*Journal of Pharmaceutical & Biomedical Analysis*
Vol. 7, No. 5, pp. 543–547, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00


# Adsorptive stripping voltammetry of $F(ab')_2$ and Fab fragments of anti-mouse immunoglobulin G*

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**Abstract**: The adsorptive stripping voltammetric behaviour of $F(ab')_2$ and Fab fragments of anti-mouse immunoglobulin G is described. Conditions were optimized for the determination of these fragments with respect to accumulation potential, accumulation time, scan rate, pulse amplitude, drop size and electrolyte composition. The $F(ab')_2$ and Fab fragments gave rise to behaviour similar to that reported previously for the intact immunoglobulin.

**Keywords**: *Adsorptive stripping voltammetry; anti-mouse immunoglobulin G; $F(ab')_2$ and Fab fragments.*

## Introduction

Studies of the electrochemical behaviour of immunoglobulins and the monitoring of immunological reactions using electrochemical techniques are becoming of increasing interest in analytical science. This is mainly due to the search for immunoassays that are more selective and safer than those currently employing radiochemical labels.

The electrochemical behaviour of immunoglobulin G (IgG) has previously been studied by Fontaine *et al.* [1, 2] using cyclic voltammetry, coulometry and polarography. These authors showed that adsorption plays an important rôle in determining the electrochemical behaviour of this protein. In recent papers, we have demonstrated how this adsorption process can be utilized to increase the sensitivity of electrochemical methods for the determination of proteins such as human serum albumin (HSA) and anti-HSA [3], and mouse IgG and anti-mouse IgG [4] using the technique of adsorptive stripping voltammetry (AdSV). In addition, we have demonstrated the possible application of this technique to monitor the reaction of an antigen and its corresponding antibody directly in aqueous solution [3, 4]. De Alwis and Wilson [5] have recently demonstrated the use of Fab fragments for a rapid heterogeneous competitive electrochemical immunoassay for IgG in the picomolar range.

In this paper, we report our results on the AdSV behaviour of $F(ab')_2$ and Fab

*Paper presented at 7th Australian Electrochemistry Conference, 14–19 February 1988, Sydney, Australia.
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fragments obtained following respective pepsin and papain enzymatic cleavages of anti-mouse IgG.

## Experimental

### *Materials*

All compounds used were of analytical grade and solutions were prepared in water obtained by passing distilled water through a Milli-Q water purification system. 0.1 and 0.01 M phosphate buffers (pH 7.4) were prepared using potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate.

The $F(ab')_2$ fragments were obtained from Serotec. These had been prepared following pepsin digestion of anti-mouse IgG (obtained from a rabbit). Fab fragments were obtained following a papain enzymatic digestion of anti-mouse IgG (obtained from a donkey) and purified according to the method reported by Dwyer *et al.* [6]. The papain was obtained from Sigma. The use of two papain digestion procedures were investigated for the preparation of Fab fragments. The first one involved the use of cysteine as an activator in the presence of EDTA (to complex any metal ions present in the solution), whereas the second method made use of 2-mercaptoethanol. Both of these methods gave satisfactory yields of Fab fragments, although neither method gave rise to a complete conversion of the anti-mouse IgG. Following digestion, the resulting Fab fragments were purified by affinity chromatographic separation on a CNBr-activated Sepharose column containing bound mouse IgG (Sigma). The presence of protein in successive 1-ml fractions eluting from the column were detected using a modified micro Bradford assay [6]. The fragments were further purified by separation from undigested mouse IgG on a Sephadex G-75 column. The purified Fab fragments were then characterized by HPLC on a Protein Pak 300 column (Waters) and by SDS–PAGE electrophoresis.

### *Apparatus*

A Waters Model 6000A HPLC pump was operated in conjunction with a Waters Model 440 absorbance detector for HPLC investigations. Adsorptive stripping voltammograms were obtained on a Princeton Applied Research Corporation (PARC) Model 264 polarographic analyser combined with a PARC Model 303 mercury electrode system, a PARC Model 305 magnetic stirrer and an Omnigraphic Model 2000 X-Y recorder.

### *Procedures*

Before each voltammetric scan, the buffer was purged with oxygen-free nitrogen for 8 min before adding the required amount of immunoglobulin fragment to the cell and further purging for 2 min. Care was taken to ensure that the purging did not cause significant frothing of the protein solution. Following the purging step, the electrode potential was set at the required accumulation potential ($E_{acc}$) for the required accumulation time ($t_{acc}$) with stirring before the stirrer was switched off automatically and the voltammetric experiment carried out.

## Results and Discussion

### *Adsorptive stripping voltammetry of $F(ab')_2$ fragments of anti-mouse IgG*

The differential pulse adsorptive stripping voltammetric behaviour of $F(ab')_2$

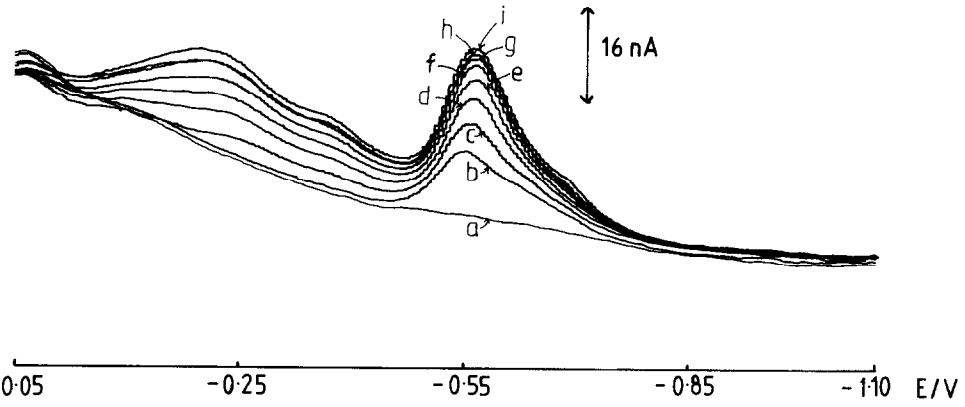


**Figure 1**
Effect of accumulation time(s) on differential pulse adsorptive stripping voltammetric behaviour of $F(ab')_2$ fragments of anti-mouse IgG in 0.1 M phosphate buffer. Conditions: $E_{acc}$ + 0.05 V versus Ag/AgCl; scan rate, 5 mV $s^{-1}$; pulse amplitude, 50 mV; $t_{acc}$ (a) 0 s, (b) 100 s, (c) 200 s, (d) 300 s, (e) 400 s, (f) 500 s, (g) 600 s, (h) 700 s, (i) 800 s.

fragments of anti-mouse IgG is similar to that reported previously for the intact immunoglobulin [4].

It can be seen from Fig. 1 that, in 0.1 M phosphate buffer (pH 7.4), under conditions of no accumulation using a starting potential of +0.05 V (versus Ag/AgCl), the $F(ab')_2$ fragments give rise to a single faradic peak at around −0.56 V. Under conditions of adsorptive accumulation at this starting potential ($E_{acc}$), an adsorption peak (peak A) appears at around −0.20 to −0.23 V, which increases in size on increasing $t_{acc}$. The current due to the peak at −0.56 V (peak B) also increases in size with $t_{acc}$, but linearity in the graph of peak current versus $t_{acc}$ is only maintained at values of $t_{acc}$ up to 300 s. At values greater than this, the electrode surface becomes fully covered with protein molecules.

The effect of varying the accumulation potential ($E_{acc}$), the accumulation time ($t_{acc}$), the pulse amplitude ($\Delta E$) and the scan rate ($\nu$) on the differential pulse adsorptive stripping voltammetric behaviour of $F(ab')_2$ fragments, is summarized in Table 1. From this it can be seen that the optimum accumulation potential is +0.15 V. At higher positive accumulation potentials, the current due to peak B decreases, a further peak appears at a more negative potential, and a large increase in background current is seen around +0.15 to −0.1 V due to the mercury oxidation response [7]. The effect of increasing the scan rate from 5 to 20 or 50 mV $s^{-1}$ had a deleterious effect on the shape of peak B, and caused a decrease in peak current. Increasing the pulse amplitude however, resulted in an increase in the peak current of peak B, and shifts in the peak potential to more positive values. Increasing the pulse amplitude to 100 mV also resulted, however, in an increase in the background current at more positive values (Fig. 2).

All of the results cited above were carried out using a Hg drop of area 0.016 $cm^2$ (medium drop). This size was chosen because when one employed a larger drop (A = 0.025 $cm^2$), a larger current was obtained, but the peak was broader and less well defined.

When a comparative study was carried out into the effect of electrolyte concentration, it was found that the sensitivity of the adsorptive stripping voltammetric method could be increased by 2–3 times using an 0.01 M phosphate buffer pH 7.4, when compared with

**Table 1**
Adsorptive stripping voltammetric data for $F(ab')_2$ fragments of anti-mouse IgG

| | | | | Peak A | | Peak B | |
|---|---|---|---|---|---|---|---|
| $t_{acc}$ (s) | $E_{acc}$ (V) | $v$ (mV $s^{-1}$) | $\Delta E$ (mV) | $i_p$ (μA) | $E_p$ (V) | $i_p$ (μA) | $E_p$ (V) |
| 200 | −0.10 | 5 | 50 | — | — | 0.019 | −0.525 |
| 200 | −0.05 | 5 | 50 | — | — | 0.019 | −0.525 |
| 200 | 0.00 | 5 | 50 | — | — | 0.024 | −0.525 |
| 200 | +0.05 | 5 | 50 | — | — | 0.026 | −0.525 |
| 200 | +0.10 | 5 | 50 | — | — | 0.031 | −0.525 |
| 200 | +0.15 | 5 | 50 | — | — | 0.031 | −0.525 |
| 200 | +0.20 | 5 | 50 | 0.003 | −0.175 | 0.028 | −0.525 |
| 100 | +0.05 | 5 | 50 | — | — | 0.014 | −0.55 |
| 200 | +0.05 | 5 | 50 | — | — | 0.018 | −0.55 |
| 300 | +0.05 | 5 | 50 | 0.008 | −0.23 | 0.022 | −0.56 |
| 400 | +0.05 | 5 | 50 | 0.004 | −0.23 | 0.024 | −0.56 |
| 500 | +0.05 | 5 | 50 | 0.006 | −0.22 | 0.026 | −0.56 |
| 600 | +0.05 | 5 | 50 | 0.007 | −0.21 | 0.027 | −0.56 |
| 700 | +0.05 | 5 | 50 | 0.007 | −0.21 | 0.028 | −0.56 |
| 800 | +0.05 | 5 | 50 | 0.009 | −0.21 | 0.029 | −0.56 |
| 200 | +0.05 | 5 | 25 | — | — | 0.019 | −0.53 |
| 200 | +0.05 | 5 | 100 | — | — | 0.019 | −0.48 |
| 200 | +0.05 | 20 | 50 | — | — | Badly defined | |
| 200 | +0.05 | 50 | 50 | — | — | Badly defined | |

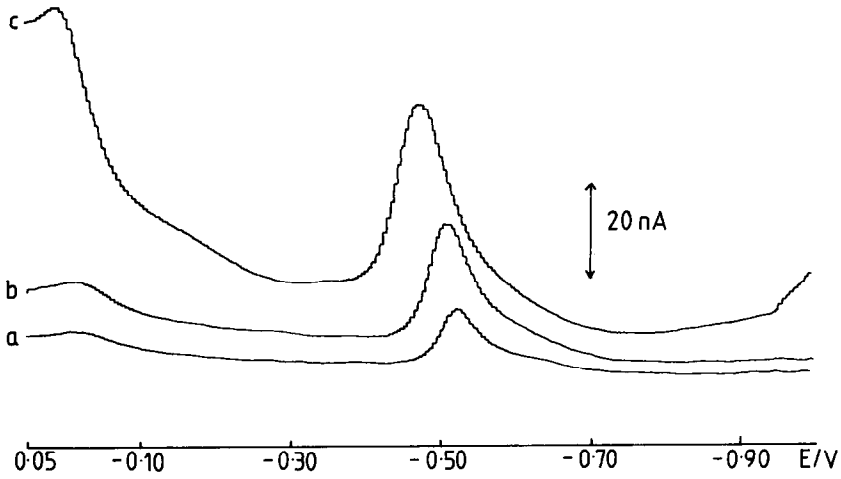


**Figure 2**
Effect of pulse amplitude on differential pulse adsorptive stripping voltammetric behaviour of $F(ab')_2$ fragments of anti-mouse IgG in 0.1 M phosphate buffer. Conditions: $E_{acc}$ + 0.05 V (versus Ag/AgCl); scan rate, 5 mV $s^{-1}$; $t_{acc}$ 300 s; pulse amplitude (a) 25 mV, (b) 50 mV, (c) 100 mV.

an 0.1 M phosphate buffer pH 7.4. This is analogous to the behaviour reported by Wang and Farias [8] for riboflavin. Under the optimum conditions cited in this paper, $F(ab')_2$ fragments could be determined down to 0.1 μg $ml^{-1}$ and a calibration curve proved linear over the range 0.2–1.0 μg $ml^{-1}$ with a slope of 11.2 nA $\mu g^{-1}$ ml.

*Adsorptive stripping voltammetry of Fab fragments of anti-mouse IgG*

In general, the Fab fragments gave rise to similar behaviour as that reported above for the $F(ab')_2$ fragments. The only difference was in the potential of peak B, which was shifted to more negative potentials by about 20–30 mV in average. Under the same optimum conditions described above, Fab fragments could be determined with similar sensitivity as for the $F(ab')_2$ fragments. The slight difference in potentials may be attributed to the effect that the terminal amino acid groups on the $F(ab')_2$ fragments have on their adsorption at the electrode surface.

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[*Received for review 22 February 1988*]