Agglutination and lysine of sheep red blood cells

They are many uses in science for agglutination for example the determination of which blood group an individual belongs to. However agglutination is commonly the adhesion of particles. Biological agglutination occurs in the clumping of cells in response to an antibody, the adhesion of small particles that are suspended in a solution, which are then (usually) precipitated in allergic reactions, when cells clump together to prevent antigens from entering, Antigens are usually proteins or polysaccharides, that could be on bacteria, but not actually the bacteria it self.

But in biology/immunology many agglutination experiments are carried out in order to identify antibodies, which are specified to go against cellular antigens. In the 1st part of the experiment we were given immune rabbit serum and non-immune rabbit serum which, was acting against sheep red blood cells as an antigen. The second part of the experiment was antibodies that produce lysins in the presence of a complement, which is similar to the agglutination process, and both processes are measured using the same technique. Aims & ObjectivesThe main objective of this experiment was to observe what actually happens in the agglutination and lysis reactions, also to help us understand the processes more clearly, and to determine the agglutination and lysine titres, making me understand how differently immune and non-immune serum would react, to sheep red blood cell. The experiment gives understanding of the importance of agglutination in immunology. MethodFirstly, the agglutination experiment with the immune rabbit serum was carried out.

But before I started the experiment, I had to wear my lab coat, safety glasses and gloves prior dealing with any of the substances for precautious

measures. To inactivate the natural complement present in the serum for this experiment it was required heating at 60 degrees for at least half an hour, this was already done by the technician, so we did not have to do it. Secondly, using 20 small sized tubes a serial dilution of the immune serum was made in the 20 tubes in which we had put 0. 5ml of phosphate buffered saline (PBS) pH 7. 2, using a micropipette.

The dilutions of the serum were made from 1 in 2 all the way to 1 in 10, 485, 760 in the PBS buffer. Next, I transferred from each of the 20 tubes 0. 2ml of solution, again using the micropipette using a clean tip, to the heamagglutination tray, in an orderly fashion starting with the least diluted first, then adding 0. 2ml of a 2% sheep red blood cell solution containing PBS into each well of the heamagglutination tray. For control, into two of the wells I added 0.

2ml buffer and 0. 2ml blood cells. Then I repeated these steps, but this time using the non-immune serum, and only producing 1 in 2 to 1 in 16 dilutions i. e.

only four dilutions, then simply agitated the tray but had to be really gentle, this was help mix the solutions. Going onto to the second part of the experiment which was the lysine titration part. For this we had to the exact same procedure as before, as in the agglutination experiment. In this part of the experiment the only big difference being was after the PBS, diluted immune rabbit serum, and the sheep red blood cells were added to the heamagglutination tray, 0. 2ml of 1 in 30 diluted complement, from a guinea pig were also added to each well. Again the guinea pig complement we also

added to the non-immune serum, with the same procedure as the non-immune serum in the agglutination process.

As for the 2 control wells, the first well had 0. 4ml of the PBS and, 0. 2 sheep red blood cells, the second control well had 0. 2ml of the complement, 0. 2ml of PBS and 0. 2ml of sheep red blood cells.

For the reactions to occur, the heamagglutination tray would require to be left for 3 hours at room temperature, however I obtained my results the day after, therefore the heamagglutination tray was left over night at a temperature of 4 degrees this would therefore slower the process. Results with discussionWe could easily tell if the agglutination had token place or if not, as if agglutination had token place the blood cell will spread around the wells, on the surface of the wells, and if agglutination had not token place the red blood cell will just go to the bottom of the wells. Looking at the results which I obtained which containing the PBS, immune rabbit serum and sheep red blood cells I could clearly see that in the first 4 wells, the least diluted wells, agglutination had token place, as this could be visualised due to the blood cells spread around the 4 wells. Well number 4, giving the end point titre to 1 in 16 dilutions for the agglutination of the immune rabbit serum. This therefore proved that the immune rabbit serum was immune against the sheep red blood cells as agglutination had occurred.

Well number 5 and onwards to well number 20, a tight button of the blood cells had formed at the bottom of each well, therefore showing us that no agglutination had taken place due to being highly diluted. The control wells containing only the PBS and sheep red blood cells, had no signs of

agglutination taking place as we would of expected, showing us that the serum was non-immune to the blood cells. According to the results I have obtained from the lysis reactions it showed it had a more immune effect against the sheep red blood cells, which were used from the same sample used in the agglutination process. However, the lysis reactions occurred in only the first 6 wells, this could also be seen more clearly there is much more spread when visualised in comparison to the agglutination reactions. Therefore the end point being the last well lysis occurred in which was in well 6, the end point titre were 1 in 64 dilutions.

From well 7 to well 20 no lysis had occurred as the sheep red blood cells had formed a tight button at the bottom of the wells. Again all the control wells had no lysis reactions taking place, as there was no serum present amongst them. Finally, the non-immune serum being present in the last 4 wells verified that it was non-immune as no reactions had occurred in any of the wells.