Short Communication

EFFECT OF STORAGE TEMPERATURE AND MULTIPLE FREEZING THAWING ON STABILITY OF GOAT POLYCLONAL ANTIBODY

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ABSTRACT

Deterioration of antibody EB07884 Goat anti- Ogg1 (mouse) antibody raised against the peptide having amino acid C-HPKTSQAKGPSPLANK by freeze and thaw method was studied. The antibody remained stable at the tested temperatures (0 to 37oC) and durations (1 to 30 days). In the western blot hybridization, relatively higher concentration of antibody was required as the number of freeze thaw cycles increased. This signifies that multiple freezing thawing deteriorates the quality of antibody; however the deterioration can be compensated by using higher volume of antibody.

Key words: Antibody, freezing and thawing, ELISA, and Western Blot

INTRODUCTION

Antibodies are the defensive glycoprotein molecules against pathogens and foreign bodies (antigen) and are found mainly in the serum of vertebrates (Madigan and Martinko, 2006). Proteins such as immunogens or antigens are most efficient in triggering the antibody production (Paniker, 2005).

Antibodies and their derivative fragments have long been used as tools in a variety of applications, in fundamental research work, biotechnology, diagnosis, and even human therapy. Incubation of conventional antibody fragments at high temperatures results in dissociation of the native structures, with subsequent exposure of the hydrophobic interfaces of both the heavy and the light chains. These exposed "sticky" areas induce aggregation and precipitation, ultimately resulting in nonfunctional molecules (Dumoulin *et al.*, 2002). Fast freezing of protein cause more damage to proteins and give lower recovery of activity after freezing and thawing, the phenomenon being explained as the ice-induced partial unfolding of proteins (Chang *et al.*, 1996). There is a lack of systematic study of the freezing rate dependence of protein denaturation and very little work has addressed how to reduce freezing damage during freezing and thawing through the optimization of operation conditions (Jiang and Nail, 1998).

The antibody production is a labour intensive and long process and it is produced in very low amount, the cost of antibody is very high. The manufacturer recommends to store at -20°C minimize freezing and thawing. This study tried to analyze the effect of multiple freezing thawing and storage at higher temperatures for different periods on antibody stability.

MATERIALS AND METHODS

Peptide reconstitution:

Peptide sequence (C-HPKTSQAKGPSPLANK) for antibody production was selected from the internal region of the protein sequence and synthesized chemically (Thermo Scientific).

Antibody preparation:

The antibody was aliquoted to different micro centrifuge tubes. Altogether 20 samples were prepared, and tubes were labeled 1-15 for temperature stability study and 1-5 for freezing thawing stability study. For temperature stability study, tube number 1,2,3,4 and 5 were kept at 0-4°C tube numbers 6,7,8,9 and 10 were kept at 25°C and tube numbers 11,12,13,14 and 15 were kept at 37°C for 1,3,7,15 and 30 days respectively. For freezing thawing stability study, tube number 1,2,3,4 and 5 were subjected to 1,5,10, 20 and 30 cycles of freezing and thawing respectively. Freezing was done at -20°C and thawed at 0-4°C. All samples after treatments were stored for further experiment at -20°C. The samples were analysed by western blotting and ELISA.

Enzyme linked immunosorbent assay (ELISA):

Individual 96 well plate were coated with 100ng peptide. Solution containing 100ng processed antibody was added to the wells in the first row, half diluted subsequently up to last row and incubated for

an hour. Alkaline phosphatase conjugated rabbit anti goat IgG secondary antibody was added to each well and incubated. 50µg p-nitrophenyl phosphate was added, color development was observed, and absorbance was taken at 450nm.

SDS-PAGE:

12% polyacrylamide gel was prepared in which tissue lysate containing 35µg protein was added to each lane and it was allowed to run at 200 constant volts for an hour. Thus separated proteins were electroblotted to PVDF membrane.

Western blotting:

PVDF membrane strips were incubated with the processed antibody for an hour. Three different concentration were tested i.e. 0.01µg/ml, 0.1µg/ml, and 1µg/ml for differential analysis. HRP conjugated rabbit anti goat IgG (Rockland) was used as secondary antibody. The detection method was chemiluminiscence. Detecting reagent (Solution A luminol and Solution B hydrogen peroxide) were mixed in 1:40 ratio and flooded over the strips for 3 minutes. Then the strips were taken to dark room and exposed to Kodak film for 1, 3 and 10 minutes. The dark bands were observed indicating binding of antibodies, if any.

RESULTS

1. Storage of antibody at higher temperature

Higher temperature is generally unfavorable for proteins in solutions so that they are stored at low temperatures. However, this study revealed that higher storage temperature does not alter the quality of antibody significantly (fig. 1). Three different concentrations were tested for each processed antibody. The highest concentration of antibody showed a strong band around 37kda, the medium concentration showed weak faint bands, while no bands were visible for the lowest concentration of antibody. The antibody was found stable even after storage at 37°C for 30 days. ELISA also showed the similar result which was consistent with western blotting (data not shown).

Multiple Freezing thawing of antibody

This study found that freezing and thawing of antibody upto 30 cycles does not alter its function in binding the rat spleen lysate protein as the western blot analysis showed only slightly decreasing intensity of band as the number of freeze-thaw cycle increases

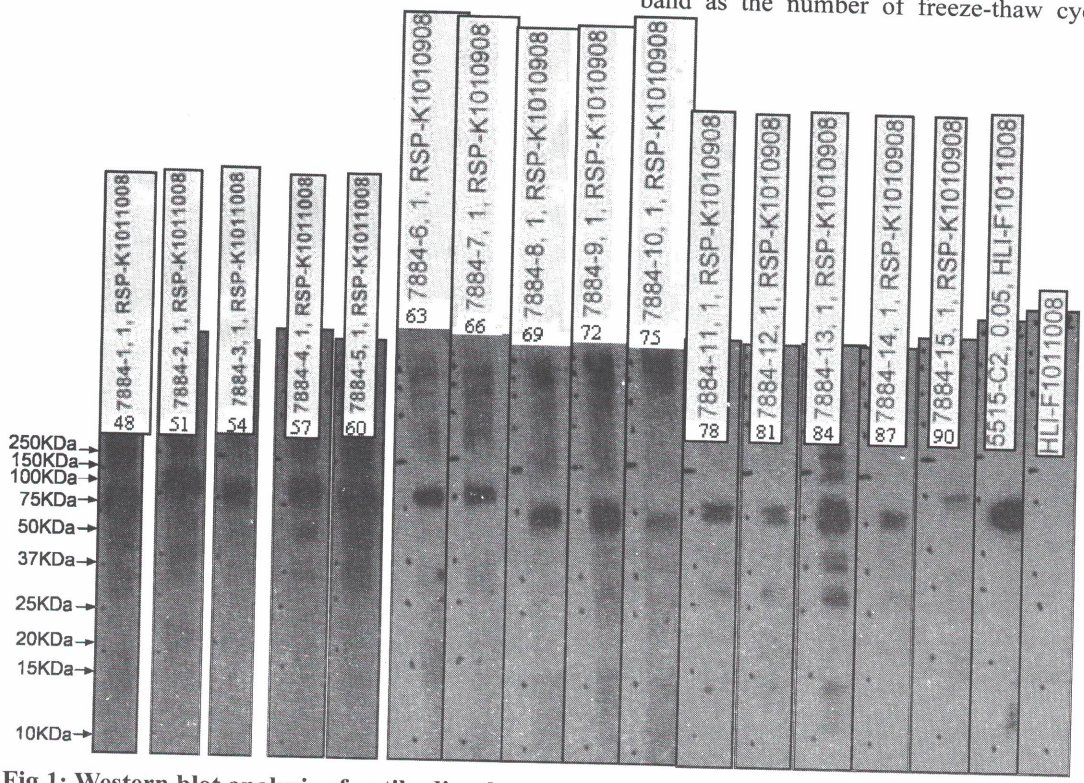

Fig 1: Western blot analysis of antibodies those were stored at different temperature for different duration. Each lane was run with 35µg rat spleen lysate protein and 1µg/ml Goat anti-OGG1 (mouse) antibody. HRP conjugated rabbit anti goat IgG diluted to 1:25000 was used as secondary antibody and detected by chemi-luminescence. Strip number 48, 51, 54, 57 and 60 shows the result for antibody stored at 0-4°C for 1, 3, 7, 15 and 30 days respectively. Strip number 63, 66, 69, 72 and 75 shows the result for antibody stored at 25°C for 1, 3, 7, 15 and 30 days respectively. Strip number 78, 81, 84, 87 and 90 shows the result for antibody stored at 37°C for 1, 3, 7, 15 and 30 days respectively. The last two strips were used as positive and negative control as indicated. Only slight variation on band intensity could be observed.

for antibody subjected for 20 and 30 cycles of freezing and thawing. The ten times more concentration showed the strong band, the background was also higher and other nonspecific bands were also visible. But the intensity of the band was found decreasing as the number of freeze thaw cycle increases (fig. 2). ELISA test for freezing thawing stability study also showed the result consistent with the result of western blotting. The titre value was found decreased for antibody subjected to maximum freezing and thawing. The titer value was first decreased, remained constant, again decreased and remained constant. Antibody showed the decreased titer value as the number of freeze thaw cycles increases. However the antibody was still capable of recognizing the peptide specific for it. These results clearly indicate that multiple freezing and thawing does not significantly deteriorate the antibody quality and it is still stable. But the slight changes might have occurred because the bands in western blot were strong only for the highest concentration of antibody used and its intensity is decreased as freezing thawing cycle increased while no bands were observed at previously used concentration for antibody subjected for higher number of freeze thaw cycle. ELISA also showed significantly decreased titre value for antibody subjected to maximum freeze thaw cycle.

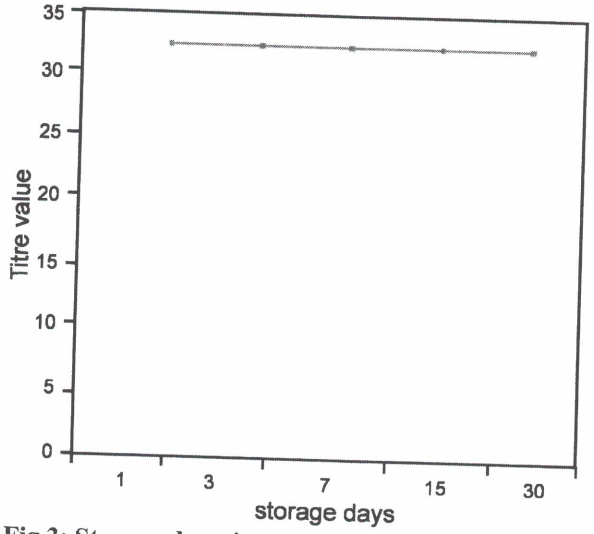

Fig 3: Storage duration vs ELISA titre value for antibody stored at 0-4°C for 1, 3, 7, 15 and 30 days. A constant titre value of 32K was observed.

These results are inconsistent with the literature studied (Petrakis NL, 1985; Helgason *et al.*, 1993) as stated that fast freezing produced more damage to proteins and lower recovery of activity after multiple freeze thaw cycles. Petrakis (1985) revealed that just 1 freeze-thaw cycle significantly reduced IgG levels and IgM activity to 25% below prefreezing levels. Similarly, Helgason *et al.* (1993) had demonstrated that IgG antibodies to cytomegalovirus were unstable after more than 5 freeze-thaw cycles, leading to significant variability in antibody testing results. But this result is consistent with the study (Fipps *et al.*, 1988) that antibodies to HIV were stable after 20 cycles of freezing and thawing and there is slight loss of antibody activity after prolonged storage at 37°C. The antibody we have used already contained bovine serum albumin. BSA might have played its role in antibody stability. Three different concentrations used in western blotting were 0.01 µg/ml, 0.1µg/ml and 1µg/ml. Antibody was found stable as it detected the specific protein in western blotting and ELISA. However, the strength of band in western blot and ELISA titre value decreased indicating the slight loss of antibody quality after storage at higher temperature for longer duration and as the number of freeze thaw cycle is increased.

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We would like to acknowledge the instant support and assistance from technical staffs of Everest Biotech. Thanks to Mr. Ishwori Adhikari for kindly providing the antibody required for the study.

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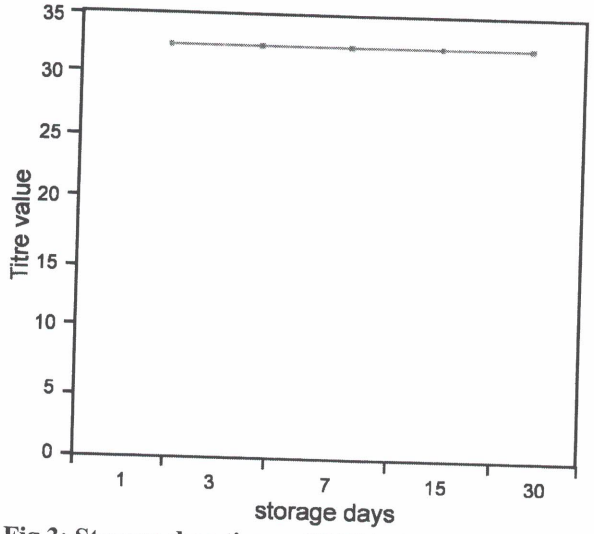

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