A THERMOCHEMICAL STUDY OF THE REACTION BETWEEN PROTEIN A FROM *S. AUREUS* AND FRAGMENT Fc FROM IMMUNOGLOBULIN G

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Received 19 March 1971

1. Introduction

The protein A–Fc reaction is similar to an antigen–antibody reaction and gives rise to precipitation [1], complement binding [2], complement activation [3] and hypersensitivity reactions [4–6]. The difference between the two types of reactions involves sites differently located on the immunoglobulin (IgG) molecule. In an antigen–antibody reaction this site is located in the Fab region of the IgG molecule whereas protein A reacts with structures in the Fc part.

The primary structure of Fc from different IgG molecules is known [cf. 7]. Fc forms part of the constant region of an antibody. In contrast, all variable regions are located in the Fab piece and are supposed to form the basis for the specificity of antibodies. Since the primary structure of Fc from human IgG_1 is known and since crystals of this fragment are easily available it is now possible to obtain the detailed tertiary structure of this part of the antibody molecule.

These arguments make it an important task to describe the protein A–Fc reaction in physico-chemical terms. In addition to its particular suitability for studies of protein–protein interactions, the protein A–Fc reaction may also provide insight into the mechanism of the antigen–antibody reaction.

In the present study the protein A–Fc reaction has been investigated by a microcalorimetric method. Measurements were in all cases performed with protein A in excess and the reaction measured therefore followed equation (1):

$$Fc + 2A \rightarrow FcA_2 \qquad (1)$$

Measurements were performed at 25, 33 and 40°. Values for the enthalpies of the reaction and the change in heat capacity as a function of temperature have been derived.

2. Materials

The Fc-fragment was isolated after papain digestion of a human myeloma IgG-1 as previously described [2]. Protein A was prepared according to methods already published [8]. Protein A and Fc were dialyzed for two days against 0.1 M sodium phosphate buffer, pH 7.0 containing 1 ppm sodium azide as preservative. Before calorimetric measurements were performed at 25, 33 and 40° aliquots of the protein solutions were further dialyzed for an additional day against the same buffer and in the same vessel at the appropriate temperatures. Protein concentrations were determined spectrophotometrically at 280 nm. The extinction coefficients used, $E^{1\%}_{280\ nm}$, for Fc and protein A were 12.2 [9] and 2.0, respectively.

3. Calorimetric equipment

The calorimeter used in the experiments was the prototype to the LKB 10700-2 batch microcalorimeter. The instrument and the measurement proce-

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Table 1
Results of calorimetric measurements for the reaction Fc + 2A → FcA_2. Molecular weight for the Fc fragment = 50,000.

Temperature (°C)	Amount of Fc (mg)	Concentration of protein A (mg/ml)	Q_{corr} (mcal)	$-\Delta H$ (kcal/mol Fc)	$-\Delta Cp$ (kcal/deg mol Fc) Mean value 25–40°
	7.34		5.65	38.5	
25.00	7.28	4.29	5.86	40.2	
	7.35		5.81	39.5	
				Mean 39.4	
33.00	7.38	4.70	6.99	47.5	1.03
	7.21		7.82	54.2	
40.00	7.44	4.05	8.15	54.8	
	7.49		8.29	55.3	
				Mean 54.8	

dure have been described previously [10]. A Keithley 150 B Microvolt ammeter was used as amplifier and the amplified signal was recorded with a Sargent SR recorder fitted with a Disc integrator. The reaction cells were made from 18 carat gold.

4. Methods

In the binding experiments the two compartments of the reaction cell were charged with 5 ml Fc and 2.5 ml protein A. The concentration of each protein solution in each experiment are listed in table 1. The amount of protein A added corresponded to a large excess of free protein A. The reference vessel was charged with 5 + 2.5 ml buffer. For each calorimetric run both cells were treated in the same manner with respect to rinsing and charging procedures.

In separate experiments, determinations of the heats of dilution of the two protein solutions were performed. 5 ml Fc and 2.5 ml protein A were diluted with 2.5 and 5 ml buffer, respectively. Buffer solutions were taken from the final solutions of the dialysis experiments.

5. Results and discussion

Results of the calorimetric experiments are summarized in table 1. The Q-values given are corrected for the heats of dilution of the protein solutions. One dilution experiment was performed for each protein solution at 25, 33 and 40°, respectively. Small endothermic heat effects were noted but no significant differences in $Q_{dilution}$ were found for the different temperatures and in the corrections mean values were used. The observed $Q_{dilution}$-values were + 0.43 mcal and + 0.49 mcal for Fc and protein A, respectively.

Table 1 shows that the enthalpy of binding is strongly exothermic and highly temperature dependent. The ΔH values are calculated on the basis of a molecular weight for the Fc fragment of 50,000. In fig. 1 these values are plotted versus the reaction temperature. The ΔCp value is calculated from the slope of the straight line and corresponds to - 1.03 kcal/deg. mol for the temperature interval 25–40°. Since the Fc fragment is made up of two identical chains it is assumed that it contains two combining sites for protein A. Hence, the enthalpy and the ΔCp values per site are half of those listed in table 1.

It is interesting to note that the same pattern of large negative ΔH and ΔCp values has recently been found by direct calorimetric measurements on a number of other specific protein coupling reactions. These involve both protein–protein coupling, hemoglobin–haptoglobin [11] and binding of low molecular substances to proteins, biotin–avidin [12] and ε-DNP-lysine antibody [13, 14].

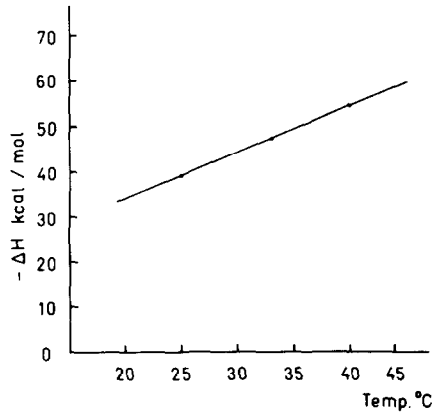

Fig. 1. Enthalpy of binding of protein A to Fc fragment as a function of temperature. ΔH-values refer to the reaction $Fc + 2A \rightarrow FcA_2$.

Results of immunochemical experiments of the protein A–Fc reaction show that a strong complex is formed [1]. It is not possible, however, from those experiments to calculate the equilibrium constant.

As with other immunochemical reactions, little is known about the mode of binding of the two components involved. Nor is there much information concerning the conformational changes involved in such interactions. The interpretation of derived thermochemical data for the present reaction must therefore be speculative.

Ionic binding, i.e. charge neutralization processes, could account for the high negative ΔH values found but this would be expected to be accompanied by a large positive ΔCp value [e.g. 15]. Enthalpy of formation of a hydrogen bond is exothermic and of the order of 7 kcal/mole. However, the formation of protein–protein hydrogen bonds in aqueous solution are likely to be preceded by breaking of corresponding protein–water bonds. Therefore we would not expect to find a large negative enthalpy value unless a considerable number of hydrogen bonds are involved. A net gain of only three hydrogen bonds could account for the enthalpy change demonstrated. Such a binding process would hardly, however, be expected to give rise to the large negative ΔCp value found. On the other hand the large ΔCp value is consistent with the formation of 'hydrophobic bonds' in the reaction between protein A and Fc. Nevertheless, the large enthalpy change implies that other kinds of interactions may also be involved.

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