Application Note – Industrial BioDevelopment Laboratory (www.ibdl.ca)

# The Cross-linking of IgG Using Two different Poly-aldehyde Cross-linking Agents

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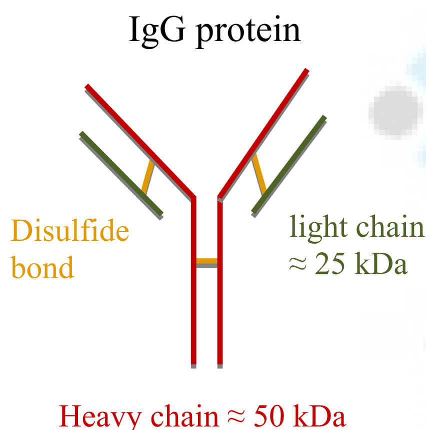
**Purpose:**

The purpose of this experiment was to use two in-house produced polyaldehyde cross-linkers to stabilize an oligomeric protein.

**Introduction**

Two carbohydrate-based protein cross-linking agents were produced in our facility. A tetra-aldehyde (MW= 278) and a hexa-aldehyde (MW= 438) were synthesized via an oxidation reaction from the sugars maltose and raffinose respectively. The utility of these polyaldehydes as protein-cross-linkers was evaluated for this application note.

The protein that was used for cross-linking was immunoglobulin g (IgG). Each IgG molecule is composed of two heavy chains of molecular weight approximately 50 kDa, and two light chains of molecular weight approximately 25 kDa. The two heavy chains are joined to each other as well as a light chain via disulfide bonds bringing the total weight of the entire antibody to 150kDa:

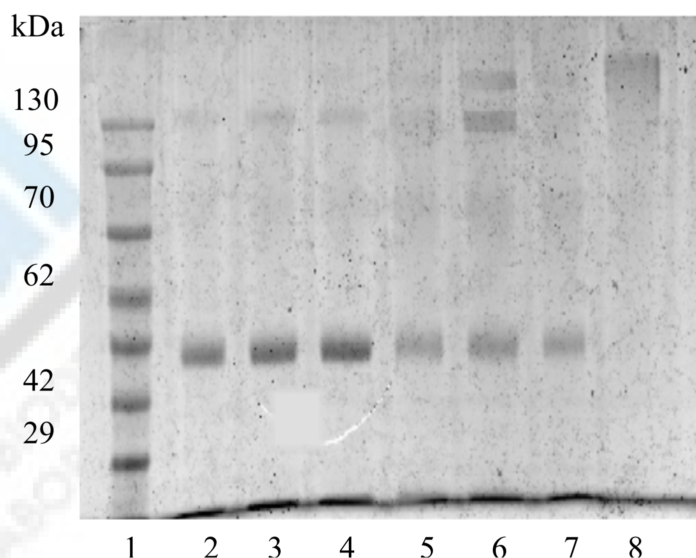



The loading buffer used in the coomassie gels contains the reducing agent beta-mercaptoethanol which reduces disulfide bonds. Thus, when placed in a reducing buffer prior to being loaded onto the gel, the IgG protein displays two bands on the coomassie gel at 25 and 50 kDa respectively. If these polyaldehyde cross-linker work as expected, these smaller protein subunits will be cross-linked via their free amino groups to form aldimine bonds. The beta-mercaptoethanol will not cleave the aldimine bonds. Therefore the larger molecular weight structures we expect to produce with the aldehyde reaction should remain intact in the reducing loading buffer.

**Method:**

A buffer was selected to ensure no reaction with the cross-linking agent. 20 mM HEPES buffer was prepared by dissolving 0.476g of HEPES in 100g of water. Following this 10N sodium hydroxide was added dropwise to obtain a final pH of 7.5. Proteins were incubated with the cross-linking agents in the following manner: an IgG solution (0.025%) was prepared in 20mM HEPES. Six other samples were prepared with the following mol ratios: 100:1 ratio of raffinose to IgG, 1000:1 ratio of maltose to IgG, 500:1 and 1000:1 ratios of tetra-aldehyde to IgG, as well as 10:1 and 100:1 ratios of hexa-aldehyde to IgG.

These samples were incubated for 18 hours at 37°C. Following incubation, the samples were placed in a reducing loading buffer and Coomassie gel electrophoresis was conducted. To do so, the samples were loaded onto an 8% gel and 2.5 μg of IgG were loaded onto each lane on the gel.




**Results:**

Gel lanes from left to right. 1) Pre-stained protein ladder. 2) IgG protein. 3) 100:1 ratio of raffinose to IgG. 4) 1000:1 ratio of maltose to IgG. 5) 500:1 ratio of tetra-aldehyde to IgG. 6) 1000:1 ratio of tetra-aldehyde to IgG. 7)10:1 ratio of hexa-aldehyde to IgG. 8) 100:1 ratios of hexa-aldehyde to IgG

**Conclusion:**

The polyaldehydes synthesized successfully cross-linked the IgG protein. A 1000:1 mol ratio of tetra-aldehyde to IgG protein (shown in lane 6) clearly shows bands of larger molecular weight. These bands indicate the cross-linking of smaller subunits present in lane 2 (IgG protein), to larger structures. Furthermore, as shown in lane 8 the smaller molecular weight band visible in lane 2 (IgG protein), has disappeared and only a band indicating a considerably higher molecular weight is visible. The higher molecular weight bands observed in lane 6 and 8 respectively, validates the cross-linking capabilities of the tetra-aldehyde and the hexa-aldehyde synthesized. These cross-linkers may be further explored and tested on other proteins.