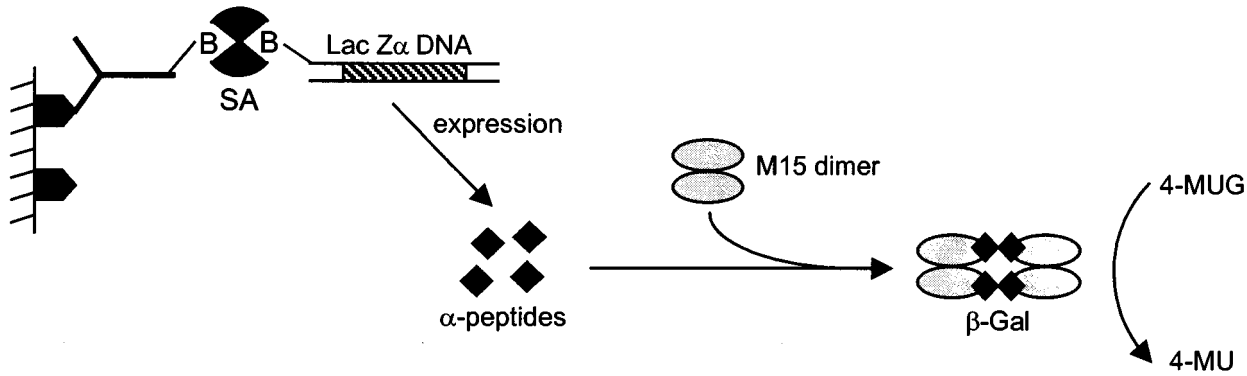

Fig. 1 Schematic presentation of the proposed assay. Analyte (an anti-thyrotropin immunoglobulin) immobilized on a solid phase is allowed to react with a specific antibody (goat anti-mouse antibody) which is then linked, through biotin (B)–streptavidin (SA) interaction, to an expressible DNA fragment (LacZαDNA) encoding the α-peptide of β-galactosidase. The solid phase bound DNA is subjected to cell-free transcription/translation and the generated α-peptide is allowed to form complexes with the M15 protein. M15 protein exists as an inactive dimer in the solution. In the presence of α-peptide, a biologically active pseudo-tetrameric enzyme is formed (complementation reaction). β-Galactosidase (β-Gal) catalyzes the conversion of 4-methylumbelliferyl galactoside (4-MUG) to 4-methylumbelliferone (4-MU) which is fluorescent.

mixture. Various dilutions of M15 extract in a total volume of 50 μl were added to an equal volume of the product from transcription/translation of 8.6 ng of DNA template and incubated for 1 h at 37 °C. The activity of the generated galactosidase was measured and the fluorescence was plotted against the protein concentration of the M15 extract (Fig. 4).

To assess the performance of the DNA template as a label, various amounts of the DNA were subjected to a coupled transcription/translation reaction (for 90 min at 37 °C) followed by the addition of M15 extract. After complementation, the mixtures were analyzed for galactosidase activity. In Figure 5, the fluorescence is plotted against the amount of DNA template introduced into the transcription/translation mixture. It is observed that 0.3 fmol of DNA template can be detected and the signal is linearly related to the amount of DNA up to 80 fmol.

The DNA template was attached to an antibody using streptavidin as a linker molecule. The first step was the preparation of a 1 : 1 complex of the biotinylated DNA template and streptavidin. The streptavidin–DNA complex was separated from the excess of streptavidin by size-exclusion HPLC. A typical chromatogram is shown in Fig. 2(*d*). The fractions containing streptavidin–DNA were pooled, concentrated and stored in the presence of 1 mmol l^{-1} EDTA. Quantification of the complex was carried out by electrophoresis followed by scanning densitometry.

The performance of the proposed immunoassay was assessed by first immobilizing various amounts of antigen (anti-thyrotropin mouse monoclonal antibody) on microtiter wells. Biotinylated goat anti-mouse antibody was used for detection. After completion of the immunoreaction, the streptavidin–DNA complex was added to the wells and allowed to bind to the immunocomplexes. The excess of reagent was washed out and the transcription/translation reaction mixture was added directly to the solid phase. The transcription/translation mixture was incubated for 90 min to allow the expression of the bound DNA template and consequently the synthesis of the α-peptide. Aliquots of the reaction mixture were subjected to heat inactivation followed by the α-complementation reaction and measurement of galactosidase activity. In Fig. 6, the fluorescence (corrected for the background) is plotted as a function of the amount of antigen in the well. The background is defined as the signal obtained when no antigen is present (*i.e.*, the zero standard). As little as 3 fmol of antigen can be detected. The RSDs (within-run) obtained at the 8 and 20 fmol levels were 10.7 and 9.3%, respectively (n = 4).

It has been suggested[5] that for a successful expression immunoassay system, the DNA label should encode a relatively small enzyme molecule which does not require post-translational modification for full activity. In contrast, β-galactosidase is a large enzyme (M 465 000) consisting of four sub-units (1023 amino acid residues per sub-unit). In the present work, instead of using the entire β-galactosidase gene as a label, a

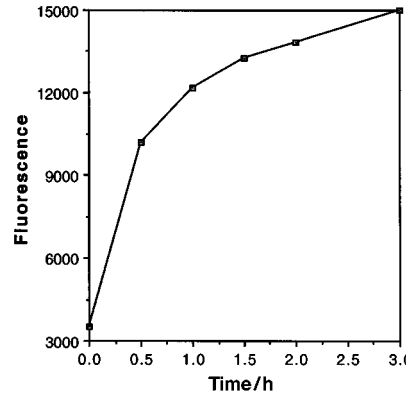

Fig. 3 Time dependence of the α-complementation reaction. Aliquots (4 μl) of a typical transcription/translation reaction mixture (8.6 ng DNA template) were mixed with an equal volume of the M15 extract and incubated at 37 °C for various times. The mixtures were then assayed for β-galactosidase activity.

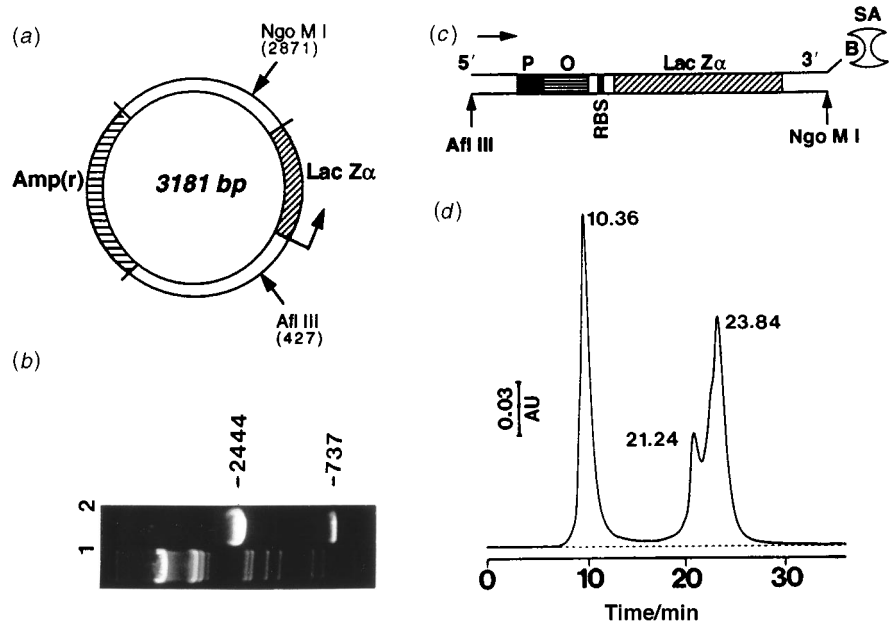

Fig. 2 (*a*) Plasmid containing the Lac Zα sequence. The relative positions of the restriction enzyme digestion sites are shown. Amp(r) is the β-lactamase encoding sequence. (*b*) Analysis of the digested plasmid by 1.2% agarose gel electrophoresis and ethidium bromide staining. Lane 1, linear DNA markers (1.4 μg); lane 2, plasmid digested with Ngo MI and Afl III (fragment sizes 2444 and 737 bp). (*c*) Structure of the streptavidin–DNA complex showing the lac promoter (P) and operator (O) sequences, the ribosome binding site (RBS) and the sequence that codes for the α-peptide (Lac Zα). The DNA is labeled with biotin at the 3′ terminus downstream of the Lac Zα sequence and is complexed to streptavidin. (*d*) Purification of streptavidin–DNA template complex by size-exclusion HPLC. Absorbance was monitored at 260 nm. The peaks at 10.4 and 21.2 min correspond to the complex and free streptavidin, respectively. The last peak is due to the EDTA contained in the solution.

DNA fragment that codes for a small inactive peptide containing 97 amino acid residues was used. The large remaining part of β-galactosidase (M15 protein, also inactive) is added as a crude extract to the reaction mixture. Furthermore, the synthesized α-peptide does not require post-translational modification and forms fully active enzyme complexes with M15 protein.

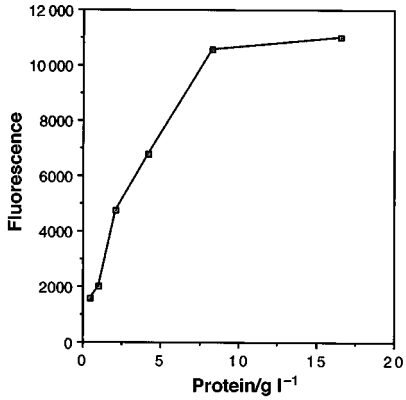

Fig. 4 Effect of the concentration of the M15 extract used in the complementation reaction. Aliquots (4 μl) of a typical transcription/translation reaction mixture (8.6 ng of DNA template) were mixed with an equal volume of various dilutions of the M15 extract in the β-galactosidase reaction buffer. The solutions were incubated for 1 h at 37 °C before being assayed for galactosidase activity.

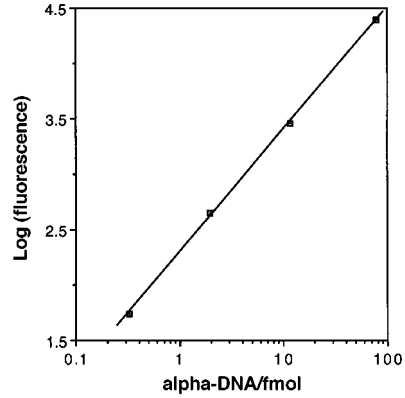

Fig. 5 Quantification of the α-peptide-coding DNA template (label) by coupled transcription/translation followed by heat inactivation of the endogenous β-galactosidase activity and complementation with M15 protein.

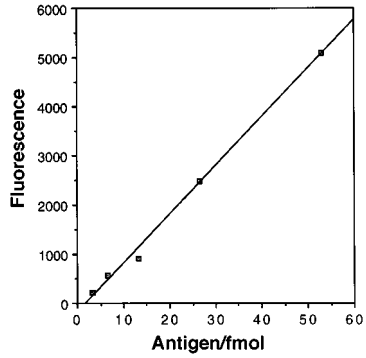

Fig. 6 Quantification of immobilized antigen (an anti-thyrotropin immunoglobulin) on microtiter wells by expression immunoassay using the expressible DNA fragment encoding the α-peptide of β-galactosidase as a label.

The sensitivity and practicality of the proposed assays could be improved further by optimizing the sequence of the DNA template used as label. This could be achieved by incorporating a T7 RNA polymerase promoter in the template and using a eukaryotic expression system. The time required for the detection step is 4 h. This includes the *in vitro* expression, the elimination of the endogenous β-galactosidase activity in the S-30 extract, the complementation reaction and the monitoring of galactosidase activity. The rather long time required for detection is mainly due to the need for heating (at 70 °C for 30 min) of the reaction mixture after transcription/translation in order to inactivate the endogenous β-galactosidase activity of the *E. coli* S-30 extract, without affecting the synthesized α-peptide. A cell-free expression system that lacks endogenous β-galactosidase activity would eliminate the heating step and allow the addition of purified M15 protein directly to the transcription/translation mixture. In this case, the expression of the DNA label and the complementation reaction would proceed simultaneously (one step).

Although the principle of the immunoassay was demonstrated using a fluorogenic substrate for β-galactosidase, the sensitivity of the assay could be further enhanced (by at least a factor of 10) by employing a chemiluminogenic substrate, *e.g.*, the 1,2-dioxetane derivative, AMPGD.[19]

The analytical system described here would also be useful for the determination of small molecules (haptens). In this case, a limiting reagent assay (competitive-type assay) would be suitable. In one possible configuration, analyte from the sample and biotinylated analyte are allowed to react with a limited amount of specific antibody which is immobilized in microtiter wells. The solid phase bound biotinylated analyte is detected with the streptavidin–DNA complex as described above. The signal is expected to decrease with increasing concentrations of analyte in the sample.

In a previous paper,[5] a luciferase-coding DNA fragment was used as a label in expression immunoassay. The chemiluminescence assay for luciferase activity is more sensitive than the fluorimetric assay of galactosidase. However, the sensitivity of the present assay might be significantly improved by using the chemiluminogenic substrate AMPGD.

To date, DNA has been used in analytical chemistry mainly as a recognition molecule (probes which bind to complementary target nucleic acid sequences) but not as a label, *i.e.*, as a signal-generating molecule. The significance of this work lies in the fact that it introduces macromolecular complexes/analytical reagents in which molecular recognition is provided by an antibody (in this case a goat anti-mouse immunoglobulin) and the signal conferring molecule is an expressible DNA fragment.

Although β-galactosidase complementation was chosen as a model, the principle can be expanded to include any DNA label that encodes a small peptide which, in turn, activates a signal generating system (enzyme, photoprotein, *etc*.).

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