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EFFECT OF 2-MERCAPTOETHANOL ON HAEMAGGLUTININ-ERYTHROCYTE AND HAEMOLYSIN-ERYTHROCYTE COMPLEX

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ABSTRACT

In rat and rabbit anti-sheep erythrocyte sera, in primary response, the haemolysin activity is associated with IgM whereas haemagglutinin activity is exhibited by both IgM and IgG. Erythrocyte-haemolysin complex, on treatment with 2-mercaptoethanol, loses its capacity to be lysed by complement, suggesting that the structural integrity of IgM is essential for complement action.

INTRODUCTION

SHEEP erythrocytes are often used as standard antigen in immunological investigations. At humoral level, the haemolytic and haemagglutinating properties of anti-sheep erythrocyte antibodies are estimated and at cellular level, the number of plaque-forming cells[1] and rosette-forming cells[2] are measured.

Deutsch and Morton[3] first described that IgM antibody is converted into 75 monomers by treatment with 2–ME (0·1 M) whereas IgG is not affected. The reduced IgM monomers are shown to retain their capacity to bind with hapten[4]. In this work using 2–ME sensitivity and Sephadex G–200 gel filtration, the haemolytic anti-sheep erythrocyte antibody in peak primary response is found to belong to IgM class whereas haemagglutinating antibody belongs to both IgM and IgG classes. Further, erythrocyte-haemolysin complex when treated with 2–ME loses its capacity to be lysed by complement, indicating that structural integrity of IgM is necessary for complement action.

MATERIALS AND METHODS

Wistar A/Iisc rats were immunised by injecting 3×10^{10} SE i.p. Blood was collected, during peak response, on 7th day after immunistation. Serum was separated by centrifugation, inactivated at 56° C for 30 min, and stored frozen. Rabbit antiserum was obtained similarly by administering 5×10^{10} SE i.v. Haemagglutination titrations were carried out in 0·15 M NaCl and haemolysin titrations in modified barbital buffer as described by Campbell *et al*[5]. Antiserum was fractionated on Sephadex G–200gel with 0·15 M phosphate-saline buffer (pH 7·2). Three ml fractions were collected and A_{280} recorded.

RESULTS AND DISCUSSION

The elution profile of rat anti-sheep erythrocyte serum is shown in Fig. 1. The haemagglutinin activity is located in two peaks, I and II, peak I being eluted with the exclusion volume of the column. The haemolytic antibody fraction is solely located in peak I. The haemolytic activity of the unfracationated antiserum as well as that of peak I was lost when incubated with 2–ME (0·1 M). Concomitantly, the haemagglutinin and haemolytic activity of the fractions in the region of peak I were lost. Under the experimental conditions employed, 2–ME is known to reduce the IgM to subunits[3].

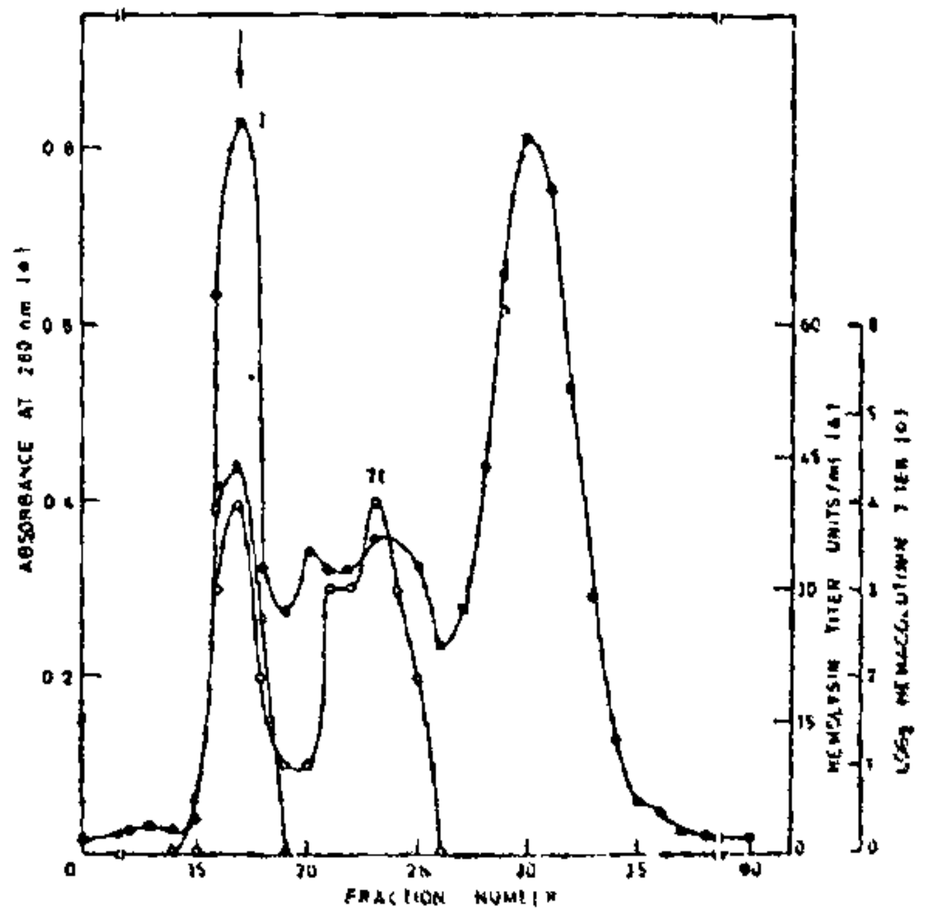

FIG. 1. Sephadex G–200 profile of rat anti-SE Serum (column 2 × 45 cm).

Removal of 2–ME from the reaction mixture was not found necessary for haemagglutination test with anti–SE serum since these haemagglutinins are not affected by 2–ME, and consequently alkylation was not required. Natural heterohaemagglutinins also have been reported to behave in similar way[6]. However, no lysis of erythrocytes was observed when 2–ME was not removed from the reaction mixture of haemolysin

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Abbreviations used : Sheep erythrocytes (SE) ; 2-mercaptoethanol (2–ME) ; modified barbital buffer (diluent).

titration. This would mean that 2–ME is interfering with one or more of the following steps : (1) Modification of erythrocytes so that they are resistant to lysis by antibody in presence of complement. (2) Destruction of anti-body-erythrocyte complex. (3) Destruction of complement activity. The following experiments were performed to check these possibilities. Rat anti-SE serum was used in following experiments.

Sheep erythrocytes were incubated with varying concentrations of 2–ME (0·001 M, 0·01 M and 0·1 M). 2–ME was removed by centrifugation, and the pellet was washed and complexed with antibody. Haemolysis was observed, as in controls where sheep erythrocytes were not incubated with 2–ME, indicating that 2–ME does not affect the erythrocytes (Table I). Next, antibody-erythrocyte complex was incubated with 2–ME and the latter removed by centrifugation and washings. In this case inhibition was observed (Table I). Thus, there are two possibilities : (1) 2–ME reduces the antibody in the complex causing either dissociation of monomeric subunits of IgM from erythrocytes or insensitivity of the resulting monomer-erythrocyte complex to complement action and (2) 2–ME dissociates the IgM from complex and subsequently reduces it to monomeric subunits. We belive it is more logical that 2–ME reduces IgM in complex and the monomer-erythrocyte complex is insensitive to complement action. It has been shown that C_1 complement fixation needs at least one IgM or two IgG molecules which would mean that initial 'polymeric' IgM structure is important for complement fixation[7]. IgM when treated with 2–ME also loses its C_1 complement fixing activity[7]. The second possibility seems unlikely as no covalent linkages are involved in antigen-antibody complex; further, IgG-erythrocyte complex is not affected by 2–ME. However, if sulfhydryl functions are involved in IgM-erythrocyte complex formation and that is affected by 2–ME then dissociation of IgM from the complex and its subsequent reduction may result.

TABLE I

Effect of 2–ME on various components of haemolysin titration of rat anti-SE serum

Material treated	% activity of haemolysin activity of antiserum	
	+ 2-ME	− 2-ME
(*a*) Antiserum	5–10	100
(*b*) SE	100	100
(*c*) Antibody-SE complex	25	100
(*d*) Complement	40–45	100

(*a*) Anti-SE serum and 2-ME (0·1 M) were incubated at room temperature for 60 min followed by incubation with SE (3×10^9 cell/ml) at 37° C for 30 min. The tubes were centrifuged, the pellet was washed, re-suspended in diluent. After this, titrations were carried out as described in text.

(*b*) SE (3×10^9 cells/ml) were incubated with 2–ME (0·001 M, 0·01 M, and 0·1 M) at room temperature for 60 min. The tubes were centrifuged and erythrocytes washed with diluent. The pellet was suspended in diluent and incubated with anti-SE serum at 37° C for 30 min followed by complement. The titrations were carried out as described in text.

(*c*) The complex was formed by incubating anti-SE serum and SE (3×10^9 cells/ml) for 30 min at 37° C. The tubes were centrifuged and pellet washed with diluent. The pellet was resuspended in diluent and incubated with 2–ME (0·1 M) at room temperature for 60 min. The tubes were then centrifuged and washed. The pellet was resuspended in diluent and titrations were carried out as described in text.

(*d*) Complement was incubated with 2–ME (0·1 M) at room temperature for 60 min. 2–ME was removed by passing the mixture through G–15 column equilibrated with isotonic barbital buffer and by eluting with the same buffer. This complement was used for carrying out haemolysin titrations.

Suitable controls were included in every experiment.

To check whether complement activity is affected by 0·1M 2–ME, the complement was incubated with 2–ME (0·1 M) and filtered through Sephadex G–15 to remove unreacted 2–ME from the complement. This preparation when added to sensitized erythrocytes showed 40–45% inhibition of lysis as compared to the control preparation which was not subjected to 2–ME treatment (Table I). The sensitivity of complement to 2–ME necessitated the modification in haemolysin titration.

The effect of varying concentrations of 2–ME (0·001 M, 0·01 M and 0·1 M) and anti-SE serum (1/10, 1/100, 1/1000) was examined (Table II). From the results it appears that reduction of IgM can be achieved at lower concentrations of 2–ME if the concentration of antibody is also reduced.

TABLE II

Effect of varying concentrations of 2–ME on different concentrations of rat anti-SE serum

Concentration of 2-ME	Concentration of anti-SE serum	% Haemolysis of control (No. 2-ME)
0·1 M	1/10	5–10
0·01 M	1/10	90
0·001 M	1/10	90
0·01 M	1/100	45
0·001 M	1/1000	100

Haemolysin titrations were carried out as described in Table I.

Similar results were obtained with rabbit anti-SE serum (Table III). Both in rat and rabbit, at the peak of primary response, haemolysin is due to IgM, and agglutination is exhibited by both IgG and IgM. These results indicate that 2–ME affects IgM both in free and complexed states.

TABLE III

Effect of 2–ME on various components of haemolysin titration of rabit anti-SE serum

	Material treated	% activity of haemolysin activity of antiserum	
		+2-ME	−2-ME
(*a*)	Anti-SE serum	10	100
(*b*)	SE	100	100
(*c*)	Antibody-SE complex	25	100
(*d*)	Complement	40–45	100

a, *b*, *c* and *d* are same as in Table I.

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DIELS-ALDER REACTION OF TETRACYCLONE WITH SOME MALEIMIDES

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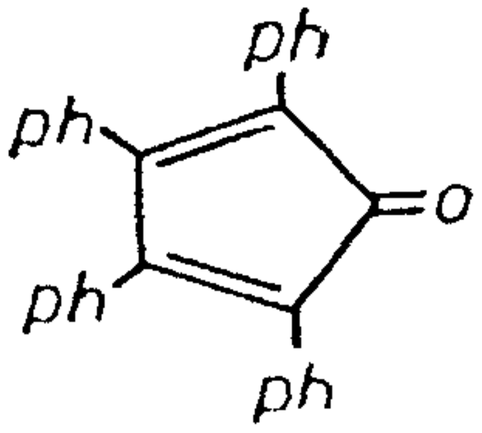
ABSTRACT

Tetracyclone I reacts with N-substituted maleimides II to give the adducts III *a–f*. Dehydrogenation of III *c*, *e*, *f* gave IV *a–c*. In a similar manner III adds another molecule of either maleic anhydride or N-substituted maleimide to give V *a–b* and VI *a–e* respectively. On the other hand, VI *b* dehydrogenates readily to VII.

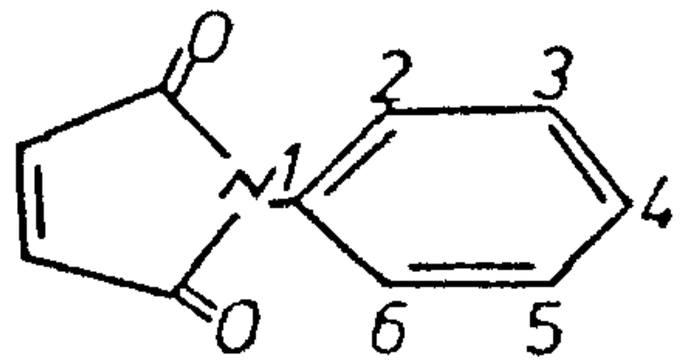
TETRAPHENYLCYCLOPENTADIENONE (tetracyclone) I and its analogues were reported to undergo Diels-Alder reaction with ethylenic dienophils[1–13].

We now succeeded to isolate the adducts III *a–f* from the reaction of one molecule of N-substituted maleimides II *a–f* with one molecule of tetracyclone in bromobenzene or in dry toluene. III *c–e* are dehydrogenated readily with bromine to give IV *a–c*.



I

The structure assigned for the addition products III *a–f* has been supported by analytical and spectral (U.V., I.R. and N.M.R.) data. III *e*, for example, shows a carbonyl two bands widely separated at 1770 cm^{-1} and 1690 cm^{-1} (for $-CO\cdot NH\cdot CO-$)[15]. The U.V. spectrum of III *c* showed an absorption band at 340 mμ.[16] The structure of the adducts obtained has been further evidenced by the N.M.R. spectrum. For example the N.M.R. spectrum of III *a* showed



II

a, 2-COOH
b, 3-COOH
c, 4-COOH
d, 2-Me, 3-Cl
e, 2-Me, 5 Cl
f, 4-Me, 3-Cl

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