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Streptococcal antigen I/II binds to extracellular proteins through intermolecular β-sheets

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Abstract One of the functions associated with the oral streptococcal surface protein I/II is to bind to human extracellular matrix molecules or blood components, which could act as opportunistic ligands in pathological circumstances. In order to understand the relative specificity of the binding repertoire of this bacterial adhesin, we examined by infrared measurements the mode of binding of the protein I/II from *Streptococcus mutans OMZ175* (I/IIf) to fibronectin and fibrinogen. This approach revealed the β-structure forming capacity of I/IIf upon interaction with both proteins. The forming of intermolecular β-structures may provide a non-selective way of interaction between I/IIf and its possible targets.

Keywords: Streptococcal antigen I/II; Extracellular matrix molecule; ATR-FTIR spectroscopy; Protein film

1. Introduction

Adhesion of oral streptococcal species to dental surfaces and oral mucosal cells represents the initial step in host colonization by these species. Subsequent opportunistic introduction of the bacteria through the circulation allows dissemination to extra-oral sites via interaction of these streptococci with extracellular matrix molecules or blood components which function as cell receptors for these bacteria [1–5]. This adhesion profile could be critical in the development of diseases such as infective endocarditis and bacteremia in neutrogenic patients [6–8]. Much interest has been focused on the antigen I/II family polypeptides expressed at the cell surface of oral streptococci [9]. Antigens I/II are multifunctional adhesins that exert diverse binding activities, i.e., with salivary glycoproteins [10], host cell receptors [11–14] and soluble extracellular matrix glycoprotein [15,16]. For example, invasion of dentinal tubules by oral streptococci is associated with collagen recognition mediated by antigen I/II polypeptide, leading to dissemination of the bacteria and expression of its cariogenic phenotype [17]. In addition, the immunomodulatory effects of the antigen on human cells may be associated with inflammatory disorders [13,18,19].

Despite some species specificity, proteins I/II display a high degree of conserved structural features. Based on sequence analyses, these proteins may be divided into three main domains: an N-terminal domain (~400 residues) containing the alanine-rich A-region (~320 residues), a central domain including the V-region (~360 residues) and a C-terminal domain (~700 residues) containing a proline-rich P-region (180 residues). Circular dichroic spectra suggest that the A-region is highly α-helical [20]. The crystal structure of the V-region revealed a distorted β-sandwich [21] and the P-region, assumed to have a β-sheet structure, seems to be important for the proper folding of the protein [22].

In order to understand the diversity and the relative specificity of the binding repertoire of these streptococcal adhesins, we examined the mode of binding of a representative of the antigen I/II family, the protein I/II from *Streptococcus mutans OMZ175* (I/IIf), to fibronectin and fibrinogen, and explored the relationship between conformational changes and binding activity, using Fourier transform infrared spectroscopy.

2. Materials and methods

2.1. Protein preparation

I/IIf was prepared, as previously described [23], from cellular extracts from *Escherichia coli* HB 101 transformed with the recombinant plasmid pHB*sr*-1 carrying the gene of protein I/IIf, by ammonium sulfate precipitation followed by immunoaffinity chromatography on an anti-protein I/IIf IgG Sepharose column. The purified protein was lyophilized together with its buffer solution for later use. Fibrinogen (Fib) and Fibronectin (Nec) were purchased in lyophilized form from Sigma.

2.2. Infrared measurements

Infrared spectra were recorded on Bruker (Karlsruhe, Germany) Equinox 55, or IFS66 Fourier transform infrared spectrometers in the ATR mode, using liquid nitrogen cooled MCT detectors, trapezoid or cylindrical ZnSe crystals. Proteins were adsorbed from a circulating solution onto the crystal surface. The progress of the adsorption was monitored by recording infrared spectra as a function of time. The adsorption having been completed, buffer solution was circulated in the ATR cell to remove any non-bound protein. When needed, a second, third, etc., layer of another protein was adsorbed onto the surface. The buffer was 10 mM PBS in D_2O, prepared from PBS pastilles (Sigma). Thus, the pH of the buffer was 6.6. For each interferogram, 1024 scans were collected at 2 cm^{-1} spectral resolutions. Spectra were calculated between 4000 and 900 cm^{-1}. Single-beam spectra were recorded at each step of the architecture construction. Absorption spectra were calculated by taking the corresponding single beam spectrum either as background or as sample spectrum. All post-run manipulations and the analysis of the infrared spectra were

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performed with the SPSERV© software (Cs. Bagyinka, BRC Szeged, Hungary).

3. Results

At the concentrations needed for transmission infrared measurements (5–10 mg/ml), I/IIf – due to its inherent aggregating character [9] – was aggregated and showed no reactivity toward other proteins. This problem was overcome by the use of attenuated total reflection infrared spectroscopy (ATR-IR) , where proteins were adsorbed onto the surface of an internal reflection element from a very dilute (50 μg/ml) solution where there was no aggregation. In addition, the different proteins could be delivered separately and the interfacial conditions would advantageously mimic in vivo situations where proteins bound to their respective cell receptors interact with bacterial cell walls.

The 1700–1600 cm^{-1} amide I region of the infrared spectra of I/IIf, fibrinogen, and fibronectin is given in Fig. 1 when adsorbed either directly onto the surface of the ZnSe ATR crystal, or onto the surface of another, earlier adsorbed protein. While minor distortions cannot be excluded upon adsorption in the protein structures, the spectra taken from proteins adsorbed directly onto the ZnSe crystal were considered as references, references in the sense that no heteroprotein interaction could be involved in these spectra. According to the particularities of the ATR-IR spectroscopy, the absorption spectrum of a newly adsorbed compound is calculated from single beam spectra recorded before and after its adsorption. Thus, the obtained absorption spectrum will reflect two aspects of a protein-protein interaction: (i) the magnitude of the absorption will be characteristic for the affinity between the two proteins in general, and (ii) the changes in the amide I region of the infrared spectrum upon protein–protein interaction when compared with the amide I region of the non-interacting protein can reveal the nature of the interaction between the proteins.

3.1. The affinity between I/IIf and its possible partners

As compared to the adsorption of protein I/IIf onto the ZnSe crystal, less I/IIf is adsorbed onto the surface of either the fibrinogen- or the fibronectin-covered crystal (Fig. 1A). Only about half amount of I/IIf was adsorbed onto fibrinogen and a further half onto fibronectin. The amount of protein I/IIf further decreased when it was administered onto a second fibrinogen layer (Fig. 1A).

For making conclusions about the affinity of protein I/IIf to matrix proteins, one has to investigate the affinity of those matrix proteins toward each other as well. The interaction between adsorbed fibrinogen and fibronectin turned out to be asymmetric. The amounts of fibrinogen and fibronectin adsorbed onto the ZnSe crystal are somewhat different (Fig. 2A). This is not asymmetry in itself as the nature of the proteins (charges, hydrophobicity of the surface of the molecules, etc.) can be different in such extent. The asymmetry appears when the second protein is adsorbed onto the layer of the first one (Fig. 2A). While considerable amount of fibrinogen is adsorbed onto the fibronectin, hardly any fibronectin adsorbs onto the fibrinogen film (Fig. 2A).

It seems that the ZnSe–fibrinogen interaction involves such parts of the fibrinogen molecules that would be needed later for fibronectin binding as well. In contrast, the interaction between fibronectin and the ZnSe crystal leaves enough attracting surface free on the fibronectin to bind the fibrinogen molecules later. This situation is sharply different from the interactions of fibrinogen/fibronectin with protein I/IIf, where both proteins adsorbed roughly in equal amounts onto the film

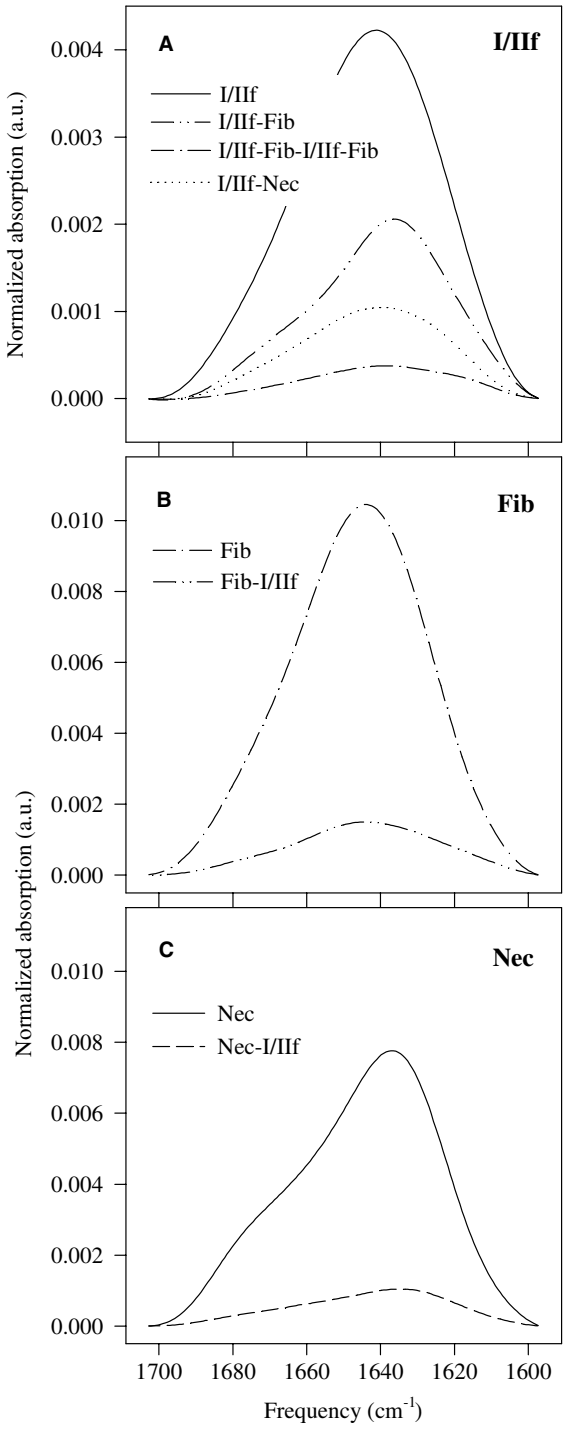

Fig. 1. Amide I regions of the infrared absorption spectra of (A) protein I/IIf when adsorbed onto ZnSe crystal or onto previously adsorbed fibrinogen or fibronectin films; (B) fibrinogen on ZnSe or adsorbed onto a protein I/IIf film; (C) fibronectin on ZnSe or adsorbed onto a protein I/IIf film.

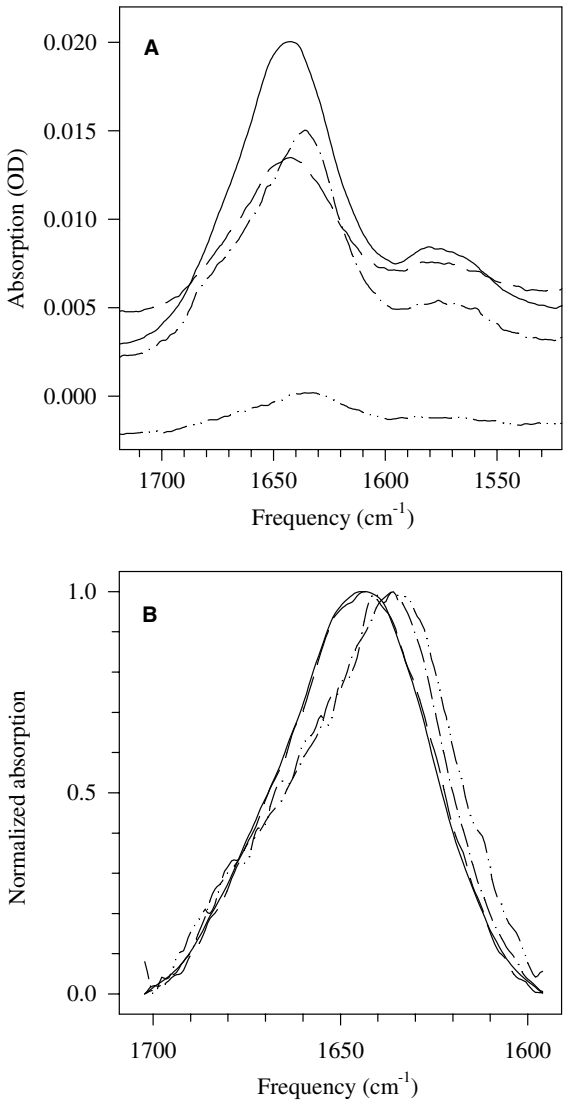

Fig. 2. Infrared absorption spectra of fibrinogen (—), fibrinogen on fibronectin (– – –), fibronectin (– · – · –) and fibronectin on fibrinogen (– · · – · · –) (A); the normalized amide I regions of the spectra shown in the upper panel (B).

of protein I/IIf (Figs. 1B and C). That means that the nature of interaction between I/IIf and its partners is very similar, regardless of the partner molecule, and this interaction is markedly different from that of between the partner molecules.

3.2. Structural changes upon I/IIf–matrix protein interactions

To learn more about the nature of the different types of interactions between these proteins, the second aspect of the infrared absorption spectra, i.e., the changes in the amide I region, should also be investigated. For this purpose, to better see the differences, the amide I regions have been normalized. The structural alterations caused by the adsorption in fibrinogen and in fibronectin are shown in Fig. 2B. It can be seen that the structure of fibrinogen in not changing upon adsorption onto a fibronectin film (see curves Fib and Fib/Nec). As it was indicated by the differences in the adsorbed amounts of the two proteins, the structure of fibronectin is indeed different from that of the fibrinogen (compare curves Fib and Nec in Fig. 2B). This is in agreement with published infrared spectra that indicated higher amount of β-structure elements in fibronectin than in fibrinogen [24]. Fibronectin was suffering a minor structural change upon adsorption onto fibrinogen, which is difficult to interpret confidently due to the very small amount adsorbed (see curve Nec/Fib in Fig. 2A). Nevertheless, it can be said that the β-structure content does not increase in fibronectin upon being adsorbed onto fibrinogen.

The situation is different when comparing the structure of protein I/IIf in different architectures. Both the shape and the

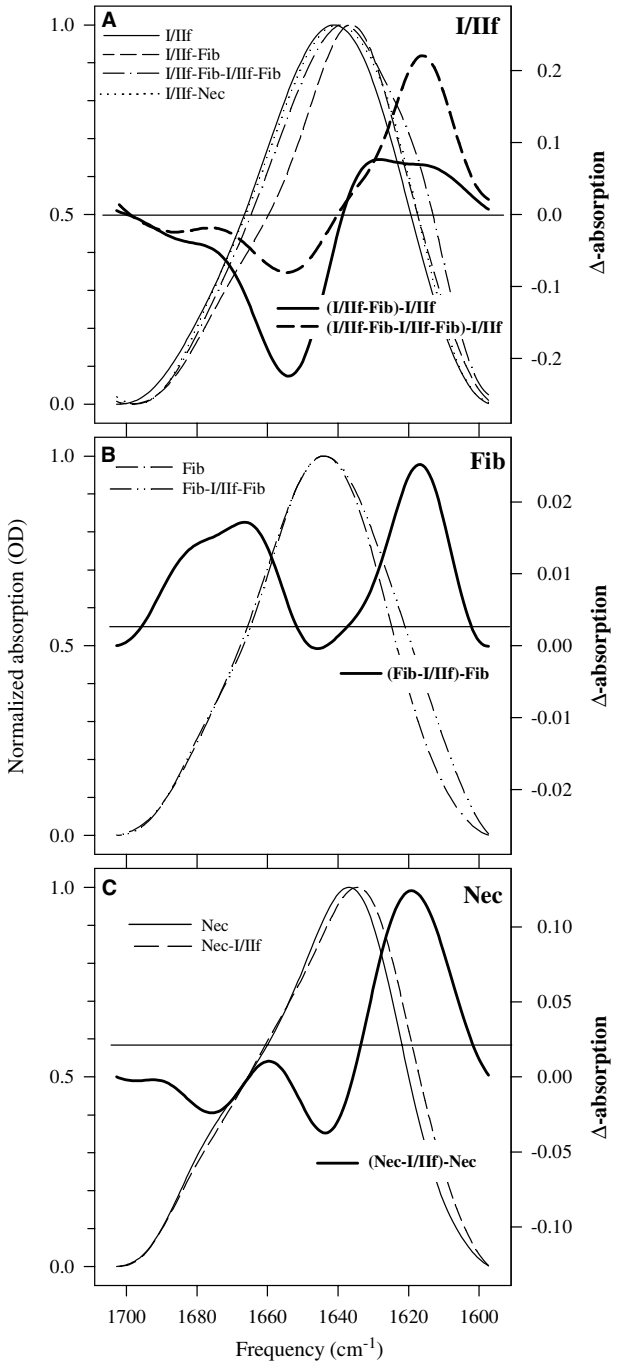

Fig. 3. Normalized infrared absorption spectra of (A) protein I/IIf on ZnSe crystal or on previously adsorbed fibrinogen or fibronectin films; (B) fibrinogen on ZnSe or on a I/IIf film; (C) fibronectin on ZnSe or adsorbed onto a I/IIf film. Thicker lines indicate difference spectra as described in the panels by bold face and belong to the right axes.

frequency of the amide I band of I/IIf is changing upon its adsorption either onto fibrinogen or fibronectin (Fig. 3A). These changes are the results of increasing β-structure content at around 1618 cm^{-1} at the expense of other structural elements. If, by adding a second bilayer, an I/IIf–Fib– I/IIf– Fib–ZnSe architecture is constructed, the structure of the terminating I/IIf will further deviate from the non-interacting I/IIf structure. In its amide I band, the twin bands at around 1618 and 1680 cm^{-1} clearly appear, being characteristic of intermolecular β-structures (Fig. 3A).

If the order of the proteins was opposite, i.e., fibrinogen (Fig. 3B) or fibronectin (Fig. 3C) was adsorbed onto protein I/IIf, the same kind of increase of β-structure elements could be observed. In the light of the very limited structural changes induced mutually in each other's structure by fibrinogen and fibronectin (Fig. 2B), the increases in the β-structure content can be accounted for changes mostly in protein I/IIf.

In general, from the infrared spectra, a detailed analysis of the secondary structure of a protein can be given. In our case, however, since we cannot assign the observed structural changes to a specific protein, only to the whole assembly, such a quantitative analysis would give no protein-specific information. The adsorption-induced structural changes can be better seen in difference spectra, which always exhibit the overall changes of the assembly. Differences were calculated for each protein between being adsorbed onto the given multiprotein architecture and onto the 'bare' ZnSe crystal. These difference spectra are depicted with thicker lines in Figs. 3A–C. The dominance of a positive difference band at around 1618 cm^{-1} is characteristic for each protein–protein interaction-induced structural change. On this basis, we believe that the primary consequence of the interaction between I/IIf and its target proteins is the formation of intermolecular β-structures. Changes in the other parts of the amide I region are probably the 'side effects' of the intermolecular β-structure formation, and these side effects can be of course protein-specific.

4. Discussion

Comparison of the structural changes induced upon the interaction of the protein I/IIf with fibrinogen or fibronectin and the changes induced upon fibrinogen–fibronectin interaction revealed that mostly intermolecular β-structure is formed between the proteins. This β-structure forming is predominantly the capacity of protein I/IIf. When adsorbed onto the surface of the ZnSe internal reflection element of the ATR-IR sample holder, I/IIf has roughly equal affinity toward fibrinogen and fibronectin. These two proteins, however, have asymmetric affinity toward each other. Adsorbed fibronectin is able to bind much more fibrinogen as compared to the opposite order, since adsorbed fibrinogen hardly binds any fibronectin. Also, the magnitudes of the structural changes in fibrinogen and in fibronectin are negligible or very small when they are adsorbed onto the other protein. Putting together these data, it seems that the protein I/IIf has a ''soft'' structure, which is making contact with other proteins by forming intermolecular β-structures, a structure that is generally formed between proteins only at elevated temperatures, during heat-denaturing. This β-structure forming capacity could provide a non-selective way of interaction between I/IIf and its possible targets. The ability of I/IIf to bind diverse host molecules, arming the opportunistic pathogen with a universal, sticky protein, suggests that it contains versatile structural motifs. This is consistent with structural data that described a flexibility of the molecule via linkers between the central domain of the molecule and the N-terminal and the C-terminal domains, respectively [21]. Indeed, the crystal structure of the central domain of the protein I/IIf revealed the presence of a structural kink acting as a hinge between the N-terminal alanine-rich domain and the central domain. From this hinge, conformational changes would propagate from one domain of the protein to another, which may result in versatility of adhesion motifs. From a physiological point of view, this feature of the protein I/II would explain in part the mode of host colonization by *Streptococcus mutans* and also would qualify the opportunistic character of these streptococci.

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