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Pepsin-mediated hydrolysis of partially purified chicken serum immunoglobulin Y under acidic conditions: A preliminary study using SDS-PAGE

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Abstract

Immunoglobulin Y (IgY) is a poultry antibody platform useful for biological and biomedical applications, but its electrophoretic response to acid proteolysis depends on digestion conditions and sample preparation. This preliminary study evaluated bovine pepsin-mediated hydrolysis of partially purified IgY from local Indonesian chicken serum. IgY was partially isolated using caprylic acid treatment followed by ammonium sulphate precipitation, quantified at 280 nm and adjusted to 1 mg/mL, and incubated with bovine pepsin at pH 2.0–2.5, 37°C for 30, 60, or 90 minutes. The reaction was stopped by rapid neutralisation using 1 M Tris and heating at 95°C for 3 minutes. The product was analysed by 12.5% SDS-PAGE using 5x Laemmli sample buffer containing beta-mercaptoethanol (BME). The pepsin-free control retained the main bands at approximately 100–135 kDa and 48–63 kDa, whereas the pepsin-treated samples showed a prominent band around 68–75 kDa with decreasing intensity over time and the loss of the band around 125–135 kDa. These findings indicate that bovine pepsin alters the SDS-PAGE profile of partially purified chicken serum IgY under acidic conditions. Further confirmation via densitometry, immunochemistry, or proteomics is recommended.

Keywords: Chicken serum; Immunoglobulin Y; IgY hydrolysis; Pepsin; Caprylic acid; Ammonium sulphate; SDS-PAGE

1. Introduction

Immunoglobulin Y (IgY) is the avian functional counterpart of mammalian IgG and is found in both chicken serum and egg yolk, where it contributes to passive immunity in developing chicken embryos. IgY-based antibodies are of interest for biological and pharmaceutical research as they can be produced economically, demonstrate useful specificity, and have been investigated for diagnostic, preventive, and therapeutic purposes in human, veterinary, and aquaculture contexts [1–6]. Although egg yolk is commonly used as a non-invasive source of IgY, IgY preparations derived from serum remain relevant for the development of laboratory-scale methodologies and for experimental work involving defined serum samples.

The purification of immunoglobulins from biological fluids often relies on simple, low-cost precipitation or fractionation procedures that are compatible with routine laboratory infrastructure. Caprylic acid has long been used to precipitate non-immunoglobulin serum proteins, whilst ammonium sulphate precipitation remains the classic salt-precipitation approach for concentrating immunoglobulins [7]. A combined caprylic acid and ammonium sulphate approach has been applied to the purification of serum immunoglobulins, and more recent studies on caprylic acid-based IgY purification support the practical value of caprylic acid as a simple, scalable reagent for IgY enrichment [8,9].

Hydrolysis of antibodies using pepsin can remove or modify Fc-associated regions whilst preserving the antigen-binding regions. In mammalian IgG, digestion by pepsin is generally associated with the formation of F(ab')2, whereas IgY has a different heavy-chain organisation and may exhibit a different fragmentation profile depending on digestion

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and electrophoresis conditions [10–12]. Pepsin activity is most optimal under acidic conditions, and IgY is known to be sensitive to low pH and digestive enzymes; however, the digestion pattern depends on sample purity, enzyme-to-substrate ratio, pH, incubation time, and whether a reducing agent is used during SDS-PAGE analysis [13,14].

This study aims to provide an initial description of pepsin-mediated hydrolysis of partially purified IgY from the serum of local Indonesian chickens and to analyse the resulting band patterns using 12.5% SDS-PAGE. The specific objective is to compare the electrophoretic profiles of IgY incubated at acidic pH with and without pepsin for 30, 60, and 90 minutes using Laemmli sample buffer containing beta-mercaptoethanol (BME).

2. Materials and methods

2.1. Study design

This laboratory study evaluated the effect of digestion time using bovine pepsin (food grade, China) on partially purified chicken serum IgY. The experimental treatments consisted of an untreated control and samples treated with pepsin, which were incubated at a pH of 2.0–2.5 and a temperature of 37°C in an incubator. The same partially purified IgY preparation was used for both the control and the pepsin-treated samples, whilst each enzymatic digestion was carried out independently at least three times. The hydrolysis results were analysed visually for the formation of protein bands on 12.5% SDS-PAGE.

2.2. Partial isolation of IgY from local Indonesian chicken serum

Chicken serum was obtained from local Indonesian chickens (*Gallus gallus domesticus*). IgY was partially isolated using a sequential protocol combining caprylic acid treatment and ammonium sulphate precipitation, modified from previously reported immunoglobulin fractionation procedures [8,9]. In brief, serum proteins were treated with caprylic acid to reduce non-immunoglobulin proteins, followed by ammonium sulphate precipitation to enrich the immunoglobulin-containing fraction. The resulting protein fraction was redissolved and dialysed against phosphate buffer (pH 6) and stored at low temperature until further processing. Protein concentration was determined by measuring absorbance at 280 nm using a NanoDrop spectrophotometer in accordance with the manufacturer's recommended procedure.

2.3. Pepsin-mediated hydrolysis of IgY

Pepsin hydrolysis was carried out using a modified antibody fragmentation procedure, based on published research on IgY/antibody fragmentation and IgY stability under acidic proteolytic conditions [10–13]. A 1 mg/mL solution of IgY in phosphate buffer (pH 6) was stored on ice until the reaction began. Fresh pepsin solution was prepared in cold 10 mM HCl without reducing agents such as DTT (Dithiothreitol) or BME (beta-mercaptoethanol). Dilute HCl or acid buffer was used to adjust the final reaction pH to 2.0–2.5. The protein sample was added last to initiate the reaction, and the mixture was incubated at 37°C for 30, 60, or 90 minutes in a laboratory incubator. The digestion process was stopped by rapid neutralisation using 1 M Tris, followed by heating at 95°C for 3 minutes.

2.4. Control treatments

Control samples contained IgY with the same protein concentration but without pepsin. Acid controls were adjusted to pH 2.0–2.5 and were either not incubated or incubated at 37°C for 30, 60, or 90 minutes in the same incubator used for the pepsin treatments. The neutral pH control consisted of IgY without incubation and IgY incubated at 37°C for 90 minutes. This control was included to distinguish pepsin-mediated proteolysis from the effects of acid exposure, heating, or incubation time alone.

2.5. SDS-PAGE Analysis

Hydrolysis products were analysed using 12.5% SDS-PAGE based on the Laemmli system with laboratory modifications [15]. Samples were mixed with 5× Laemmli sample buffer containing beta-mercaptoethanol (BME) prior to loading. A commercial protein ladder (BLUelf Prestained Protein Ladder, GeneDireX, Inc.) was used as a molecular weight marker. Following electrophoresis, the gel was stained and photographed. Band interpretation was performed visually by comparing the control lane and the pepsin-treated lane against the molecular weight marker.

2.6. Determination of lanes for SDS-PAGE

The distribution of samples and the protein molecular weight marker in each lane of the 12.5% SDS–PAGE gel are shown in Table 1.

Table 1 Sample loading arrangement for the 12.5% SDS–PAGE gel

Lanes	Sample conditions
1	Control IgY without pepsin; no incubation; pH 2.0–2.5
2	IgY control without pepsin; pH 2.0–2.5; 30 minutes at 37°C
3	IgY control without pepsin; pH 2.0–2.5; 60 minutes at 37°C
4	IgY control without pepsin; pH 2.0–2.5; 90 minutes at 37°C
5	IgY + pepsin; pH 2.0–2.5; 30 minutes at 37°C
6	IgY + pepsin; pH 2.0–2.5; 60 minutes at 37°C
7	IgY + pepsin; pH 2.0–2.5; 90 minutes at 37°C
8	IgY control; no incubation; neutral pH
9	IgY control; neutral pH; 90 minutes at 37°C
Mr	BLUelf Prestained Protein Ladder

3. Results

3.1. Electrophoresis profile of the IgY control

In the neutral control lane (lane 8), two main bands were visible in the molecular weight range of approximately 100–135 kDa and 48–63 kDa. A generally similar pattern was also observed in the control samples exposed to acid without pepsin (lanes 1–4), including the unincubated acid control and samples incubated at 37°C for 30, 60, and 90 minutes. Therefore, incubation under acidic conditions alone did not eliminate the upper band in the range of approximately 125–135 kDa and did not produce the pepsin-associated banding pattern observed in lanes 5–7.

3.2. Time-dependent changes in SDS-PAGE following bovine pepsin treatment

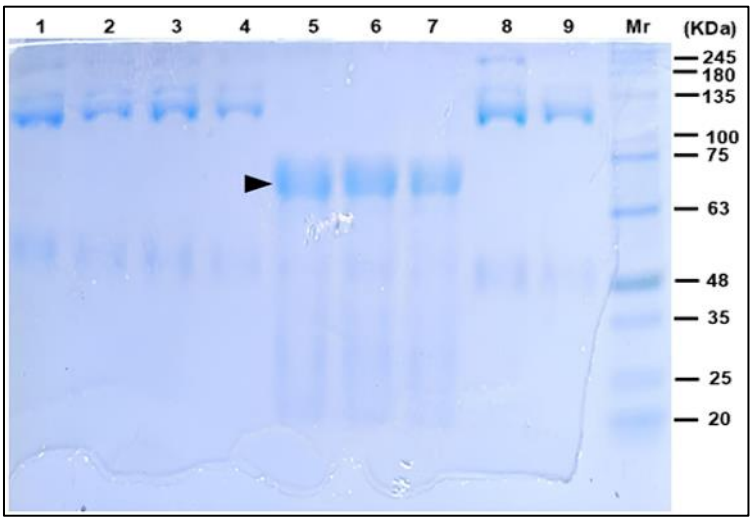

Figure 1 Representative SDS-PAGE profiles of partially purified local chicken serum IgY following acid incubation with or without pepsin. Lanes 1–4, IgY control without pepsin at pH 2.0–2.5 for 0, 30, 60, and 90 minutes; lanes 5–7, IgY plus bovine pepsin at pH 2.0–2.5 for 30, 60, and 90 minutes; lane 8, neutral pH IgY control without incubation; lane 9, neutral pH IgY control after 90 minutes at 37°C; Mr, BLUelf Prestained Protein Ladder (GeneDireX, Inc.). Lanes treated with pepsin showed prominent bands at approximately 68–75 kDa (arrowhead) with decreasing intensity over time, accompanied by the loss of a band at approximately 125–135 kDa. This figure represents at least three independent replicates

Samples treated with pepsin (lanes 5–7) showed a prominent band at approximately 68–75 kDa. The intensity of this band appeared to decrease with increasing incubation time from 30 to 90 minutes, indicating progressive proteolytic processing during prolonged exposure to pepsin under acidic conditions. A striking change in the pepsin-treated lanes was the disappearance of a band of approximately 125–135 kDa, which remained visible in the neutral and acidic controls. This pattern suggests that pepsin treatment, rather than simply incubation at low pH, is associated with the conversion or degradation of high-molecular-weight IgY-containing material into low-molecular-weight species under the SDS-PAGE conditions tested using Laemmli sample buffer containing BME.

4. Discussion

The current gel profile supports the interpretation that bovine pepsin alters the SDS-PAGE profile of partially purified chicken serum IgY under acidic conditions. The persistence of the main bands in the 100–135 kDa and 48–63 kDa ranges in the neutral and acid-only controls suggests that the loss of the band around 125–135 kDa in lanes 5–7 is primarily due to the enzyme, rather than low pH or incubation at 37°C alone. This interpretation is consistent with recent ex vivo evidence suggesting that acidic conditions alone may be less damaging to IgY than an acidic proteolytic environment, whilst gastric/stomach conditions containing proteases can significantly degrade IgY, as demonstrated by SDS-PAGE and immunoblotting [13].

The molecular weights reported here should be interpreted with caution. Samples were prepared with Laemmli loading buffer containing beta-mercaptoethanol prior to SDS-PAGE; however, electrophoretic mobility may be influenced by sample preparation, the state of partial purification, and the heterogeneity of proteolytic fragments. Furthermore, the IgY preparation is only partially purified from serum; therefore, residual serum proteins or co-precipitated proteins may affect band clarity or contribute to background bands. For this reason, this study does not establish definitive protein identities for individual SDS-PAGE bands.

Several lines of investigation would strengthen and extend these preliminary findings in future work. First, densitometric analysis of replicate SDS-PAGE gels would enable semi-quantitative tracking of the 125–135 kDa and 68–75 kDa bands across incubation times. Second, additional replicates under identical sample-preparation conditions (in the presence of BME) would establish the reproducibility of the observed band patterns. Third, confirmatory analysis by Western blotting with anti-IgY antibodies or by mass spectrometry would verify whether the bands are IgY-derived, resolving the uncertainty inherent to a partially purified sample. Fourth, consistent reporting of key parameters—enzyme source (bovine pepsin), protein quantification (OD_{280} via NanoDrop), incubation conditions (37 °C), and sample-buffer composition (5× Laemmli buffer with BME)—would support reproducibility across laboratories.

Within these limits, the present study provides a practical preliminary protocol for evaluating IgY hydrolysis using readily available reagents and conventional SDS-PAGE. The combined use of acid-only controls, neutral-pH controls, and sequential bovine-pepsin digestion supports the interpretation that the loss of the ~125–135 kDa band and the emergence of the ~68–75 kDa band are attributable to enzymatic hydrolysis. Considered alongside recent IgY literature on purification, stability, and antibody-fragment generation, these findings underscore the value of further optimising IgY hydrolysis conditions [6,9,12,13].

5. Conclusion

Treatment of partially purified chicken serum IgY with bovine pepsin at pH 2.0–2.5 and 37 °C altered the 12.5% SDS-PAGE profile: the ~125–135 kDa band was lost, and a prominent ~68–75 kDa band appeared, its intensity declining as incubation extended from 30 to 90 minutes. The pepsin-free control retained its principal bands at ~100–135 kDa and ~48–63 kDa. These results indicate that the modified protocol produces electrophoretic changes consistent with IgY hydrolysis within 30 minutes, with further processing during extended incubation. Confirmatory characterisation by densitometry, immunochemical detection, or proteomic analysis is recommended before definitive structural conclusions are drawn.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Statement of ethical approval

This study was approved by the Research Ethics Committee of the Faculty of Animal Science, University of Mataram (Approval No. 303/FapetUN/ETIK/2025).

Statement of informed consent

Informed consent was obtained from all individual participants included in the study."

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