Collagen at interfaces II: Competitive adsorption of collagen against albumin and fibrinogen

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Adsorption of chemically radiolabeled [^{14}C] collagen from binary mixtures with albumin or fibrinogen was studied on the solution/air and solution/polyethylene interfaces and revealed the preferential adsorption of albumin. This phenomenon is confirmed by the data of surface tension measurements of single protein, collagen–albumin, and collagen–fibrinogen solutions. Desorption experiments clearly show that more irreversibly adsorbed collagen was found on polyethylene surfaces when adsorption was performed from collagen–fibrinogen than from collagen–albumin solutions. The combined adsorption–desorption and the surface tension data show that competitive adsorption of collagen at the hydrophobic surfaces is strongly influenced by the surface tension properties of the proteins in solution.

INTRODUCTION

In an earlier article we have described the behavior of collagen molecules at the solution/air and polymer/solution interfaces. In particular the existence of the reversibly adsorbed protein layer on polymers has been demonstrated by adsorption/desorption studies.

In the complex process of adsorption of flexible macromolecules, such as proteins, the adsorption phenomenon is influenced by specific surface properties and by the presence of other proteins in the mixture. The protein affinity to a particular surface will indicate greater number of protein-to-surface bonds for a selectively adsorbed protein molecule, or a greater mean binding energy per bond, or both.

In the work reported here, the *in situ* adsorption of binary solutions of collagen–albumin and collagen–fibrinogen was investigated on solution/air and solution/polyethylene interfaces. As it has already been pointed out in the first part of this work,[1] the experiments were performed at pH = 2.7 in order to minimize the self-association of collagen molecules in solution. The choice of proteins (albumin, fibrinogen) was dictated by their abundant presence in mixtures with collagen in the interstitial space of the skin dermis.[2] Although this pH does not correspond to the physiological conditions and adsorption of proteins at the interfaces may be pH dependant, the adsorption data reported here give an information relevant to the understanding of *in vivo* interactions in which collagen molecules are involved.

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MATERIALS AND METHODS

Proteins

Collagen was isolated from rat tail tendons and acetylated with (1 − ^{14}C) acetic anhydride. The extraction and labeling procedures were described in.[1] The specific activity of (^{14}C) collagen used in this study was 14.9 μCi/mg.

Rat albumin (Fraction V) and rat blood fibrinogen (Fraction I containing approximately 73% of protein) were obtained from Sigma Chemical Company (St. Louis, MO) and were used without further purification.

Reagents

All reagents used were Merck (Darmstadt, F. R. G.) analytical grade. Water was tridistilled from a permanganate solution using Pyrex apparatus.

Adsorption and desorption procedures

The *in situ* adsorption measurements were performed using binary collagen–albumin and collagen–fibrinogen mixtures. In these experiments the collagen concentration in solution was maintained constant and the second protein concentration was varied. The *in situ* adsorption measuring techniques were described in details in our previous article and it was shown that no preferential adsorption of either labeled or unlabeled collagen molecules occurred at the interfaces studied.[1] All experiments were performed using 0.2 *M* NaCl–0.1 *M* CH_3COOH buffer adjusted with concentrated HCl to give pH = 2.7.

The desorption experiments were performed after 20 h of adsorption on low-density polyethylene films (Cryovac L Film, manufactured by Grace France, thickness 19 μm, density 0.929 g/cm^3). In these experiments a protein-containing solution in the cell was replaced by the 0.2 *M* NaCl–0.1 *M* CH_3COOH buffer solution. Details are given in Ref. 1.

Surface tension measurements at the air/protein solution interface

The surface tension was determined by the Wilhelmy plate method. The decrease of the surface tension with time due to the protein adsorption at the air/protein solution interface was continuously recorded and the values after 20 h were taken. They corresponded to the steady state values. As in the adsorption experiments, the subphase consisted of 0.2 *M* NaCl and 0.1 *M* CH_3COOH adjusted with concentrated HCl to give pH = 2.7. The range of sensitivity of measurements was $\simeq$0.01 mN/m.

For the adsorption and the surface tension measurements the protein solutions were freshly prepared each day.

RESULTS

Surface tensions of single and of binary protein solutions

Table I summarizes the surface tension values of single collagen, albumin, and fibrinogen systems and of binary albumin–collagen and fibrinogen–collagen mixtures measured after 20 h equilibration. It can be noted that adding albumin or fibrinogen to the collagen solutions brings about the decrease of the surface tension of these solutions. At both collagen solution concentrations studied, the decrease of the surface tension was always higher with albumin than with fibrinogen.

Adsorption at the solution/air interface

Figure 1 shows a typical time course of adsorption at the solution/air interface for an experiment in which collagen–albumin and collagen–fibrinogen were in equal (0.005/0.005 mg/mL) solution concentrations. The inflection in the first minutes of adsorption is reproducible, and was also observed on polymer/protein solution interface.

Collagen adsorption in competition with albumin and fibrinogen is illustrated in Figure 2. In these experiments collagen concentration in solution was maintained constant (0.005 mg/mL and 0.025 mg/mL) and albumin or fibrinogen solution concentration was varied in 0.001–0.02-mg/mL range.

Adsorption at the solution/polyethylene interface

Competitive adsorption of albumin–collagen and of fibrinogen–collagen binary mixtures at the solution/polyethylene interface is shown in Figures 3 and 4. For these systems, as for the adsorption experiments at the

TABLE I
Surface Tension in (mN/m) of Collagen, Albumin, and Fibrinogen, Single Systems and of the Albumin–Collagen and Fibrinogen–Collagen Binary Mixtures after 20 h Equilibration at 22°C.

Albumin (mg/mL)	Collagen (mg/mL)		
	0	0.005	0.025
0		66.6	61.0
0.001	55.6	52.5	48.0
0.01	48.0	48.2	48.0
Fibrinogen (mg/mL)	**Collagen (mg/mL)**		
	0	0.005	0.025
0		66.6	61.0
0.001	70.0	59.6	53.4
0.01	53.4	51.6	49.0

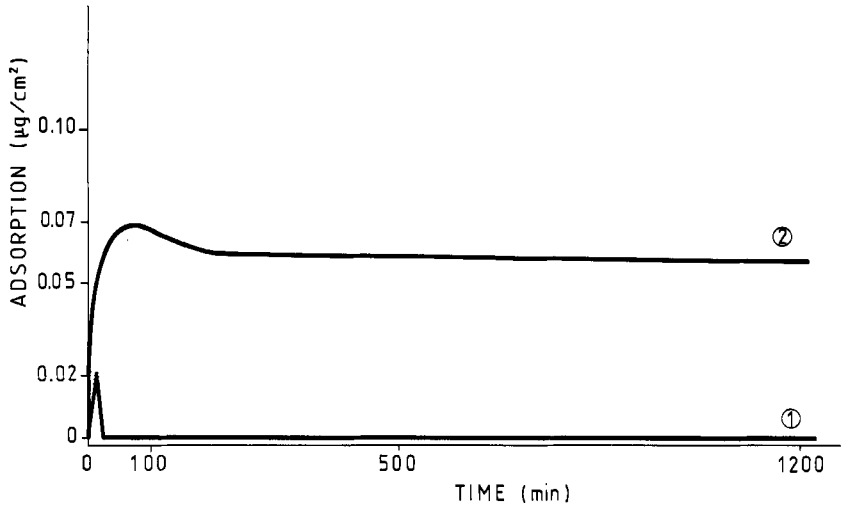

Figure 1. Continuously recorded time courses for the adsorption of collagen at the solution/air interface in presence of albumin (1) and fibrinogen (2). Collagen solution concentration 0.005 mg/mL. Albumin or fibrinogen solution concentration 0.005 mg/mL; pH = 2.7.

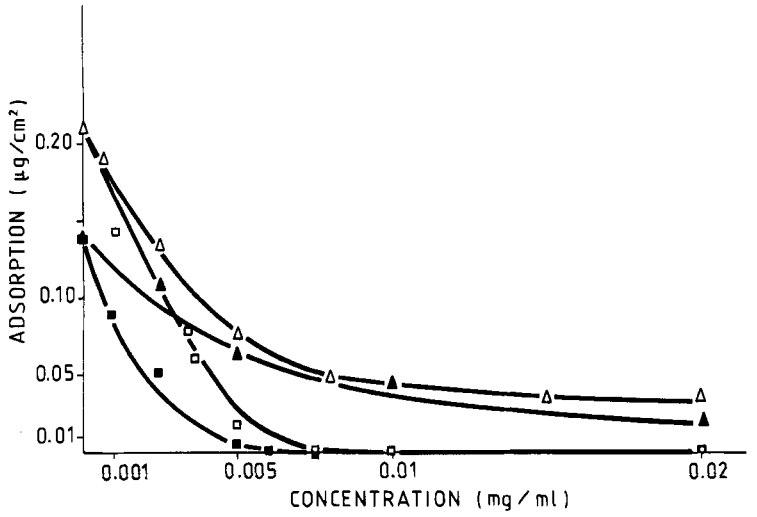

Figure 2. Collagen adsorption at the solution/air interface versus albumin (□, ■) or fibrinogen (△, ▲) concentration. Open symbols: collagen concentration 0.025 mg/mL. Closed symbols: collagen concentration 0.005 mg/mL. Equilibration time 20 h; pH = 2.7.

solution/air interface, collagen concentration in solution was maintained constant (0.005 mg/mL and 0.025 mg/mL). It may be noticed (Figs. 3, 4) that the increase of albumin concentration in solution above 0.005 mg/mL provoked a rapid decrease of adsorbed collagen molecules at the interface. With the albumin concentration of 0.005 mg/mL, collagen adsorption at the interface was almost negligible at the system with 0.005 mg/mL collagen solution

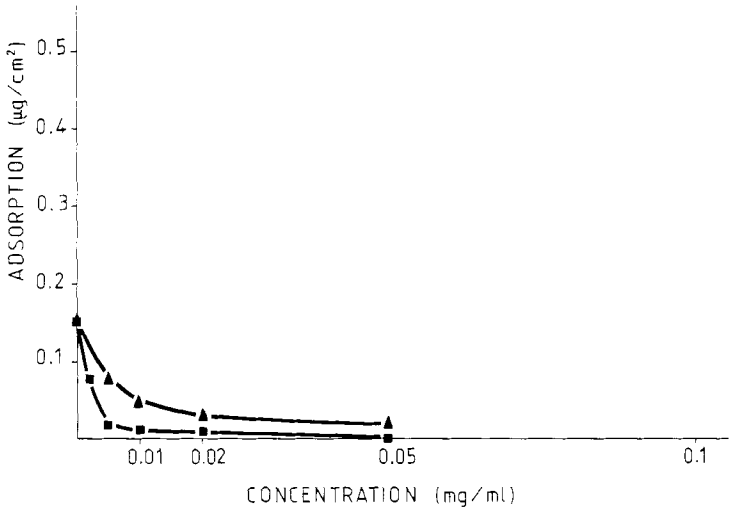

Figure 3. Collagen adsorption at the solution/polyethylene interface in presence of albumin (■) and fibrinogen (▲). Collagen solution concentration 0.005 mg/mL; equilibration time 20 h; pH = 2.7.

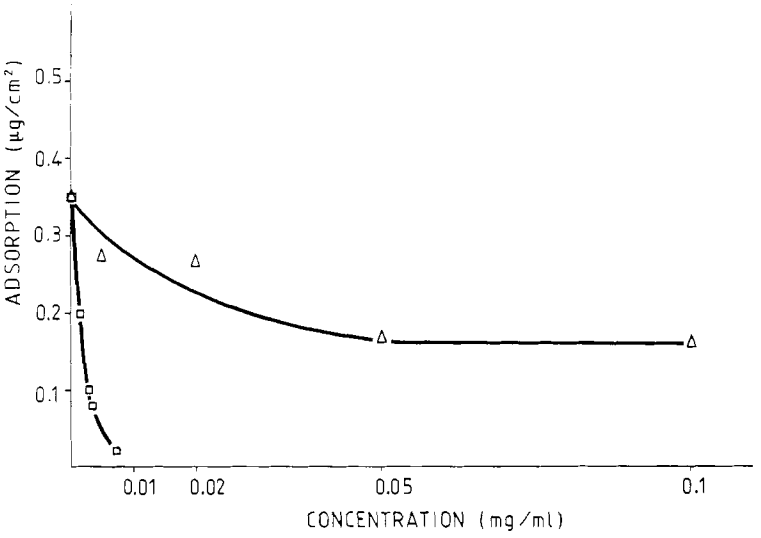

Figure 4. Collagen adsorption at the solution/polyethylene interface in presence of albumin (□) and fibrinogen (△). Collagen solution concentration 0.025 mg/mL; equilibration time 20 h; pH = 2.7.

concentration. At the 0.025 mg/mL collagen solution concentration (Fig. 4) the presence of albumin at the concentrations higher than 0.0075 mg/mL led to non reproducible results.

Finally, in Figure 5, the amounts of irreversibly adsorbed collagen after desorption with a buffer are plotted against albumin or fibrinogen concentration in the adsorption solution.

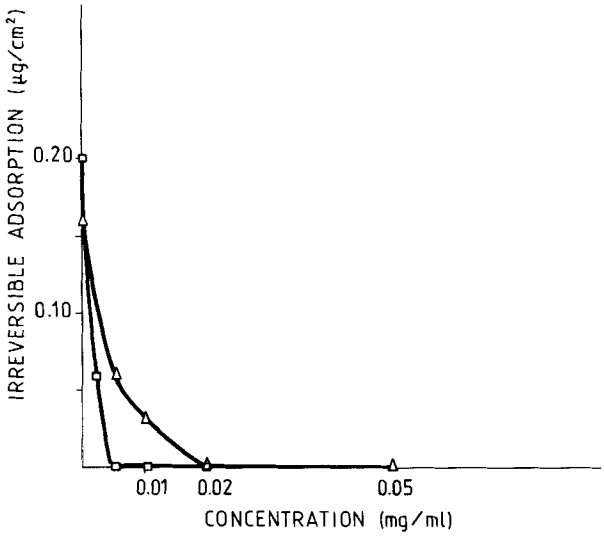

Figure 5. Irreversibly adsorbed collagen amounts on polyethylene surface versus albumin (□) or fibrinogen (△) concentration in the adsorption solution. Collagen solution concentration 0.025 mg/mL.

DISCUSSION

A striking difference of collagen adsorption at the solution/air and at the solution/polyethylene interfaces is observed between the experiments carried out from collagen–albumin and collagen–fibrinogen binary solutions. At the same solution concentration of albumin or fibrinogen, the presence of albumin in a binary mixture considerably lowers collagen adsorption. For example, at the solution collagen concentration equal to 0.005 mg/mL and at the solution/air interface (Fig. 2), after 20 h of adsorption, the presence of 0.005 mg/mL of albumin or fibrinogen in solution lowers collagen adsorption up to 0.06 $\mu g/cm^2$ with fibrinogen in the mixture and to almost zero with albumin in the mixture. These values should be compared with the value of 0.14 $\mu g/cm^2$ corresponding to adsorption of collagen from single-protein solution. A similar effect can be observed at the polyethylene/solution interface (Fig. 3).

In order to get a better insight of whether the surface enrichment of albumin can be related to its surface activity, or adsorption/desorption behavior at interfaces, let us analyze the data in Table I. These data clearly show the differences in behavior of the three proteins. The surface activities of these proteins are in the order albumin > fibrinogen > collagen and as a general rule, as their solution concentration increased, surface tension decreased. The values for albumin are in good agreement with those reported by Katona et al.[3] via the Wilhelmy plate technique and corresponding to the steady state surface tension values after equilibration. The data in Table I indicate also that the surface tension of all studied fibrinogen–collagen mixtures are lower than the surface tension of single fibrinogen or collagen

solutions. Albumin–collagen mixtures present two distinct cases. At the low albumin concentration (0.001 mg/mL) the mixtures with collagen, have the surface tension lower than the surface tension of either albumin or collagen single solutions. At the higher albumin concentration (0.01 mg/mL), the surface tension of the mixture is the same (about 48 mN/m) and equal to the surface tension of the single albumin solution at this concentration. It is interesting to notice that for the (0.001 mg/mL/0.025 mg/mL) albumin–collagen mixture the surface tension is also 48.0 mN/m in spite of the fact that albumin solution at this concentration has the surface tension of 55.6 mN/m.

The lowering of the surface tension of binary solutions below the values corresponding to their single protein solutions would reflect the presence of the mixed collagen-competing protein adsorbed layers. In the opposite case (albumin at 0.01 mg/mL or higher concentrations) albumin is the only constituent of the adsorbed layers. The data of collagen adsorption in the presence of either fibrinogen or albumin (Figs. 2–4) at the interfaces entirely confirm such a situation.

Another evidence of albumin being more efficient than fibrinogen to displace collagen from the interface is given by the desorption experiments (Fig. 5). The irreversibly bound collagen amounts adsorbed from the mixtures containing fibrinogen are higher than those with albumin. The complete desorption of collagen from the adsorbed layers takes place at albumin concentrations in solution higher than 0.005 mg/mL, while more than 0.02 mg/mL of fibrinogen is needed in solution to obtain the same effect.

The appearance of a maximum in the first minutes of the adsorption course (Fig. 1) has to be discussed in more detail and can be explained as follows. At the beginning of the adsorption protein molecules are not present at the studied surface and the adsorption is a diffusion controlled process. Each protein arrives at the surface according to its own diffusion coefficient and its concentration in solution. After a certain period of time (which is different for each studied system) the exchange process takes over the diffusion-controlled protein adsorption and at equilibrium, protein concentration at the surface would depend only upon the protein exchange. If the adsorbing protein reaches a surface concentration higher than that of the equilibrium, then through the exchange process its surface concentration would necessarily be reduced. When this protein is a labeled protein (in our case collagen) one can monitor a maximum in the adsorption curve. Similar adsorption patterns have been reported by Lemm and Unger[4] for albumin–fibrinogen and albumin–γ-globulin systems.

The polyethylene/solution and the air/solution interfaces may be considered as hydrophobic interfaces bearing no specific adsorption sites. The main conclusion which may be drawn from the collagen adsorption experiments on such interfaces is that the adsorption process appears to be strongly influenced by the surface activities of other proteins present in solution. The experimental data clearly show that the higher the surface activity of

the competing protein, the higher is its hindering effect upon collagen adsorption.

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