MAGNETIC PARTICLE SEPARATION TECHNIQUE: A RELIABLE AND SIMPLE TOOL FOR RIA/IRMA AND QUANTITATIVE PCR ASSAY

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Abstract

Five types of magnetic particles without or with aldehyde, amino and carboxyl functional groups, respectively were used to immobilize first or second antibody by three models, i. e. physical adsorption, chemical coupling and immuno-affinity, forming four types of magnetic particle antibodies. The second antibody immobilized on polyacrolein magnetic particles through aldehyde functional groups and the first antibodies immobilized on carboxylic polystyrene magnetic particles through carboxyl functional groups were recommended to apply to RIAs and/or IRMAs. Streptavidin immobilized on commercial magnetic particles through amino functional groups was successfully applied to separating specific PCR product for quantification of human cytomegalovirus.

In the paper typical data on reliability of these magnetic particle ligands were reported and simplicity of the magnetic particle separation technique was discussed. The results showed that the technique was a reliable and simple tool for RIA/IRMA and quantitative PCR assay.

1. INTRODUCTION

In procedures of radioimmunoassay (RIA), immunoradiometricassay (IRMA) and quantitative polymerase chain reaction (PCR), separation of determined target molecules is an essential step. In addition to being technically demanding and time-consuming, the step is the major source of imprecision in the assays. Application of solid phase separation techniques is an important improvement in analytical methodology. Introduction of magnetic particle separation technique is more interesting because the magnetic particles bound to the target molecules can be quickly and clean separated from mixture solution, thus providing a solid phase methodology which avoids many disadvantages of other solid phase techniques while retaining their advantages.

In the paper six types of magnetic particles with or without functional groups were used to immobilize antibodies or streptavidin by three types of model, forming five types of magnetic particle ligands. The solid phase ligands were applied to the procedures of RIA/IRMA or quantitative PCR assay. Reliabilities of the separation techniques were breifly reported.

TABLE Ⅰ. MAGNETIC PARTICLE AND MAGNETIC PARTICLE LIGAND

Magnetic particle and magnetic particle ligand	Description
Magnetic Particle	
1. Fe_3O_4 particle (Magnetic nucleus, MN)	Prepared by a chemical precipitation method, 10nm ± 34% in diameter
2. Polyacrolein magnetic particle (AMP)	Aldehyde functional groups were coated on MN by ^{60}Co-irradiation polymerization of acrolein
3. Silanized magnetic particle (SMP)	Amino functional groups were coated on MN by acidic aqueous silanization using 3-amino-propyltrimethoxysilane
4. Carboxylic polyacrylamide magnetic particle (CAMP)	Carboxyl functional groups were coated on MN by emulsion polymerization of mathacrylic acid and acrylamide (CAMP) or of mathacrylic acid and styrene (CSMP)
5. Carboxylic polystyrene magnetic particle (CSMP)	
6. Magnetic Affinity Particle (MAP)	Purchased from Paesel/Lorei Co., Germany, 0.5~1.5μm in diameter, amine-terminated
Magnetic Particle Ligand	
1. Magnetic second antibody-P (MSA-P)	Donkey anti-rabbit (D×R) serum was immobilized on MN by physical adsorption (P)
2. Magnetic second antibody-C (MSA-C)	D×R serum was immobilized on AMP by chemical coupling (C)
3. Magnetic first antibodies-C (MFA-C)	Anti-T_3, T_4 or TSH serum was immobilized on SMP, CAMP or CSMP by chemical coupling (C)
4. Magnetic first antibody-I (MFA-I)	Anti-T_3 rabbit serum molecules were immobilized on the D×R serum molecules of MSA-C by immuno-affinity (I)
5. Magnetic particle streptavidin (MP·SA)	Streptavidin (SA) was immobilized on MAP by chemical coupling

2. MATERIALS AND METHODS

2.1. Magnetic particles and magnetic particle ligands

In Table Ⅰ were listed six types of magnetic particles and five types of magnetic particle ligands, which were developed in the former researches[1-3] except that the Magnetic Affinity Particle (MAP) was from a commercial source and the magnetic particle streptavidin (MP·SA) was prepared in our laboratory according to the procedure presented by the manufacturer of MAP. [4]

2.2. Use of magnetic particle antibodies in RIAs and IRMAs

Magnetic second antibody of MSA-P or MSA-C was used as an immuno-separation reagent in RIAs. After the reaction between an antigen and its first antibody was completed, add the magnetic second antibody into the reaction solution and incubate for 10 min. The magnetic solid phase was separated by a magnet, and then washed and counted. The procedures of incubation, separation and washing may be finished within 20 min. [1]

Magnetic first antibody of MFA-C or MFA-I were used as an immuno-separation reagent while as an immuno-reaction reagent in RIAs and/or IRMAs. Add the magnetic first antibody to a sample serum and incubate until the reaction was finished. Separate and wash as above, and then continue following assay procedures. [1,2]

2.3. Use of MP·SA in quantitative PCR assay

MP·SA was used to separate specific PCR products. The separation was based on the extremely high affinity of streptavidin (SA) to biotin (B). A nested PCR (nPCR) procedure was performed.[5] In the second PCR run one of B-labelled primer and [α-^{32}P] dATP were used. The amplified specific products which were labelled by B at an end of one strand of the double strands while by [α-^{32}P] dATP into the DNA sequences were separated by adding MP·SA to the amplified mixture and incubating for 15 min. Magnetically separate and wash the magnetic solid phase. The no B-labelled strand of the specific products was eluted by using NaOH solution from the magnetic solid phase for 5 min and then counted. The procedures of separation may be finished within 1 h.

3. RESULTS

3.1. Application of magnetic particle antibodies to RIAs/IRMAs

Magnetic second antibodies of MSA-P and MSA-C have been successfully applied to RIAs for triiodothyronine (T_3), reverse T_3(rT_3), free T_3(fT_3), thyroxine (T_4), free T_4(fT_4), thyroid-stimulating hormone (TSH), thyroglobulin (TG) and TG-antibody (TG-Ab).[1] In Table Ⅱ was listed the correlation of levels of quality control (QC) serum obtained with MSA-C assay and liquid phase double antibody (LDA) assay (As a control). The results showed a better correlation of the results between both of assays thus the reliability of MSA-C. The MSA-P presented the similar performance to MSA-C but unsatisfactory stability of storage at surrounding temperature.[1]

TABLE Ⅱ. CORRELATION OF LEVELS OF QC SERUM OBTAINED WITH LDA(X) AND MSA-C (Y) ASSAYS*

RIA item	Y = a + bX			
	N	a	b	r
T_3	156	0.043	0.977	0.988
T_4	159	2.266	0.973	0.993
TSH	159	0.371	0.897	0.982

* Tests of the correlation were performed on three levels of QC serum monthly from 1992 to 1996. N: number of determination, r: correlation coefficient

TABLE Ⅲ. PARAMETERS OF STANDARD CURVE OF IRMA FOR BLOOD SPOT TSH USING MAGNETIC ANTI-TSH ANTIBODIES

Magnetic particle immobilized by anti-TSH antibody	Binding, %		LogY = a + bLogX			QC serum, μIU/ml		
	B_0	B_{160}	a	b	r	L	M	H
Latex-M (Control)	0.29	8.94	1.82	0.941	0.9975	6.3	26.3	52.5
CSMP	0.29	8.25	1.79	0.940	0.9982	8.7	27.8	53.6
CAMP	0.29	7.14	1.73	0.943	0.9996	9.8	29.1	58.3
SMP	0.29	7.56	1.81	0.891	0.9955	10.1	31.8	52.9

B_0 and B_{160}: ^{125}I-binding at TSH of 0 μIU/ml and 160 μIU/ml, respectively; L, M and H: Low, middle and high values

Magnetic first antibodies of MFA-C and MFA-I could be applied to RIAs of T_3, fT_3, T_4, fT_4 or blood spot T_4.[1,2] A standard curve of T_3 RIA obtained by using MFA-I was shown in Fig. 1 and compared to those by using MSA-C and LDA under the same conditions. The results showed that the standard curve of MSA-C was comparable with that of LDA, but the standard curve of MFA-I showed a lower slope than that of LDA. The difference may result from unsatisfactory suspension ability of the magnetic first antibody since it needed to react for a longer time (1 h) than the magnetic second antibody (10 min).

The anti-TSH antibodies immobilized on SMP, CAMP and CSMP could be applied to IRMAs of TSH and blood spot TSH.[2] In Table Ⅲ were listed parameters of standard curves of the blood spot TSH obtained by using these magnetic anti-TSH antibodies and were compared to that obtained by using Latex-M (A commercial magnetic anti-TSH antibody purchased from Rhone-Doulenc Co., France). The results showed that the anti-TSH antibody based on CSMP presented better performances similar to Latex-M and should be recommended.

3.2. Application of MP·SA to quantitative PCR assay

Biotinylated PCR products were able to be separated quantitatively by MP·SA as shown in Table Ⅳ. The results showed that ^{32}P-binding of MP·SA decreased to the level of its non-specific binding when secondary separation was done.

In a quantitative PCR assay initial amount of determined templet is determined by detection of accumulated amount of ^{32}P-labelled PCR products from a standard curve which may be prepared by using a standard templet. The standard curve was established by an external standard method for quantification of human cytomegalovirus (HCMV) using MP·SA separation technique[5], as shown in Fig. 2. The standard curve was a straight line on Log to Log scales between initial templet amounts from 1.3×10^{-4} amol to 1.3×10^{-1} amol and its intercept, slope and correlation coefficient (r) were 1.071, 0.517 and 0.986, respectively.

Three levels (low, middle and high) of QC templet were determined by the method and the results were listed in Table Ⅴ. The results showed that coefficients of variation (CVs) were less than 39% for intra-assay and less than 73% for inter-assay, and were decreasing with increasing of levels of QC templet. Compared the detected average values ($\bar{X}$) with the reference values (X_0), there were differences up to three times between both of relevant values. Above results were comparable to that reported in some literatures[5] and the method could be applied to relative quantification of HCMV.

4. DISCUSSION

An ideal magnetic solid phase should fulfill the following requirements: 1) Its preparation should be simple, reproducible and inexpensive and should use readily available reagents and equipments. Its ligands should be immobilized by covalent linkage, preferably without prior acitve step, 2) It should have high lig-

TABLE Ⅳ. SEPARATION OF ^{32}P-BOUND PCR PRODUCT

Item	^{32}P-binding, % *
Non-specific binding	0.23±0.04
Specific binding, First	3.92~8.38
Second	0.15±0.06

* Ratio of ^{32}P-counts separated to added

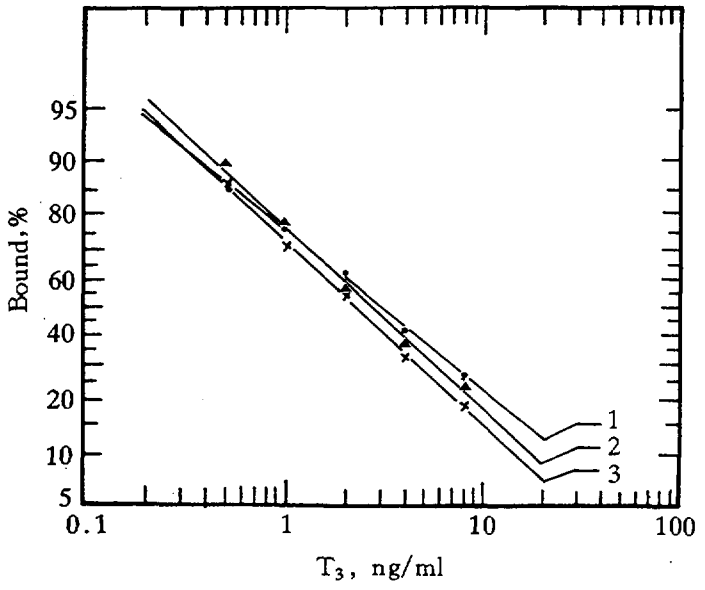

Fig.1. Standard curves of RIA for T_3 using MFA-I ("·", curve 1), MSA-C ("▲", curve 2) and LDA ("×", curve 3) assays*. 1 ng/ml is equal to 1.54 nmol/L for T_3

* As control

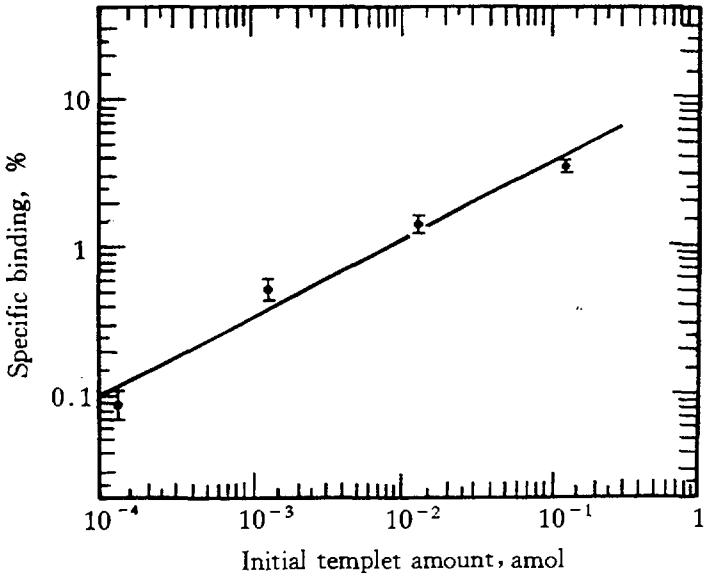

Fig.2. Standard curve for quantification of HCMV by nPCR using MP·SA separation technique

TABLE V. QUANTITATIVE RESULT OF QC TEMPLET BY NPCR

QC templet		Intra-assay			Inter-assay		
No	Xo, amol	N	$\bar{X}$, amol	CV, %	N	$\bar{X}$, amol	CV, %
L	2.6×10^{-4}	4	1.3×10^{-4}	38.9	5	1.6×10^{-4}	72.5
M	2.6×10^{-3}	4	7.8×10^{-3}	17.8	5	4.4×10^{-3}	54.9
H	6.4×10^{-2}	4	6.6×10^{-2}	14.5	5	6.6×10^{-2}	39.0

Xo and $\bar{X}$: Reference level and detected average value; CV: Coefficient of variation

and-bound capacity but low non-specific binding, thus providing a complete and clean separation of target molecules from impurities. It can remain stable suspension for a longer time during incubation and keep a high magnetic response to ensure rapid separation, 3) It should be stable in aqueous solution at 4℃ and/or surrounding temperature without change of its physical, chemical and affinity characteristics, 4) It should be applicable to the majority of assay items and suitable for automation of the assay procedures.

It is difficult to meet all these requirements, where unsatisfactory suspension ability is a main obstacle to application of the magnetic solid phase. Magnetic materials, which have to be used, have a high density hence the size of magnetic particle should be controlled. But too small particle used will require a longer time of separation or a stronger magnetic field. The density of magnetic particle can be decreased by means of coating a polymer with low density on surface of magnetic nucleus while amount of the magnetic material in the particle should be enough to ensure its high magnetic response. It is possible to resolve these problems technically.

To date, the magnetic solid phase technique is being increasingly applied to many scientific aspects. In China the technique is being applied to invitro assays clinically instead of the traditional methods. Introduction of the technique has made our work get twice the result with half the effort and seemed to make radionuclide-based operation easy be accepted since its simplicity, time-saving and suitability to automation. With improving of the performance of the magnetic solid phase, the unique technique will undoubtedly have a substantial future impact on the practice of radionuclide-based laboratory diagnoses.

5. CONCLUSION

A second antibody immobilized on polyacrolein magnetic particles through aldehyde functional groups and first antibodies immobilized on carboxylic polystyrene magnetic particles through carboxyl functional groups were recommended to apply to RIAs and/or IRMAs. Streptavidin immobilized on commercial magnetic particles through amino functional groups was successfully applied to separating specific PCR products for quatification of HCMV. The results showed that the magnetic particle separation technique was a reliable and simple tool for RIA/IRMA and quantitative PCR assay.

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