Electronic Supplementary Material (ESI) for Analyst.

SUPPORTING INFORMATION

Competitive Immunochromatographic Assay for the Detection of Thiodiglycol Sulfoxide: Degradation Product of Sulfur Mustard

Manisha Sathe, Shruti Srivastava, S. Merwyn, G.S. Agarwal, M.P. Kaushik

Discovery centre, Defence R & D Establishment, Gwalior, 474002 India

CONTENTS:

1). Spectral data:

Compound 2 ESI–MS 139 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 2,7 (t, 2H), 3.87 (t, 2H), 3.65 (br OH).

Compound 3 ESI–MS 239 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 1.3 (s, 9H), 2.76 (t, 2H), 2.85 (t, 2H), 3.85 (t, 2H), 4.50 (t,. 2H).

Compound 4 ESI–MS 339 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 1.3 (s, 9H), 2.73 (t, 2H), 2.83 (t, 2H), 2.85 (m, 4H), 4.41-4.50 (m, 4H),11.0 (br, COOH).

Compound 5 ESI–MS 239 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 2.73 (m, 4H), 2.83 (m, 4H), 3.86 (t, 2H), 4.42 (t, 2H), 3.65 (br OH), 11.0 (br, COOH).

2). Conjugation of hapten to carrier protein

N-hydroxy succinamide (5.7 mg, 50 µmol) and EDC (9.5 mg, 50 µmol) was added to a solution of hapten (50 µmol) in DMF. The reaction mixture was incubated for 1 h at RT. The protein stock solution (10 mg mL^{-1}; 0.15 µmol mL^{-1}) was prepared in borate buffer (pH 9.0). The activated hapten (120 µL 6.0 µmol) was then added dropwise to protein solution (0.13 µmol, 8.8 mg, 880 µL) using hapten: protein molar ratio equal to 40:1. The final reaction volume of the protein–hapten conjugates was kept constant at 1 mL for each preparation. The conjugates were incubated overnight at room temperature and centrifuged for 5 min at 10,000 rpm to remove the precipitate. It was further purified by passing through a P10 gel filtration column (Pharmacia, Sweden). Fractions with the highest protein concentration were determined by absorbance measurements at 280 nm using a molar extinction coefficient of 43,824$M^{-1}cm^{-1}$ on a UV spectrometer.

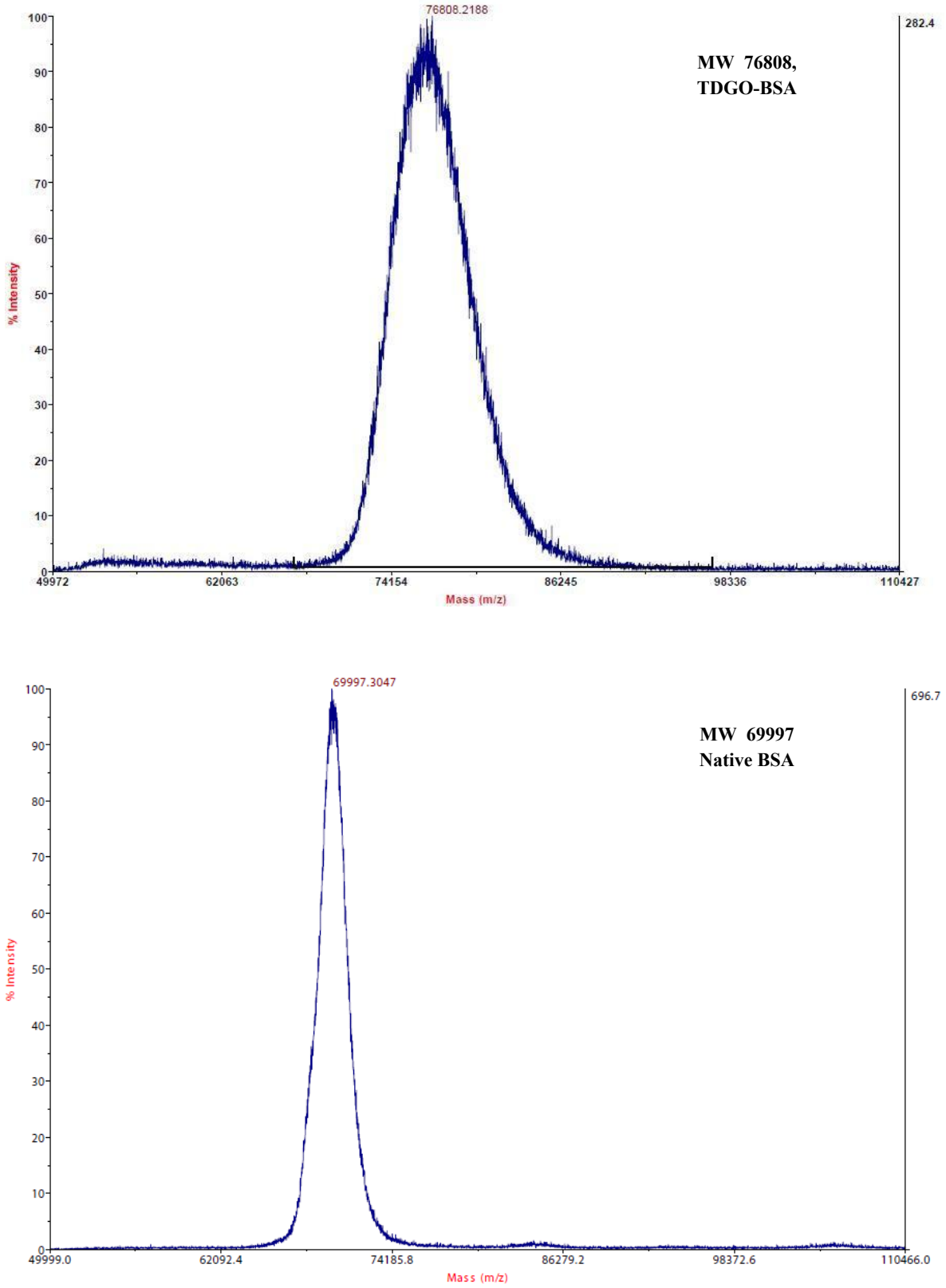
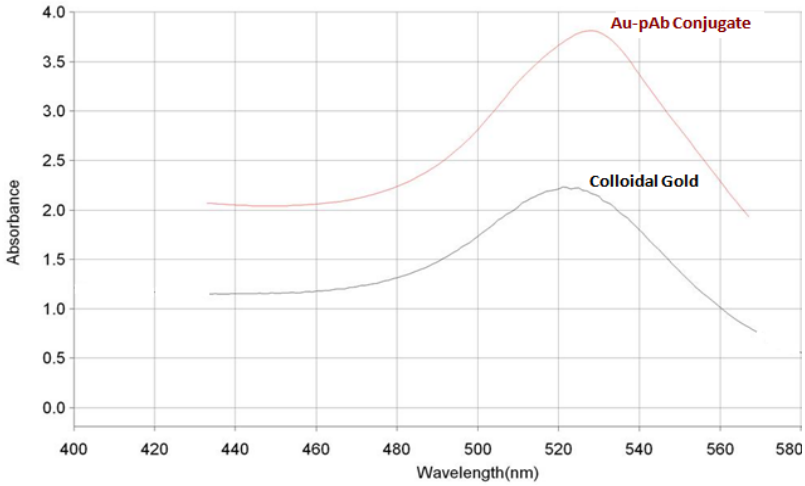


Fig. 1. MALDI-MS spectra of TDGO-BSA conjugates and native BSA. On each graph X-axis represent the mass (m/z) and Y-axis count relative intensity (%).

3). Preparation of colloidal gold

In brief, in 500ml round bottom flask, 250ml of 0.01% $AuCl_4$ in doubly distilled water was brought to boil with vigorous stirring. To this solution was added 3.75ml of 1% trisodium citrate. The solution turned deep blue within 20 sec. and finally colour changed to wine red in 60sec. boiling was pursued for additional 10 min. and then heating source removed and stirred for another 15 mins. This solution was stored in dark bottles at 4^0C. Finally, the gold colloidal solution supplemented with 0.05% sodium azide (used as an antiseptic) was stored at 4 ◦C for further study.

4). UV-Visible spectra of gold colloidal particle



5). Preparation of Gold -BSA conjugate

A definite amount of BSA was required to stabilize the gold nanoparticles solution which was determined, by titration of gold (250 µL, pH- 9.0) with different volumes (0, 5, 10, 15, 20, 25 µL) of BSA stock solution of 1mg/mL concentration. Mixtures were then incubated for 15 min at RT and 100 µL of 10% NaCl solution was added in all the vials. The color of the samples changed from brilliant red to blue as the concentration of BSA decreased. The optimum concentration of pAb for colloidal gold labeling was the lowest concentration of pAb solution that did not change color. The concentration at which the gold retained its color was 5 µg ml^{-1}. Consequently, 20 µL of BSA (1mg/mL) was mixed with 1 ml of colloidal gold solution and incubated for 30 minutes at RT. After the addition of 625 µL of 10% BSA solution in 20 mmol L^{-1} sodium borate (pH 9.0), the mixture was again incubated at RT for another 10 minutes. then washed using repeated centrifugation at 10,000 rpm, 4 °C for 30 min with 20 mmol L^{-1} sodium borate buffer (pH 9.0) containing 1% BSA and 0.1% sodium azide. The precipitate was then resuspended in 500 µl of resuspension buffer and stored at 4 °C for use.

6). Assay Parameters:

Table 1. Effects of methanol concentration, pH and buffer concentration on assay parameters of the ELISA[a].

		Abs_{max}	Slope	IC_{50} (μg mL^{-1})
Methanol (%)	10	0.834	0.578	0.09
	20	0.776	0.764	0.21
	30	0.823	0.674	0.5
	40	0.713	0.664	1.6
pH	6.5	0.785	0.874	0.52
	6.0	0.798	0.856	0.41
	7.5	0.881	0.734	0.09
	8.5	0.854	0.983	0.31
Buffer[b]	10 mM	1.66	0.654	0.09
	20 mM	1.63	0.632	0.12
	30 mM	1.39	0.692	0.4
	40 mM	1.37	0.767	0.2

[a]ELISA conditions: antiserum to TDGO-BSA, diluted 1/2000 with 10 mM PBST; coating antigen, TDGO 5-OVA, 10 μg $well^{-1}$; goat antirabbit IgG-HRP diluted 1/3000. Data were obtained from the four parameter sigmoidal fitting. Each set of data represents the average of three replicates. [b]Incubation time required for colour development; 10 mM = 10 min., 20 mM = 13 min., 30 mM = 20 min., 40 mM = 23 min.