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ORIGINAL PAPER

Enzyme solid-state support assays: a surface plasmon resonance and mass spectrometry coupled study of immobilized insulin degrading enzyme

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Abstract Solid-support based assays offer several advantages that are not normally available in solution. Enzymes that are anchored on gold surfaces can interact with several different molecules, opening the way to high throughput array format based assays. In this scenario, surface plasmon resonance (SPR) and mass spectrometry (MS) investigations have often been applied to analyze the interaction between immobilized enzyme and its substrate molecules in a tag-free environment. Here, we propose a SPR-MS combined experimental approach aimed at studying insulin degrading enzyme (IDE) immobilized onto gold surfaces and its ability to interact with insulin. The latter is delivered by a microfluidic system to the IDE functionalized surface and the activity of the immobilized enzyme is verified by atmospheric pressure/matrix assisted laser desorption ionization (AP/MALDI) MS analysis. The SPR experiments allow the calculation of the kinetic constants involved for the interaction between immobilized IDE and insulin molecules and evidence of IDE conformational change upon insulin binding is also obtained.

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Introduction

Insulin degrading enzyme (IDE) (Farris et al. 2003) is a zinc metalloprotease, able to degrade several different substrates besides insulin (e.g., β-amyloid) that are involved in many pathological conditions such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Vepsäläinen et al. 2007; Kurochkin and Goto 1994; Blomqvist et al. 2004). Very recently, the structures of human IDE in complex with four different substrates have been reported and some insights into the interaction mechanism have also been given (Shen et al. 2006). IDE has a buried catalytic site in the structure and access to this chamber is kinetically controlled by a closed–open conformational switch, so IDE probably conforms to a complex kinetic model where catalysis does not lead automatically to product release. Instead, an additional step is required in which the protease opens up to allow the products to escape and a "latch" system has been proposed in order to explain the experimental results obtained so far for this enzyme (Leissring and Selkoe 2006). Recent developments on IDE conformational changes suggest that, because of the extensive interaction between N- and C-terminal domains, IDE could exist in its closed conformation without requiring the binding energy contributed by its substrate (Im et al. 2007). Moreover, it has been reported that in solution IDE exists as a mixture of monomers, dimers, and tetramers and the equilibrium between the different forms is concentration-dependent, with the dimer the more active form (Song et al. 2003). Very recently, (unpublished data) we have also found that the various IDE

oligomeric forms produce different insulin fragmentation patterns. The latter can also be altered by the presence of other molecules such as ubiquitin (Grasso et al. 2008).

In this scenario, although the exact molecular mechanism of the interaction between IDE and insulin is still unclear, it is likely that IDE has an open conformation in the active state and a closed one when is inactive or is bound to its substrates. Similar behaviors have been observed in the past for other enzymes which undergo a hinge twist motion between two separate domains (Sharff et al. 1992). Although optical and spectroscopic techniques, like CD, fluorescence, NMR, and X-ray scattering, are routinely used to investigate the conformational state of proteins in solution or crystal (Drobny et al. 2003; Andrade et al. 2004), a valid contribution toward a better understanding of IDE-substrates interaction mechanism is also expected from alternative experimental approaches such as solid-state assays. The development and optimization of an immunocapture-based assay for the specific measurement of IDE activity in brain tissue homogenates has already been described (Miners et al. 2008). However, such method requires a fluorescent tag to be present in the substrate and the investigation of the proteolytic action of the enzyme is limited to the only cleavage site where the fluorescent tag is attached.

Surface plasmon resonance (SPR) is able to detect variation in physical parameters (for instance, dielectric constant) caused by volume changes of surface bound proteins and it is usually applied to study biomolecular interactions in real time to obtain kinetics parameters (Homola 2006). In 1998, the first investigation of pH-induced structural transitions of immobilized proteins by SPR was reported (Sota et al. 1998). This was the first description of a correlation between resonance angle shifts and conformational changes of immobilized proteins, opening the way to several indirect observations of protein conformational changes using SPR (Kim et al. 2005; Kang et al. 2006; Geitmann and Danielson 2004).

Our group has already shown the advantages offered by coupling SPR and mass spectrometry (MS) for studying a certain class of enzymes (Grasso et al. 2005, 2007a) in a solid-state format. However, in the latter cases no insights into the molecular mechanism of the interaction were given and the analysis was limited to the calculation of enzyme activity or the identification of the immobilized biomolecules (Grasso et al. 2006).

In order to overcome the above mentioned limitations, in this paper we propose an SPR-MS combined experimental approach that gives an insight into the molecular mechanism of the interaction between an enzyme and one of its substrate. The proposed method is able to detect if the interacting immobilized enzyme undergoes a conformational change upon substrate binding. For this reason, the interaction between IDE and insulin molecules is here scrutinized. We immobilized IDE onto a gold substrate by the amino coupling approach and used atmospheric pressure/matrix assisted laser desorption ionization (AP/MALDI)-MS to monitor the activity of the anchored biomolecules. The latter are arrayed in a spatially resolved manner by coupling the SPR imaging (SPRI) technique with a home-made microfluidic system, allowing the calculation of the kinetic constants involved for the IDE-insulin interaction. The proposed SPR experimental approach produces evidences of the enzyme conformational changes upon insulin binding, in accordance with the view that active substrate-free IDE is in its open conformation (Im et al. 2007). In this way, the study of the monomeric form of the wild type enzyme interacting with one of its natural substrates is feasible and insight into kinetic details of the interaction between immobilized IDE and insulin is provided.

Materials and methods

Reagents

IDE, his-tag, rat, and recombinant from *Spodoptera frugiperda* was purchased from Calbiochem. Insulin from bovine pancreas, phosphate buffer solution (PBS), α-cyano-4-hydroxycinnamic acid (CHCA), trifluoro acetic acid (TFA), acetonitrile (C_2H_3N), ethanol solution, ethanolamine-HCl 1M, guanidine-HCl 8M, sucrose and dithiobis (*N*) succinimidyl propionate (Lomant's reagent) were all purchased from Sigma-Aldrich, while ZipTip$_{SCX}$ pipette tips were from Millipore, and dithiol tethers SPT-0013 and SPT-0014C were purchased from Sensopath. Gold substrates (GWC Technologies, USA) were obtained by thermally evaporating a gold layer (450 Å) onto SF-10 glass slides (Schott, USA). Chromium (50 Å) was used as the adhesion layer.

Surface plasmon resonance

Two different immobilization procedures were scrutinized for IDE and positive results were obtained in both cases. Specifically, similar SPRI signals (see Fig. 1) were indeed registered after a 40 min injection at 5 µl min^{-1} of a 36 nM IDE solution into a microchannel in contact with a gold surface previously functionalized with:

1. Lomant's reagent (Grasso et al. 2005);
2. Dithiol tethers (SPT-0013:SPT-0014C = 10:1 mixed ethanol solution; Lahiri et al. 1999).

We found that the pH of the PBS buffer used for sample dilution is crucial for a positive result of the activity measurements and a pH of 7.4 was chosen for all experiments. Ethanolamine-HCl 1M was used for deactivation of the

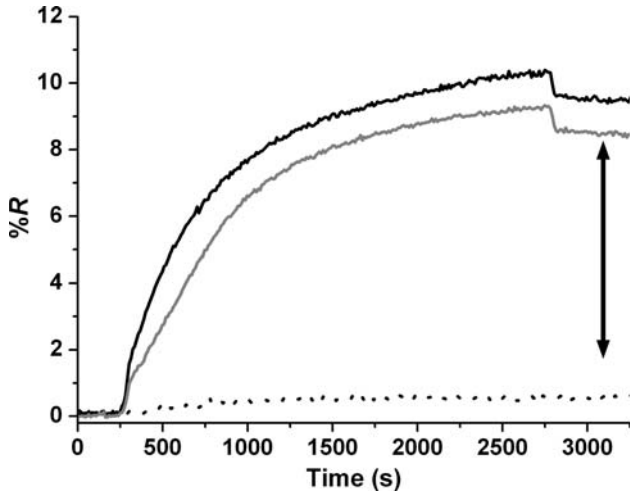

Fig. 1 SPRI response registered for the immobilization of IDE obtained by injecting a 36 nM IDE solution in a microchannel in contact with the pre-functionalized gold surface. The reported *curves* refer to IDE immobilization on a gold surface pretreated with Lomant's reagent (*black line*) or dithiol tethers (*gray line*) and to reference (*dotted line*) have been obtained by mediating the SPRI response of five different channels in two separate experiments. The *double arrow* indicates the amount of immobilized enzyme that differs slightly for the two experimental procedures (about 10%). However, the observed difference is within the reproducibility range (12% is the largest calculated standard deviation of the SPRI signal for the immobilization) and no appreciable difference was detected for the two cases in the following SPRI interaction experiments

unreacted NHS groups, while 5 min injection at 5 µl min^{-1} of guanidine-HCl 8M was used for denaturation of IDE. In order to estimate the number of IDE molecules anchored on 1 cm^2 of gold surface, we followed the method previously described (Grasso et al. 2005). Briefly, the adlayer thickness (d) is obtained from the measured SPR response (R), which is the shift in wavenumber of the SPR minimum in reflected light intensity associated with changes in the index of refraction of the medium in contact with the metal surface of the SPR device, $\Delta\eta$. Once a calibration curve that correlates R with the change in refractive index $\Delta\eta$ is obtained (sucrose solutions were used for this purpose), it is possible to estimate the adlayer thickness (d) from the following equation:

$$d = (l_d/2) \times (R/R_{max}) \quad (1)$$

where l_d is the decay length of the evanescent electromagnetic field into the specific medium, that is usually 25–50% of the wavelength of the light. R_{max} is the SPR response that is the maximum response that would be measured for an infinitely thick adlayer. In the special case where d is very small compared to l_d, Eq. 1 reduces to:

$$d = (l_d/2) \times \{R/[m(\eta_a - \eta_s)]\} \quad (2)$$

where η_a is the refraction index of the adsorbate (the pure IDE protein in our case), while η_s is the refraction index of the buffer. The SPR shift due to the anchoring of IDE was determined (Fig. 1) and a value for the adlayer thickness $d = 0.9$ nm was obtained, using Eq. 2. It is then straightforward (Darnell et al. 1990; Leslie and Lilley 1985) to convert the adlayer thickness into the surface concentration, θ, in molecules per cm^2:

$$\theta(\text{molecules/cm}^2) = d(\text{cm}) \times N(\text{molecules/cm}^3) \quad (3)$$

where N is the bulk number density of the adsorbate and can be estimated from the bulk density of the adsorbate, ρ, in units of g cm^{-3}, just by dividing by the molecular weight and multiplying by the Avogadro's number. In our case, $\rho = 1.30$ g cm^{-3} was obtained from literature (Jung et al. 1998) and $\theta \cong 6 \times 10^{11}$ (molecules cm^{-2}) was calculated, using Eq. 3.

The SPRI apparatus (GWC Technologies, USA) was the same as reported in some of our previous works (Grasso et al. 2005). SPR images were analyzed, using the V++ software (version 4.0, Digital Optics Limited, New Zealand) and the software package Image J 1.32j (National Institutes of Health, USA). SPRI provides data as pixel intensity units (0–255 scale). Data were converted in percentage of reflectivity (%R), using the formula:

$$\%R = 100 \times (0.85Ip/Is)$$

where Ip and Is refer to the reflected light intensity detected using p- and s-polarized light, respectively. The experiments were carried out by sequentially acquiring 15 frames averaged SPR images with 5 s time delay between them. Kinetics data were obtained by plotting the difference in percent reflectivity (%R) from selected regions of interest (ROIs) of the SPR images as a function of time. All the SPRI experiments were carried out at room temperature.

The rate constants reported in Table 1 were calculated by fitting adsorption/desorption kinetics data through numerical integration analysis (Myszka and Morton 1998). After insulin interaction immobilized enzyme could be recycled for other SPRI analyses by flowing buffered solution onto the surface for about 20 min in order to allow complete insulin dissociation and restoring of the SPRI baseline. As long as the functionalized surface was kept in contact with the buffered solution, immobilized enzyme

Table 1 Kinetics parameters obtained by fitting the sensorgrams recorded for the interaction between immobilized IDE and insulin according to the conformational model described in the text (see Fig. 5)

	Rate constants
k_{a1}	1.0 (±0.3) × 10^3 M^{-1} s^{-1}
k_{d1}	1.3 (±0.2) × 10^{-2} s^{-1}
k_r	2.4 (±0.2) × 10^{-3} s^{-1}
k_{-r}	1.5 (±0.7) × 10^{-3} s^{-1}

The calculated standard deviations are indicated. The low reported value of k_{a1} is discussed in the text

Fig. 2 SPR image of the gold surface arrayed with six microchannels. While one of the latter was used for referencing, five insulin solutions at different concentrations could be monitored in real time simultaneously. Dimension of microchannels were 0.2 μm (width) × 80 μm (depth) × 1.6 cm (length)

degradation was observed as an altered SPRI baseline only after several hours (data not shown).

PDMS microfluidic channels fabrication

Microfluidic channels were fabricated in poly(dimethylsiloxane) (PDMS) polymer as described elsewhere (Grasso et al. 2007a). Briefly, PDMS microchannels having a volume of about 0.7 μl were created by replication from masters in polyvinyl chloride (PVC), with a pattern of parallel microchannels (80 μm depth, 1.4 cm length, 400 μm width), featuring circular reservoirs (diameter 400 μm) at both ends of each channel. A six microchannels microfluidic device was used in this case for following the interaction between IDE and insulin at different concentrations (see Fig. 2). PEEK tubes (Upchurch Scientific) were inserted in such reservoirs in order to connect the PDMS microfluidic cell to a Masterflex L/S (Cole-Parmer, USA) peristaltic pump, operating at 100 μl min^{-1}. Replicas were formed from a 1:10 mixture of PDMS curing agent and prepolymer (Sylgard 184, Dow Corning, USA). The mixture was degassed under vacuum and then poured onto the master in order to create a layer with a thickness of about 3–4 mm. The PDMS was then cured for at least 2 h at 60°C before it was removed from the masters.

The evaluation of the kinetic constants for biomolecular interactions by SPR experiments can be affected by diffusion problems (D'Agata et al. 2008). In order to estimate the contribution of diffusion to the kinetic parameters obtained, k_m is normally used to describe the diffusion of the protein to the surface and it can be related to the diffusion coefficient of the protein. The dimensions of the above described microchannels ensured that, at the flow rate used in our SPRI experiments for the interaction between IDE and insulin (100 μl min^{-1}), the value of k_m describing the diffusion of insulin to the gold surface was 1.4×10^8 M^{-1} s^{-1}. The latter is well above the rate constants values reported in Table 1 and therefore the results obtained are not affected by diffusion problems.

Mass spectrometry

AP/MALDI-MS experiments were carried out, using a Finnigan LCQ Deca XP PLUS (Thermo Electron Corporation, USA) ion trap spectrometer which was fitted with a MassTech Inc. (USA) AP/MALDI PDF-source. The latter consists of a flange containing a computer-controlled X–Y positioning stage and a digital camera, and is powered by a control unit that includes a pulsed nitrogen laser (wavelength 337 nm, pulse width 4 ns, pulse energy 300 μJ, repetition rate up to 10 Hz) and a pulsed dynamic focusing (PDF) module that imposes a delay of 25 μs between the laser pulse and the application of the high voltage to the AP/MALDI target plate. Laser power was attenuated to about 55%. The target plate voltage was 1.8 kV. The ion trap inlet capillary temperature was 220°C. Capillary and tube lens offset voltages of 30 and 15 V, respectively, were applied. Other mass spectrometer parameters were as follows: multipole 1 offset at −3.75 V, multipole 2 offset at −9.50 V, multipole RF amplitude 400 V, lens at −24.0 V and entrance lens at −88.0 V. Automatic gain control (AGC) was turned off and instead the scan time was fixed by setting the injection time to 220 ms and using five microscans. Although there is the risk of loosing resolution, the latter experimental conditions were chosen as sensitivity was the main goal in all MS experiments carried out on the immobilized enzyme.

Results and discussion

Immobilization of biomolecules on solid supports and use of microfluidic systems have shown to offer several advantages not available in the standard solution assays as mentioned above. In this work, MS and SPRI experiments on immobilized IDE were performed. Particularly, the activity of the immobilized IDE was verified by AP/MALDI-MS on the functionalized gold surface by sampling

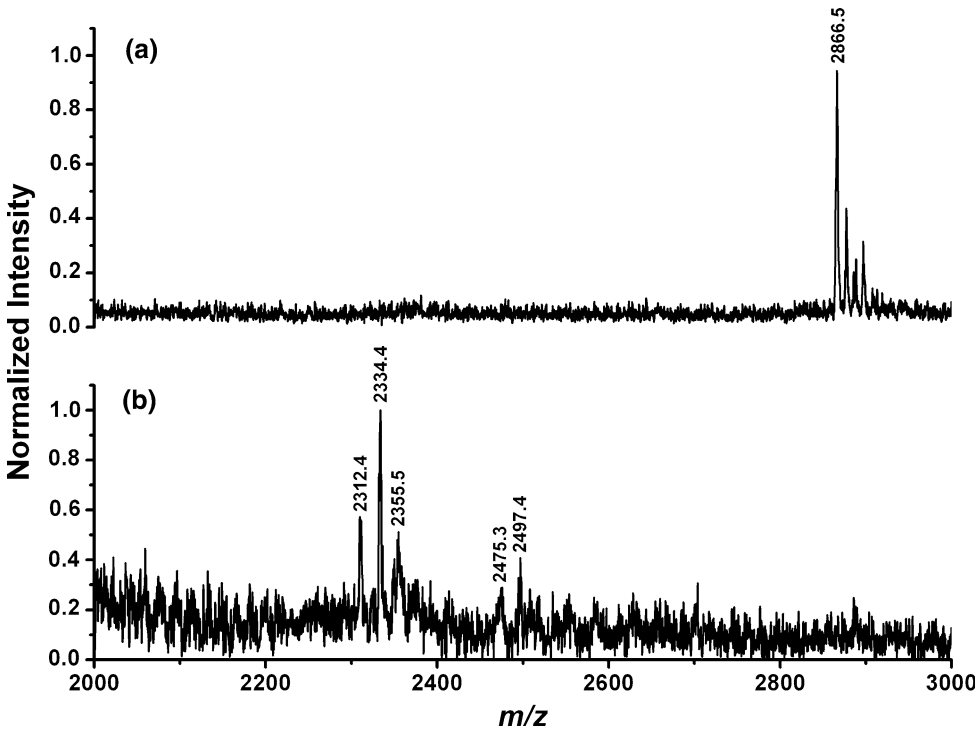

Fig. 3 AP/MALDI-MS data recorded for a supernatant insulin solution (18 μM) sampled from a clean gold surface (**a**) and from IDE functionalized surface (**b**). The insulin doubly charged molecular peak at *m/z* 2866.5 in (**a**) is substituted with peaks attributed to insulin fragments in (**b**)

the supernatant solution in contact with the functionalized surface as previously described for other enzymes (Grasso et al. 2005), and representative spectra are reported in Fig. 3. Fig. 3a shows the spectrum of the doubly charged insulin molecular peak which is detected at *m/z* 2866.5 together with the associated sodiated peaks (presence of sodium salt is necessary in order to maintain enzyme activity). Fig. 3b shows the mass spectrum registered for the supernatant solution that had been in contact with the IDE functionalized surface for about 20 min. In this case, the molecular peak at *m/z* 2866.5 disappeared, while new peaks at *m/z* 2312.4 and 2475.3 assigned to the insulin fragments reported in Table 2 appeared together with associated sodiated peaks at *m/z* 2334.4, 2355.5, and 2497.4. From these data, we concluded that the immobilized IDE is able to cleave insulin molecules, producing some of the expected insulin fragments (Grasso et al. 2007b). It has already been found by our group that it is possible to have information on the IDE oligomeric forms distribution from the produced insulin fragmentation patterns (unpublished data). Particularly, the peaks observed in the case of immobilized IDE are mainly produced by the monomeric form of the enzyme. Therefore it is possible to conclude that, if the immobilization of IDE is carried out according to the above described experimental protocol, anchored IDE exists mainly as a monomer and it is able to cleave insulin molecules.

In Fig. 4 the change in percent reflectivity over time obtained for the interaction between immobilized IDE and a 9 μM insulin solution (black line) is shown together with

Table 2 Insulin fragments detected from the supernatant solution in contact with the IDE functionalized surface

Fragments combinations	Calculated peaks (*m/z*)	Experimental peaks (*m/z*)
A(1–13) + B(1–9)	2313.0	2312.4
A(1–14) + B(1–9)	2476.0	2475.3

For explanation/discussion see text

the SPRI curve obtained by putting the same solution in contact with denatured IDE (gray line). IDE denaturation was achieved by injecting guanidine-HCl 8 M at 5 μl min^{-1} for 5 min (Di Venere et al. 2000; Yamaguchi et al. 2003). Although some non-specific interactions between the insulin molecules and the functionalized surfaces were observed, a large difference was recorded between active and denatured IDE. Particularly, no shift in the SPRI response was recorded when the insulin solution (experiments were carried out up to 80 μM insulin concentration) flowed into the microchannel in contact with denatured IDE functionalized surface, indicating that insulin molecules did not interact with the immobilized denatured enzyme. Furthermore, non-specific insulin interactions were not observed in this case, probably because unfolded denatured IDE molecules completely blocked the gold surface that is sampled by SPRI (Jung et al. 1998). This last observation reinforces the conclusion that the SPRI data recorded in the case of active IDE-insulin is due to a physiological interaction between the two biomolecules.

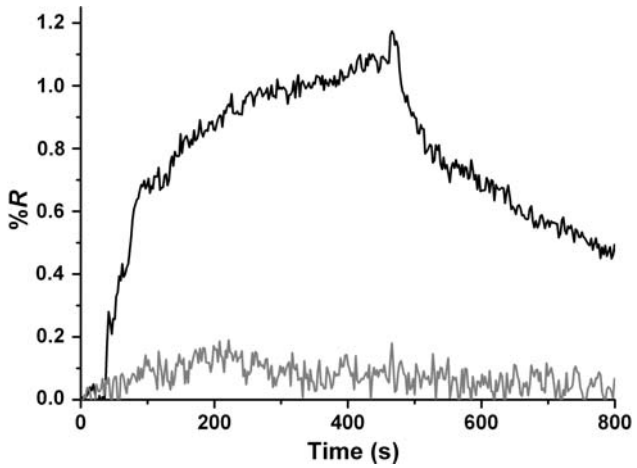

Fig. 4 SPRI response registered when a 9 μM insulin solution is flowed for 9 min into two differently modified microchannels: immobilized active (*black line*) and denatured (*gray line*) IDE. The latter functionalized surface was achieved by injecting guanidine-HCl 8 M at 5 μl min^{-1} for 5 min onto the active immobilized IDE molecules

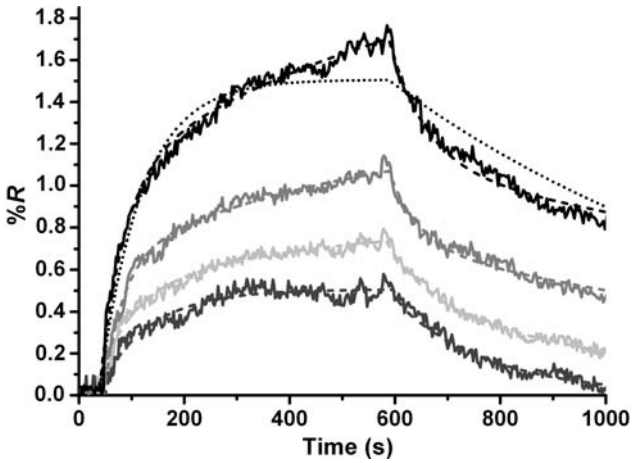

Fig. 5 SPRI sensorgrams (*solid lines*) obtained by flowing insulin solutions for 550 s at the following different concentrations onto active immobilized IDE: 4 μM (*dark gray*), 6 μM (*light gray*), 9 μM (*gray*), and 18 μM (*black*). Fitted lines according to the conformational change model are shown in *dashed lines*, while only in the case of the 18 μM insulin solution the line fitted according to the simple biomolecular model is also shown (*dotted line*)

Therefore, immobilized IDE interacts with insulin molecules only in its active conformation. In order to elucidate the mechanism of such an interaction a common procedure is to propose a plausible model and to fit the experimental kinetic data accordingly (Honjo et al. 2002; Yowler and Schengrund 2004). For this reason, SPRI kinetic curves obtained with four insulin solutions at different concentrations were fitted, assuming that IDE undergoes a conformational change upon substrate binding (see Fig. 5). Kinetic parameters obtained from the fitting of the experimental data are shown in Table 1, while the reactions describing the model used in order to fit the kinetic data are:

$$L + A \underset{k_{d1}}{\overset{k_{a1}}{\rightleftarrows}} LA \underset{k_{-r}}{\overset{k_{r}}{\rightleftarrows}} (LA)_{r}$$

where L represents the immobilized IDE, A is the free insulin molecules in solution and LA is the IDE-insulin complex before and after the conformational change $(LA)_r$. The poor results obtained by fitting data assuming pseudo first-order kinetics are evident from Fig. 5 (dotted black line). Analogously, other interaction models (surface heterogeneity, etc.) were used but the best fit of the kinetic data was obtained by applying a conformational change interaction model. The calculated value of k_{a1} reported in Table 1 is low if compared to the values normally reported in the literature for various biomolecular interactions (Ji et al. 2003). In order to interpret this result correctly, it is important to consider that the values reported in Table 1 are meaningful only for the interaction between immobilized IDE and insulin. In fact, although immobilized enzymes have often similar kinetic parameters as in solution (Grasso et al. 2007a), in the case of IDE the interaction mechanism is more complicated as it involves different oligomeric forms of the enzyme (Shen et al. 2006; Im et al. 2007; Song et al. 2003). AP/MALDI-MS results show that IDE is mainly immobilized as a monomer and therefore it is expected that the kinetic parameters for the interaction with insulin are somehow different from the ones referring to the solution state where all the possible oligomeric forms of the enzyme are present (the k_{a1} value is expected to be higher in solution). Therefore, our results serve to characterize the interaction between the monomeric immobilized form of the enzyme and insulin, opening the way to high throughput solid-state assays. Moreover, in our working buffer (PBS at pH 7.4) insulin exists in equilibrium as a mixture of monomers, dimers, hexamers, and possibly higher oligomeric species, and we refer to the abundance of work in the literature addressed to study such equilibria in solution (Manno et al. 2007). However, the interaction between IDE and insulin does not seem to be affected by the substrate oligomeric distribution, as revealed by the same insulin fragmentation patterns detected by MS (Grasso et al. 2007b).

A further SPRI experiment was performed in order to support the hypothesis that the conformational change model is correct to describe the IDE-insulin interaction. The experiment consisted of varying association times and analyte concentrations in order to have always the same amount of analyte interacting with the immobilized molecules (Myszka et al. 1999; Aguilar and Small 2005). In fact, the amount of insulin bound to the same IDE surface

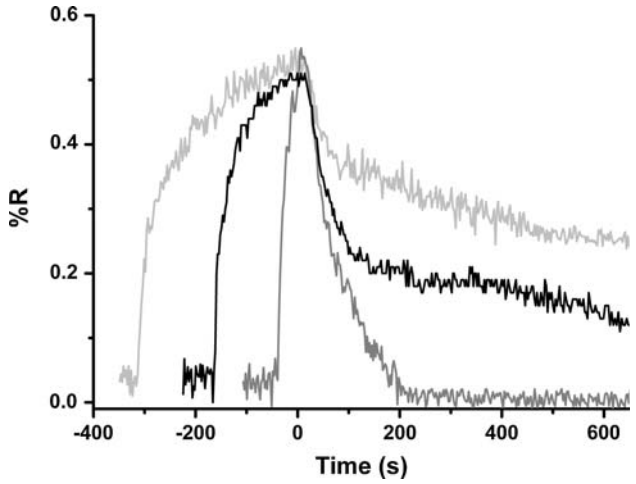

Fig. 6 Varying contact time and insulin concentration: 6.3 μM and 325 s (*light gray line*); 15.6 μM and 182 s (*black line*); and 25 μM and 44 s (*dark gray line*), respectively. The concentration and contact time of insulin solutions flowing into the microchannels were varied with the intent of keeping the overall amount of insulin bound to the immobilized IDE constant at *%R* of 0.53 ± 0.02 at the beginning of the dissociation phase

is dependent on both association time and insulin concentration. The *%R* variation detected by SPRI is proportional to the amount of bound insulin, thus different combinations of association time and insulin concentration were used to reach the same *%R* value (0.53 ± 0.02). Results from the experiments are reported in Fig. 6 and they have been interpreted as follows. In the case of high concentration of analyte molecules and short contact time (25 μM and 44 s), it is insufficient for immobilized IDE molecules to change their conformation. Therefore, the insulin molecules exhibit mainly non-specific interactions with the IDE surface and give rise to a rapid decrease of the SPRI response in the dissociation phase (see Fig. 6, dark gray line). In contrast, when a low concentration solution is flowed into the microchannel for a prolonged period (6.3 μM and 325 s), there is sufficient time for the immobilized IDE molecules to change their conformation and the dissociation phase is characterized by a slower dissociation rate (see Fig. 6, light gray line). In fact, in the latter case, IDE has to change its conformation back to the open state in order to release bound insulin molecules, a process requiring more time than a simple surface desorption.

The above described SPRI approach is applied to verify the conformational change of immobilized enzyme molecules occurring upon substrate binding, giving an insight into the IDE-insulin interaction mechanism. In fact, it is important to highlight that the MS results showed that the enzyme is mainly immobilized on the surface as a monomer and therefore the conformational change observed by applying our SPRI approach refers only to this specific oligomeric form of IDE, conferring an advantage to the use of the solid-supported coupled SPRI-MS approach over other solution-based investigation tools. Particularly, we confirmed a model where, in the absence of substrate, the inactive resting state of monomeric IDE is in a closed conformation, since substrates cannot gain access to the catalytic chamber when the protease is in this state. Only when the enzyme switches to its open conformation the substrate molecules can properly interact with IDE (see Table 1 for the rate constants values). Thus, the closed conformation is critical for regulating the catalytic cycle of monomeric IDE while a conformational switch to the open state needs to occur if the enzyme is to carry out its catalytic activity.

Conclusions

A solid-support based assay that allows a multiplexed approach to study IDE-insulin interaction was proposed. We immobilized IDE molecules on gold surfaces and monitored their activity by AP/MALDI-MS, demonstrating the ability of the anchored monomeric form of the enzyme to cleave insulin molecules. SPRI experiments subsequently were carried out on the immobilized enzyme molecules, producing evidence of a conformational change of IDE upon insulin binding. Kinetic parameters were calculated and SPRI experiments based on different ratios of insulin concentrations and contact times have also been carried out to give an insight into the IDE-insulin interaction. Particularly, the results confirmed a model where the closed conformation is the inactive resting state of monomeric IDE while the open conformation is its active state that switches to a closed state upon substrate binding. According to this model IDE should shrink its hydrodynamic radius upon insulin binding.

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