Formation of a Complex of Human Plasma Myeloperoxidase and Opsonins

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 120, № 9, pp. 320-322, September, 1995
Original article submitted November 22, 1994

Human neutrophil myeloperoxidase adsorbs on immobilized fibronectin or IgG under conditions approaching the physiological ones. Immobilized myeloperoxidase binds liquid-phase plasma fibronectins and thermoaggregated IgG, but not native IgG. Protein-myeloperoxidase interaction is thought to be the mechanism arming phagocytes with this enzyme in the course of phagocytosis of pathogenic microorganisms and to be one means of preventing tissue injury by oxidants generated by extracellular myeloperoxidase.

Key Words: *myeloperoxidase; opsonins; protein-protein interaction*

Macrophages possessing no myeloperoxidase (MP) of their own are known to appear in a focus of inflammation directly after neutrophils, and to acquire there both strong antibacterial properties [6] and the capacity to halogenate proteins (which is characteristic of peroxidase catalysis) [10]. On the other hand, in the course of phagocytosis and in the process of activation neutrophils may release MP into the extracellular space [9]. Since no specific receptors for this enzyme have been detected on the surface of macrophages, we proposed that proteins capable of transferring MP from the extracellular space to macrophages may be present in the plasma. Plasma fibronectin (FN) and immunoglobulin G (IgG), that is, opsonin proteins specifically interacting with the receptor system of phagocytes, were selected as potential MP-transferring proteins. Whereas IgG as an antibody is aimed at a certain antigenic determinant of phagocytosed material, FN, as a nonspecific opsonin, may interact with a broad spectrum of compounds, which is important at the nonspecific stage of development of defense reactions [13]. The possibility of MP interaction with these opsonic proteins was studied by quantitative affinity chromatography.

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MATERIALS AND METHODS

FN and MP were isolated by methods described previously which make it possible to obtain homogeneous preparations of these proteins, as shown by polyacrylamide gel electrophoresis and immunoelectrophoresis [8]. IgG was obtained by the standard method, including the removal of pseudoglobulins by dialysis against H_2O and 40% ammonium sulfate precipitation followed by ion-exchange chromatography on DEAE-cellulose [2]. Then IgG was filtered through a column packed with IgG-agarose equilibrated with phosphate buffer saline (0.01 M phosphate buffer, pH 7.4, with 0.15 M NaCl) to remove any antiimmunoglobulin admixture. After this, IgG was purified by affinity chromatography in a column with immobilized protein A of *Staphylococcus aureus* (manufactured by the Pasteur Research Institute of Epidemiology and Microbiology). Aggregated IgG was

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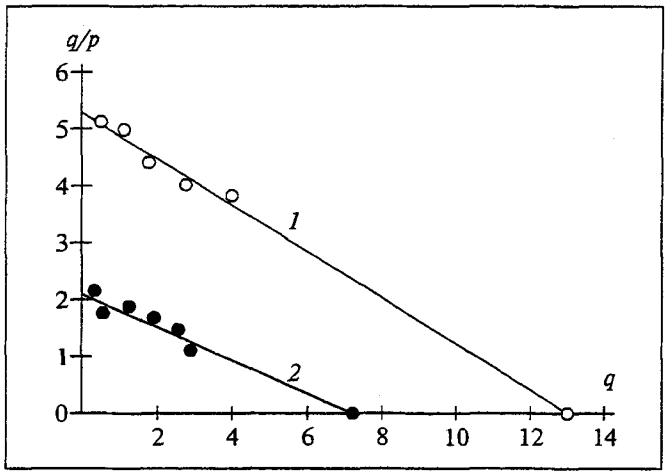

Fig. 1. Scatchard plot showing MP interaction with solid-phase FN (*1*) and IgG (*2*). *p*: concentration of free circulating MP; *q*: concentration of fixed MP on adsorbent. m_f=13.05 μM (MP on FN-agarose) and m_f=7.22 μM (MP on IgG-agarose). Here and on Fig. 2: volume of adsorbent 2 ml, t=20°C, circulating phosphate buffer saline, eluting solution 0.05 M glycine-HCl, pH 2.8, in 0.5 M NaCl, volume of eluate 3.5 ml.

obtained as described previously [14]. The concentration of protein preparations was measured by spectrophotometry using known coefficients: $A_{280}^{1\%}$= =12.8 cm^{-1} (FN), $A_{280}^{1\%}$=13.4 cm^{-1} (IgG), $A_{280}^{1\%}$= =14.5 cm^{-1} (MP).

The resultant protein preparations were immobilized routinely on BrCN-activated agarose [1]. The association of dissolved proteins with immobilized protein was assessed using affinity chromatography in the distributive equilibrium variant. For this purpose a recirculating system was employed which included a Microperpex (LKB) peristaltic pump, a column with protein immobilized on BrCN-activated agarose (V=2 ml), and a column with ethanolamine-inactivated BrCN-agarose (V=2 ml) to assess the level of nonspecific binding of circulating protein, all these being connected by flexible supply tubes. The volume of free liquid in the structure of the gel matrix was assessed in the gel-filtration regimen using phenol red and a column with intact agarose (V=50 ml). The experiment, performed by adding the test protein in various concentrations to the recirculating system equilibrated with the phosphate buffer saline, was carried out overnight (for 16 to 18 h) at 20°C. The concentration of unbound protein was measured in the circulating fluid, and that of adsorbed protein after its elution in 0.05 M glycine-HCl, pH 2.8, in 0.5 M NaCl, a neutral pH being immediately attained by adding 0.5 M NaOH. For this purpose the unbound protein concentration was subtracted from the protein concentration in the eluate with due consideration for the effect of the sample dilution. FN and IgG concentrations were assessed by rocket immunoelectrophoresis against the relevant antisera [3], while the MP concentration was assessed from the specific activity of the enzyme. The activity of MP was determined as described elsewhere [11].

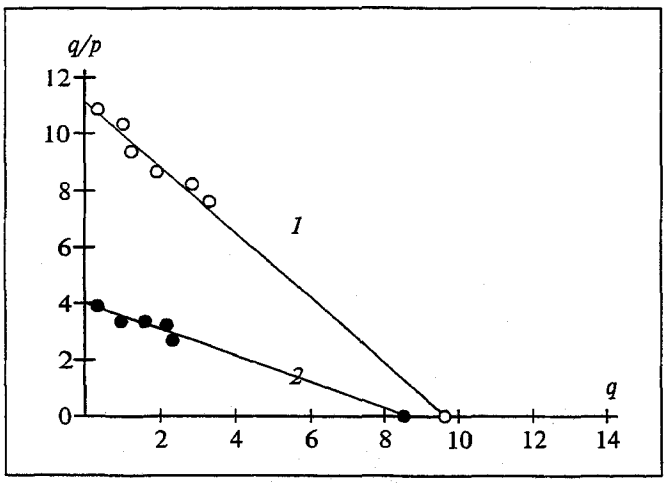

Fig. 2. Scatchard plot showing interaction between FN (*1*) and thermoaggregated IgG (*2*) with solid-phase MP. *p*: concentration of free FN or aggregated IgG in circulation; *q*: concentration of fixed FN or aggregated IgG on agarose-MP adsorbent. m_f=9.77 μM (FN) and m_f=8.67 μM (IgG).

RESULTS

Individual adsorbents which resulted from protein immobilization contained proteins in the following concentrations: FN-agarose, 4.2 mg/ml gel; MP-agarose, 5 mg/ml gel; IgG-agarose, 2.6 mg/ml gel. With the internal volume of gel (free liquid in the matrix structure) amounting to 86%, these values are, respectively, 4.9 mg/ml (10.9 μM) for FN, 5.8 mg/ml (38.7 μM) for MP, and 3.02 mg/ml (20.1 μM) for IgG. As is seen from Fig. 1, opsonic proteins are capable of associating soluble MP: FN with a dissociation constant (Kd) of 2.43 μM and IgG with a Kd of 3.45 μM. The values of these constants are within the range corresponding to specific protein-protein interaction [7]. On the other hand, immobilized MP behaves differently toward FN and IgG (Fig. 2). The association is enhanced during interaction with FN (Kd=0.88 μM), but no complex is formed during interaction with native IgG, whereas interaction with thermoaggregated IgG is well expressed (Kd=2.13 μM). Comparison of the concentration of binding sites (m_f) for soluble proteins with the concentrations of immobilized partners of these proteins revealed that for immobilized FN these values were virtually the same (with a slightly increased m_f): m_f=13.06 μM at a 10.9 μM concentration of FN in the column. Bearing in mind empirical data that an appreciable portion of bind-

ing sites during protein immobilization is inaccessible to ligands [7], we would expect the FN molecule to possess several MP-binding sites. The same follows from the known structural similarity of both polypeptide chains of this protein [13]. Analysis of the interactions of soluble FN and aggregated IgG with immobilized MP shows m_f values equal to 26.4 and 22.7%, respectively, of the immobilized protein concentration. Since aggregated IgG imitates many characteristics of an immune complex [5], we may assume that IgG as an antibody binding the object of phagocytosis acquires the ability to fix MP on it. In the case of immobilized IgG, however, its capacity to fix MP is evidently a result of demasking of the respective sites of this protein's molecule in the course of its covalent modification during interaction with the agarose matrix. The data on the ability of FN and IgG to fix soluble MP under conditions approaching the physiological ones indicate that these proteins can contribute to macrophage arming by delivering this enzyme to cells. This may be of special importance in cases where pathogenic microorganisms in the neutrophil phagolysosome prevent the latter's fusion with MP-containing lysosome-like granules (for example, *M. tuberculosis,* etc.). Moreover, there is another important aspect to the capture and delivery extracellular MP to phagocytes. MP, a potent destructive factor for defense cells, is also a key agent of oxidative destruction of tissues during the massive migration of neutrophils to the focus of inflammation [12]. Removal of MP from the extracellular space must have an effect on the diminution of tissue damage by oxidants generated by this enzyme.

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