Optimization of Antibody Isolation Collected From Hyperimunized Rabbit using Ammonium Sulphate With and Without Centrifugation

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Abstract
Antibody utilization for many purposes are increased nowadays especially in medical applications such as desease therapy and biochemical asssay for diagnosis as well as in research applications. Antibody produced in animal previously purified by various process in order to achieve good quality including isolation, purification and poolishing step. This study aim at gaining optimal condition for antibody isolation from rabbit serum after hyperimunization. Hyperimun serum collected from rabbit immunized by HCG was isolated using various concentration of ammonium sulfat from 20% to 80% with centrifugation while another serum collected from rabbit immunized by IgY was isolated without centrifugation. Pellets of each groups were dialysed and characterized by SDS-PAGE. We found that optimum concentration of ammonium sulphate to isolate serum antibody with centrifugation was achieved at 60%. Additionally, we found that optimum concentration to isolate antibody without centrifugation was achieved at 40% after incubation for 8 hours stepwise.

Key words: ammonium sulphate, antibody isolation, HCG, IgY, optimization

Introduction

Antibody nowadays has been widely used for many purposes such as medical diagnosis applications, theurapeutic, and immunochemical techniques in general research. Antibody can be produced by immunize animal using spesific antigen and harvested after some incubation times through animal‘s blood collection and serum preparation known as hyperimun serum (Graham, 1995). There are many kinds of protein in serum including antibody therefore some processes have to be conducted in order to obtain ready to use antibody such as isolation, purification and poolishing.

The method of antibody isolation depend on several consideration in purpose of using, type of assay to be perform and source of antibody as well as expected level of purity (Suhartono, 1989). Besides, it is important to recognize some biochemical and physical properties of antibody while sustaining its native strucutre and reactivity againts spesific antigen. One common technique for isolating proteins including antibody is salt precipitation. The solubility of antibody is related to the salt concentration of the antibody-containing solutions. This is commonly called salting out so the differences in composition and shape of antibody among other proteins mean that antibody precipitate at certain concentration of salt. Darmawi et.al (2009) used concentration of 60% Ammonium sulphate to isolate IgY from chicken egg yolk immunized by L3 A. galli and obtained 0,875 mg/ml yield of IgY. Different protein composition in rabbit serum from those in chicken egg yolk lead us to this study which aim at achieving optimal condition for isolating antibody from two kinds of rabbit serum immunized by human chorionic gonadotropin (HCG) and chicken IgY. We modify the concentration range of Ammonium sulphate (w/v) from 10% to 80% as well as centrifugation steps in order to avoid intervention on antibody precipitation during the isolation process.

Method

Hyperimun serum from rabbit immunized by HCG prepared then 1 mL was dispensed into microtube. 0,1 gr of Ammonium sulphate (10% w/v) was added into serum while being shaked gently. The solutions was incubated one hour at 4^{O}C and centrifugated 1000 rpm for 10 minutes.

The pellet was collected while supernatan was followed by Ammonium sulphate addition to achieve intended concentration (w/v) namely 20% to 80% with the same treatment as the first. Each of pellets then were dialysed in aquadest and analysed using SDS-PAGE technique.

Hyperimun serum from rabbit immunized by chicken IgY prepared then 1 mL was dispensed into microtube. 0,2 gr of Ammonium sulphate (20% w/v) was added into serum while being shaked gently. The solutions was incubated four hours at 4^{O}C with no centrifugation step. The pellet was collected while supernatan was followed by Ammonium sulphate addition to achieve intended concentration (w/v) from 40% to 80% with the same treatment as the first. Each of pellets then were dialysed in aquadest and analysed using SDS-PAGE technique. Longer incubation time for eight, twelve, and sixteen hours have also been observed in this study.

include the design, population, sample, data sources, techniques/instruments of data collection and data analysis procedures. Methods should make readers be able to reproduce the experiment. Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described. Do not repeat the details of established methods.

Results

Results of first optimization on anti-HCG-containing serum with centrifugation step are discribed in figure 1 as below:

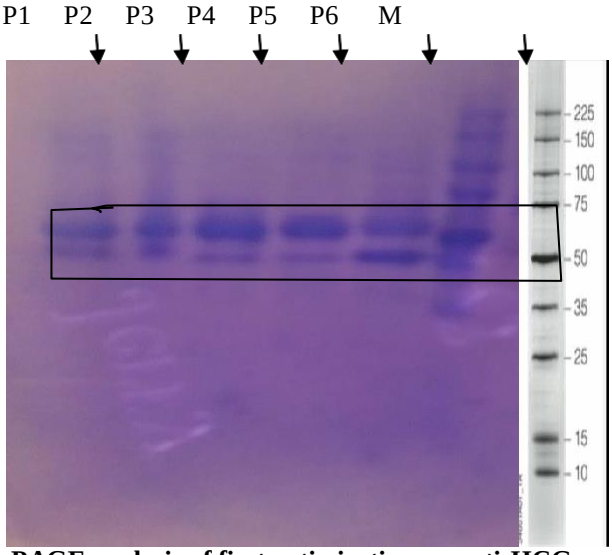

Figure 1. SDS-PAGE analysis of first optimization on anti-HCG-containing serum. (P1) 10% pelet, (P2) 20% pelet, (P3) 40% pelet, (P4) 60% pelet, (P5) 80% pelet (P6) Marker

Optimization on anti-HCG-containing serum have been shown in Figure 1. A distinct number of band in P4 (60% Ammonium sulphate) indicates less protein can be isolated including antibody (equal to 50 kD). While in other wells there are more bands indicate more proteins precipitate during incubation time. These data might be influenced by centrifugation step that interfere precipitation process of antibody and other proteins. Therefore we continue to next optimization with no centrifugation step.

Results of second optimization on anti-IgY-containing serum with no centrifugation step are discribed in figure 2 as below:

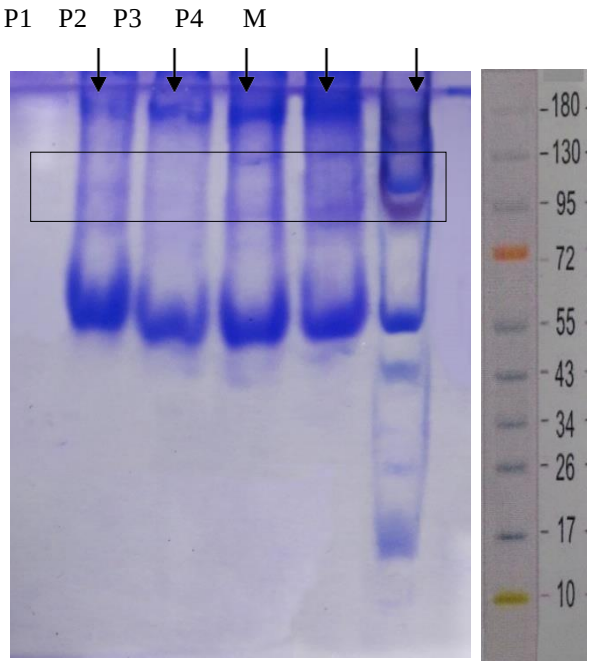

Figure 2. SDS-PAGE analysis of second optimization on anti-IgY-containing serum. (P1) 20% pelet, (P2) 40% pelet, (P3) 60% pelet, (P4) 80% pelet, (M) Marker

Optimization on anti-IgY-containing serum have been shown in Figure 2. A distinct number of band in P2 (40% Ammonium sulphate) indicates only one protein can be isolated namely antibody (equal to 55 kD). While in other wells there are more bands indicate more proteins precipitate during incubation time.

Results of whole optimization on anti-IgY-containing serum were summarized in table 1 as below:

Table 1 Optimization on anti-IgY-containing serum

No	Optimization	20%	40%	60%	80%
1	4hrs	150 kDa	150 kDa	150 kDa	150 kDa
		100 kDa	50 kDa	125 kDa	125 kDa
		75 kDa		100 kDa	100 kDa
		50 kDa		50 kDa	50 kDa
2	8 hrs	150 kDa	150 kDa	150 kDa	150 kDa
		100 kDa	50 kDa	125 kDa	125 kDa
		75 kDa		100 kDa	100 kDa
		50 kDa		75 kDa	75 kDa
				50 kDa	50 kDa

3	12 hrs	150 kDa	150 kDa	150 kDa	150 kDa
		75 kDa	100 kDa	100 kDa	125 kDa
		50 kDa	50 kDa	75 kDa	100 kDa
				50 kDa	75 kDa
					50 kDa
4	16 hrs	150 kDa	150 kDa	150 kDa	150 kDa
		75 kDa	100 kDa	100 kDa	100 kDa
		50 kDa	50 kDa	50 kDa	75 kDa
					50 kDa

Compilation of proteins isolated from each of incubation time were described in table 1. Interesting results seen at 4 hours and 8 hours with 40% concentration of ammonium sulphate, there were two bands of 150 kD and 50 kD that indicate the same protein namely rabbit antibody in native formation (150 kD) and heavy chain fragmen (50 kD). We choose 4 hours as an optimum condition than 8 hours to cover the limitation of method in this study.

Discussion

These datas from different treatment of optimization confirm that centrifugation step influence precipitation process of antibody after incubation time. Without centrifugation antibody fall down naturally following the gravitation as well as being isolated from other proteins in appropriate concentration of Ammonium sulphate. More efficient salt was obtained (40%) in second optimization due to less proteins precipitate during incubation time and pellet collection. Less bands of protein indicates only antibody precipitate at the time (4 hrs) therefore we can use these results as potential early step in antibody purification from rabbit hyperimun serum. For instance, antibody purification using hydrophobic cromatography technique will give better yields if we use better sample namely isolated antibody from other proteins in crude sample.

Conclusion

In Conclusion, we found that centrifugation step give different results on yields of antibody precipitation using ‑salting out‖ technique therefore in our study optimum concentration to isolate antibody from rabbit hyperimmun serum was achieved at 40% concentration of ammonium sulphate after incubation for 4 hours without centrifugation.

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