Development of amino acid conjugated highly sensitive and simple C-reactive protein (CRP) latex test and its clinical significance in liver cirrhosis

○ Hideki KOHNO

College of Industrial Technology, Nihon University

Background

C-reactive protein (CRP) is one of acute-phase proteins, and it is important of inflammatory response. CRP levels elevate rapidly in various disease resulted from tissue injury, infection and/or inflammation. To elucidate more accurate functional status of liver disease in detail, we developed novel type of ultra sensitive CRP latex test using amino acid spacers with high sensitive and wider assay range. Furthermore, to pursue more evaluation of CRP in Clinical diagnosis, we studied CRP level in chronic liver disease and liver cirrhosis at the event of possible recurrence of liver cirrhosis into acute phase.

Purpose

Latex reagents are considered to be effective immunoassay method. In order to evaluate small change of CRP in liver disease, we developed ultra high sensitive CRP reagent using amino acids conjugated latex beads which enabled us to measure 1-10ng/ml CRP in patient of various type of liver disease.

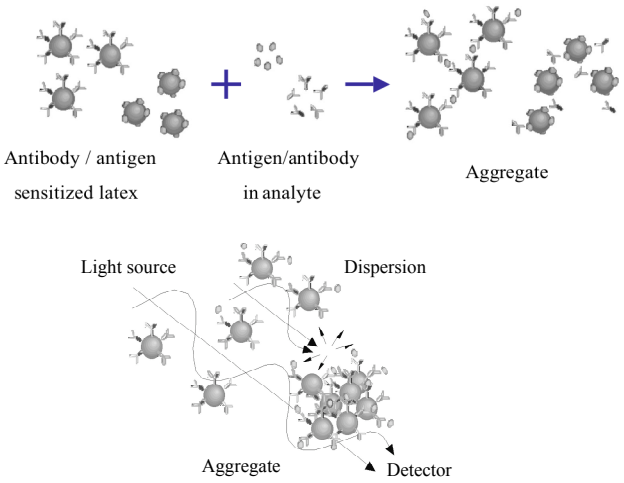

Fig. 1 Principle of latex photometric immunoassay using near infrared nephelometry

Materials and Methods

Latex reagents were prepared in novel manner using amino acids (glycine, arginine and others) conjugated polystyrene beads (200-400nm in diameter). The beads were coupled with various

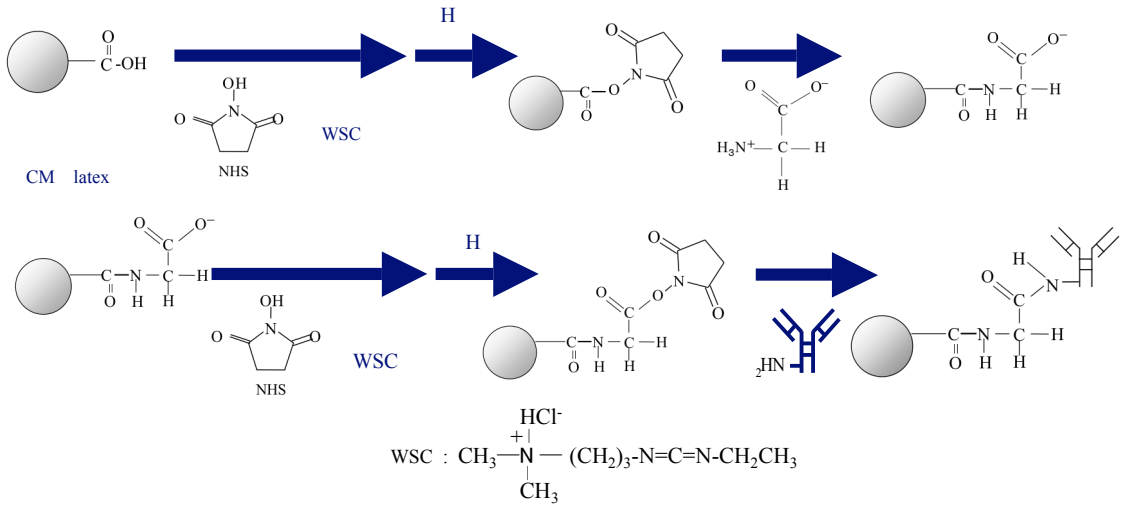

Fig. 2 Conjugation of an antibody to Carboxyl Modified (CM) latex by carbodiimide

amino acids and then, our sensitive anti CRP immunoglobulin fragments were conjugated to the spaced amino acid. In comparison, we prepared direct chemically coupled reagent without spacer amino acids. Using these reagents, the normal subjects and patients of cirrhosis were measured. During the course of study, we evaluated sensitivity and specificity in the use of our novel type of our CRP reagent in Latex Photometric Immunoassay System (LPIA).

Results and Discussions

Capability of our developed CRP latex test showed averaged value of 422ng/ml (max. 1825ng/ml, min.199ng/ml) in normal subjects sera (n=30).

On the other hand, cirrhosis group (n=400) showed the CRP level in averaged value of 1159ng /ml (max.13187ng/ml, min.342ng/ml). We evidenced that our latex agglutination test could measure accurately the low concentration CRP and it showed that cirrhosis patient group indicated higher level of CRP. Among the chronic hepatitis, it was found that the rapid increase of CRP indicated the recurrence hepatitis.

Other aspect of clinical significance in CRP, AMD group (n=9) showed the CRP level in averaged value of 931ng /ml (max.2694ng/ml, min.327ng/ml). Although, the measured number are small, we evidenced that our latex agglutination test measured accurately low concentration CRP and it showed that AMD patient group indicated higher level of CRP.

Conclusion

We developed ultra high sensitive latex agglutination test using amino acids spacer conjugation method. These reagents were evaluated in clinical samples. The reagent showed simple and stable in the use of clinical investigation and it discriminated between the normal and patients. Our conclusion is that our amino acid spaced latex reagent can measure 1-10ng/ml serum CRP in normal subjects and cirrhosis patient as short as in 3min.

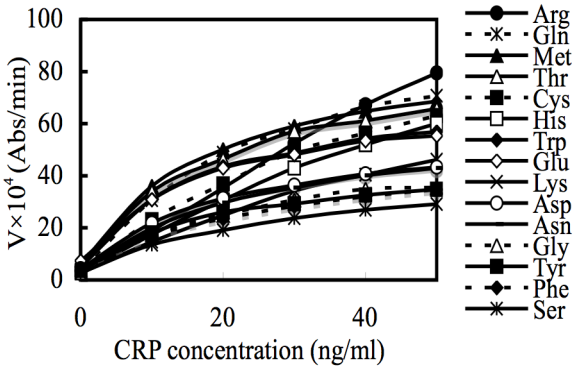

Fig 3. Aggregation of various amino acid spacers latex in Comparison various amino acid spacers

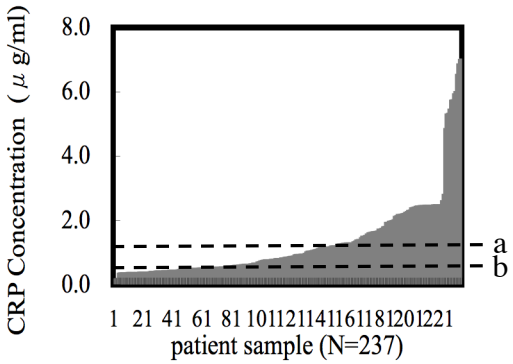

Fig. 4 CRP concentration of lever cirrhosis group
a : average of cirrhosis patient
b : average of normal subjects

References

1) Hideki Kohno, Patent file Japan 2005-345713
2) Wan Beom park, etc. Diagnostic Microbiology and Infectious Disease, Vol.51, pp.227-230, 2005
3) Andrew K. Vine, etc., The American of Ophthalmology Published, Vol.112, 2076-2080, 2005