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The construction, fouling and enzymatic cleaning of a textile dye surface

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ABSTRACT

The enzymatic cleaning of a rubisco protein stain bound onto Surface Plasmon Resonance (SPR) biosensor chips having a dye-bound upper layer is investigated. This novel method allowed, for the first time, a detailed kinetic study of rubisco cleanability (defined as fraction of adsorbed protein removed from a surface) from dyed surfaces (mimicking fabrics) at different enzyme concentrations. Analysis of kinetic data using an established mathematical model able to decouple enzyme transfer and reaction processes [Onaizi, He, Middelberg, Chem. Eng. Sci. 64 (2008) 3868] revealed a striking effect of dyeing on enzymatic cleaning performance. Specifically, the absolute rate constants for enzyme transfer to and from a dye-bound rubisco stain were significantly higher than reported previously for un-dyed surfaces. These increased transfer rates resulted in higher surface cleanability. Higher enzyme mobility (i.e., higher enzyme adsorption and desorption rates) at the liquid–dye interface was observed, consistent with previous suggestions that enzyme surface mobility is likely correlated with overall enzyme cleaning performance. Our results show that reaction engineering models of enzymatic action at surfaces may provide insight able to guide the design of better stain-resistant surfaces, and may also guide efforts to improve cleaning formulations.

1. Introduction

The cleaning of stained fabric is a common activity in human daily endeavour, and is facilitated by the treatment of surfaces with dyes that resist the irreversible adsorption of staining agents, including proteins, to the fabric proper. Reactive dyes, such as cibacron blue F3GA (CB), are a major class of commercial textile dyes with growing application not only in textile dyeing but also in other fields such as affinity chromatography [1]. The dyeing process chemically and physically modifies a surface, so that protein stain mainly interacts with the dye rather than the underlying surface. Improved fundamental understanding of the interaction between proteins and dye surfaces might therefore enable the design of better stain-resisting surface coatings, as well as insights into better cleaning methods and formulations. While the displacement of surface-bound protein stains by enzymatic reactions has been widely studied [2–5], there is a lack of fundamental studies of the enzymatic cleaning of protein stains from dyed surfaces.

Cibacron blue F3GA (CB) is a textile reactive triazine dye [6] that can be easily immobilized onto any functionalized support bearing amino, hydroxyl or carboxyl groups [7,8]. It is a derivative of monochlorotriazine, containing four basic primary and secondary amino groups and three acidic sulphonate groups [6]. CB has been the subject of many studies aimed at understanding the chromatographic purification of proteins. However, from a detergency standpoint, there is a lack of information about how the utilization of dyes, including CB, affects the interaction of cleaning agents, such as enzymes, with a protein stain on the dyed surface. In this work we are particularly interested in understanding the action of proteases which can degrade a dye-bound protein stain using formulations having low environmental impact. This situation is complex [3], but amenable to mathematical modelling which can be used to decouple the transfer and reaction rates occurring at the surface. Nevertheless, to the best of our knowledge, a detailed analysis that decouples the transfer and reaction rates for the degradation of a protein stain bound to a dye surface, by a protease, has not been published.

The main objective of this study is, therefore, the creation of a biosensor-linked dye surface and its use to quantitatively study the cleaning of a model rubisco protein stain. We chose to link dye molecules to biosensor chips useful for Surface Plasmon Resonance, thus constructing a dyed surface able to accurately measure the protein cleaning process in real time and in a tractable way. A key objective of this study was to decouple the transfer and reaction parameters for this system, as mentioned above.

To achieve a decoupling of the rates of transfer and reaction we use a recently-published mathematical model [9]. The model

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included enzyme activity loss, which is an important issue in many enzymatic applications, especially for proteases, mainly due to autolysis [3,10,11]. Briefly, the model assumes enzyme in bulk adsorbs onto the immobilized substrate and forms enzyme–substrate complexes that dissociate into washable fragments (products) and released enzyme. Concurrently, the enzyme can be deactivated both in bulk and on the surface. With a series of simplifying assumptions [9], the following model equations have been shown to adequately represent the coupled transfer-reaction processes:

$$\text{Enzyme adsorption}: \quad \frac{d\Gamma_{E_T}}{dt} = k_a[E_B](\Gamma_{E_{Max}} - \Gamma_{E_T}) - k_d\Gamma_{E_T} \tag{1}$$

$$\text{Surface-proteolysis reaction}: \quad \frac{d\Gamma_S}{dt} = -k_c\Gamma_S\Gamma_{E_A} \tag{2}$$

$$\text{Enzyme deactivation}: \quad \frac{dx}{dt} = -k_{de}x, \quad \text{where } x = \frac{[E_A]}{[E_B]} = \frac{\Gamma_{E_A}}{\Gamma_{E_T}} \tag{3}$$

$$\text{Enzyme adsorption isotherm}: \quad \Gamma_{E_T} = \frac{[E_B]\Gamma_{E_{Max}}}{[E_B] + K_{diss}} \tag{4}$$

In Eqs. (1)–(4), $[E_B]$ is the total bulk concentration of the enzyme, Γ_S is the surface concentration of the stain, x is the fraction of active enzyme, and t is time. The total adsorbed enzyme (active + inactive), the maximum amount of adsorbed enzyme (active + inactive) and the active enzyme are indicated by the symbols E_T, E_{Max} and E_A, respectively. Constants k_a, k_d, k_c and k_{de} are, respectively, the adsorption, desorption, proteolysis and enzyme deactivation rate constants, while K_{diss} ($=k_d/k_a$) is the dissociation constant of the overall adsorbed enzyme. In this work, we report a novel dye-modified biosensor and use it to collect new data for the cleaning of a rubisco-stained dye surface. The kinetic data are analysed using the above model to reveal how dyeing changes the transfer properties of the protease between the surface and bulk, in such a way that cleaning is improved relative to un-dyed surfaces.

2. Materials and methods

2.1. Rubisco purification and sampling

Rubisco was purified from spinach leaves as described previously [12], and was divided into small aliquots that were kept frozen at −80 °C until use (an aliquot was used for every experiment). Frozen samples (stored up to 6 months) gave similar results to fresh samples. Rubisco aliquots were diluted to 0.5 mg mL^{-1} using 20 mM Na^+Phosphate buffer pH 8.0 before use.

2.2. Design of a Surface Plasmon Resonance (SPR) biosensor chip with upper dye surface

A 10^{-4} M ethanolic mixture of β-mercaptoethanol (Merck, 805740, Kilsyth, Australia) and α-lipoic acid (Sigma, T5625) at a molar ratio of 9:1 was prepared. The mixed SAM solution was then added to the jar containing cleaned [9] gold slides. The self-assembly reaction was allowed to proceed for at least 1 d at room temperature. The liquid mixture of the two components is expected to produce a mixed layer comprising the two components on the gold surface. While this does not necessarily mean that same ratio is present on the surface, no significant difference between the molar ratio of the mixed SAM and the mixed solution has been found [13]. The prepared surface was then derivatised by dye coupling into the SAM as described by Schlereth and co-workers [14–18], except that 4-morpholineethanesulfonic acid (MES) buffer was used instead of phosphate buffer. MES buffer has been reported to be an excellent choice, while buffers containing phosphate, amines or carboxylic acids cause deactivation of N-(3-Dimethylaminopropyl)-N-ethyl-carbodiimide hydrochloride (EDC) [19], which is used to activate the SAM carboxyl group, leading to its covalent binding to the primary amino group of the dye anthraquinone [20,21]. To achieve dye coupling, the SAM solution was first removed and slides were sequentially washed with 1 M sodium hydroxide (NaOH), MQW, and 70% HPLC ethanol in Milli-Q water (MQW), several times. Cibacron blue F3GA (CB, 27319, Sigma) was covalently linked onto the carboxyl group of a self-assembled monolayer [14–18] as schematically shown in Fig. 1. Coupling was achieved by incubating 0.1 mg mL^{-1} CB solution in 50 mM MES (M3671, Sigma) buffer in the presence of 15 mg mL^{-1} Na_2CO_3 and 2 mg mL^{-1} EDC (03450, Sigma). The addition of salts enhances the dye fixation efficiency due to charge screening effects. Carbonate salt (e.g., Na_2CO_3) is usually used in textile dyeing. The reaction temperature was set to 70 °C since monochlorotriazine dyes, such as CB, have an optimum dyeing temperature of *ca.* 70 °C. The reaction residence time was about 24 h. After that, the dye solution was removed and the slides were rinsed with MQW, 1 M NaOH, and 70% HPLC ethanol in MQW to remove physically-adsorbed dye molecules. Slides were then kept in HPLC ethanol until use. Every functionalised surface was used only once. Prior to use, the contact angle of MQW droplets on the dyed surface was measured using a Krüss drop shape analysis system DSA10 (Krüss GmbH, Hamburg, Germany).

2.3. Rubisco adsorption

Surface Plasmon Resonance (SPR) spectroscopy [22] (Resonant Probes GmbH, Göettingen, Germany) with a 632 nm He–Ne laser in a Kretschmann configuration [23] was used in this study. The time-course of rubisco adsorption onto the dyed surface was recorded by monitoring the change of the reflectivity signal at a fixed incident angle (approx. 1° smaller than the SPR angle), which typically gives an initial reflectivity of 30% (maximum reflectivity (100%) occurs after reaching the critical angle). The flow cell mounted with the functionalized SPR chip was washed with phosphate buffer (20 mM Na^+Phosphate, pH 8.0, ionic strength 56 mM) at a flow rate of 1 mL min^{-1} (the flow path volume is 0.4 mL) using a peristaltic pump (ISMATEC SA, Glattbrugg, Switzerland) until a stable baseline was established. An SPR angular scan was performed to determine the SPR angle prior to rubisco adsorption. Changes in the reflectivity signal were monitored for about 1 h after rubisco injection. An angular scan was then performed to find the shift in SPR angle. Finally, buffer wash, to remove rubisco from the flow path and any weakly bound molecules at the interface, was carried out for 10 min at a flow rate of 1 mL min^{-1}.

2.4. Adsorption isotherm of SA onto immobilised rubisco

To decouple enzyme adsorption from surface reaction, and hence obtain reliable values of the adsorption parameters for subtilisin A (SA) onto immobilised rubisco, the proteolysis reaction rate must be negligible. This objective was achieved by a combination of phenylmethanesulfonyl fluoride (PMSF) treatment of SA and glutaraldehyde (GA) cross-linking of rubisco [9]. Measurements of the adsorption isotherm of partially deactivated SA onto cross-linked rubisco were conducted as follows. First, a baseline was established in 20 mM Na^+Phosphate buffer to ensure stability of the interface before the addition of enzyme solution. An angular scan was performed in the buffer before injecting the enzyme solution and after washing the enzyme solution from the flow path. Different enzyme concentrations were injected in a sequential addition after every plateau. A buffer wash was then performed to measure the desorption rate. The pseudo surface equilibrium

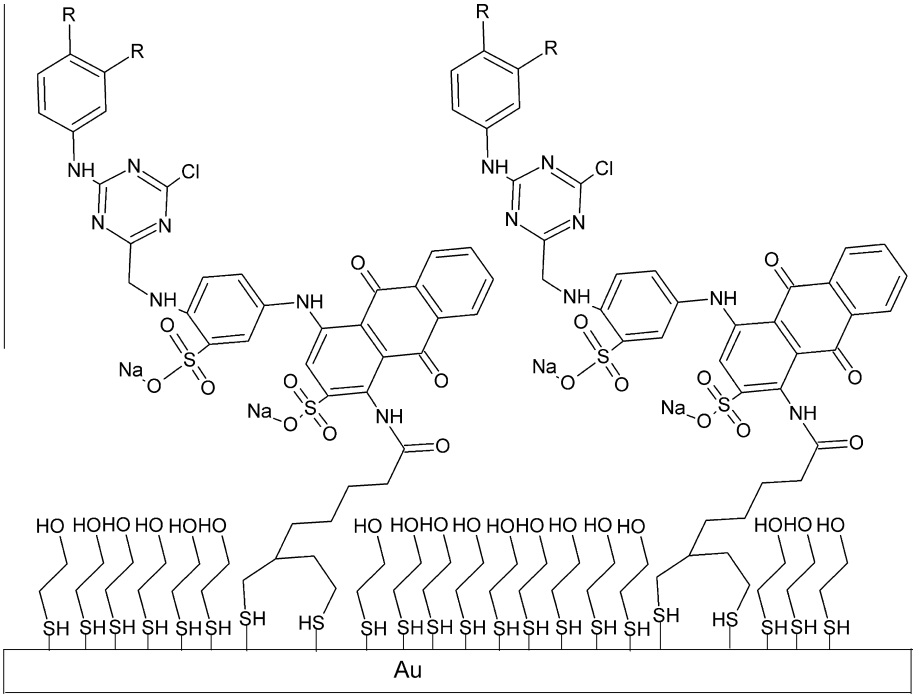

Fig. 1. Idealized structure of the functionalized SPR chip showing the self-assembled monolayer (SAM) of β-mercaptoethanol and α-lipoic acid on a gold surface. The coupling of CB molecules to the carboxylic functional group of α-lipoic acid is also shown.

concentrations at the corresponding enzyme bulk concentrations were used to estimate the maximum adsorbed amount of SA ($\Gamma_{E_{Max}}$) onto the immobilized rubisco and also to estimate the apparent dissociation constant (K_{diss}) from the non-linear fit of Eq. (4) using a program code in SigmaPlot, v 10.0. The apparent desorption rate constant (k_d) was estimated from the fit of the buffer wash data (desorption) using Eq. (1) with $[E_B] = 0$. From K_{diss} and k_d the apparent adsorption rate constant ($k_a = k_d/K_{diss}$) was calculated.

2.5. Rubisco surface proteolysis

The proteolysis of a dye-bound rubisco stain was performed using active subtilisin A (SA) at five different bulk concentrations (0.05, 0.1, 0.5, 2 and 5 ppm, all freshly prepared in 20 mM Na^+Phosphate pH 8.0 before injection). Washout of protein fragments from the interface by the enzyme solution was continuous over the 1 h reaction time at a flow rate of 1 mL min^{-1}. Enzyme adsorption parameters along with the previously estimated reaction rate constants [9] were incorporated into Eqs. (1)–(3) to enable a *priori* prediction of rubisco surface proteolysis at every enzyme concentration using user-defined program codes in SigmaPlot, v 10.0. These codes were built based on the Fourth Order Runge–Kutta Method.

2.6. Data analysis

Winspall Software (v 2.20, MPI-für Polymerforschung, Mainz, Germany) simulations were used to convert the shifts in SPR angle into an average film thickness, assuming that both refractive indices of rubisco and SA are 1.45 [24]. The large area interrogated by the SPR laser (of mm size) suggests the effect of localised variations in the optical properties of the surface (if any) on the overall (average) film thickness will be minimal. The shift in the film thickness is linearly proportional to both the shift in the SPR angle and the shift in the reflectivity signal (at a fixed incident angle) [25–27]. The average film thickness and protein density were used to calculate the surface mass concentration (mg m^{-2}) of the films; rubisco and SA densities were assumed to be the same as that reported in literature for proteins, which is 1.33 g cm^{-3} [28,29].

3. Results and discussion

A novel biosensor for analysis of protein stain cleaning from a dyed surface was prepared by the covalent bonding of dye mole-

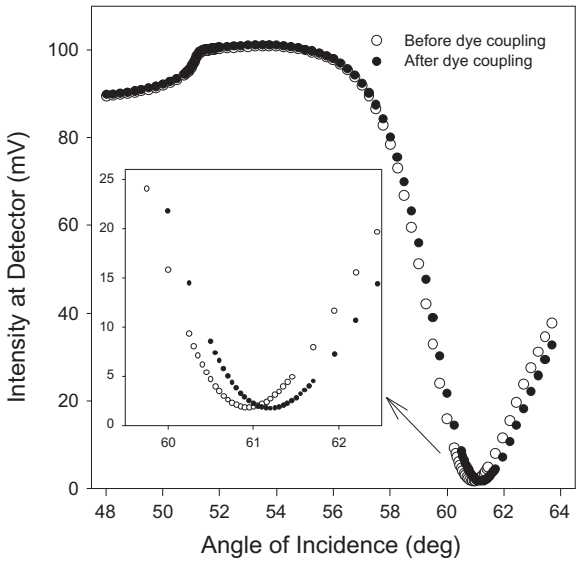

Fig. 2. SPR angular scans before and after dye coupling to the mixed SAM. Dye molecules were coupled from 0.1 mg mL^{-1} dye solution in 50 mM MES buffer containing 2 and 15 mg mL^{-1} EDC and Na_2CO_3, respectively, at 70 °C. After dye coupling the physically-adsorbed molecules were removed by washing several times with 1 M NaOH, 70% ethanol and MQW, prior to an SPR angular scan.

cules onto SPR biosensor chips modified with a self-assembled monolayer (SAM). Fig. 2 shows the SPR angular shift due to the coupling of the dye molecules into the SAM surface. The shift in SPR angle is linearly proportional to the film thickness of the covalently linked materials. The predicted dye film thickness is 1.5 nm, assuming the dye refractive index to be 1.45. Dye coupling onto different surfaces with different functional groups has been reported by several authors [30–39]. However, none of these studies reported the thickness of the coupled dye, nor utilised dye surfaces to investigate detergency (i.e., the studies did not examine protein stain removal from these surfaces by the action of an enzyme).

The successful preparation of a dyed surface suitable for SPR allowed a kinetic investigation of the fouling and enzymatic cleaning of a model surface relevant to detergency. Fig. 3 shows the kinetics of the dye surface fouling with rubisco protein. The kinetics of fouling of the SAM surface, which lacks a conjugated dye layer, are also shown in Fig. 3. A significant change in original surface characteristics through dye coupling has been achieved. Rubisco adsorbs more on the dyed surface, suggesting higher protein affinity for the dye, which is in agreement with observations by other researchers for other proteins [18,30,39–46]. Fig. 3 also shows the buffer washout step after 1 h of rubisco adsorption. The objective of this step was to probe the irreversibility of rubisco adsorption at the solid–liquid interface and to examine the effect of surface dyeing on the desorption process. A further objective was to remove rubisco from the bulk flow path, leaving only irreversibly bound rubisco molecules at the interface. No significant decrease in the adsorbed amount was observed, indicating irreversible rubisco adsorption at both solid–liquid interfaces. Such behaviour has been observed for other solid–liquid interfaces [9,47] as well as the air–liquid interface [12], making rubisco a good model protein stain, since any measured protein removal will only be due to the action of the imposed cleaning solution.

To analyse the cleaning process we first sought to quantitatively establish the transfer properties of enzyme to the dye-adsorbed protein stain. Our objective was to independently establish three key model parameters ($\Gamma_{E_{Max}}, k_a, k_d$) using experimental data for the adsorption and desorption of enzyme from the rubisco stain bound to the dyed surface. Although there are more advanced and complex models for protein adsorption [48–50], for simplicity we used the Langmuir isotherm and found it to be adequate, particularly in light of the observed reversibility of enzyme adsorption (e.g., Fig. 4 washout). Fig. 4 shows the adsorption kinetics of partially deactivated SA onto cross-linked rubisco adsorbed to a CB-dyed surface. This combination of SA deactivation and rubisco cross-linking reduces proteolysis rates to almost zero [9], meaning that the transfer processes can be probed without confounding reaction. In Fig. 4, each plateau represents an equilibrium surface coverage; these equilibrium data points were used to establish the experimental adsorption isotherm (Fig. 5). Data points in Fig. 5 were regressed using Eq. (4) (Langmuir isotherm), providing the best-fit Langmuir adsorption isotherm (solid curve in Fig. 5).

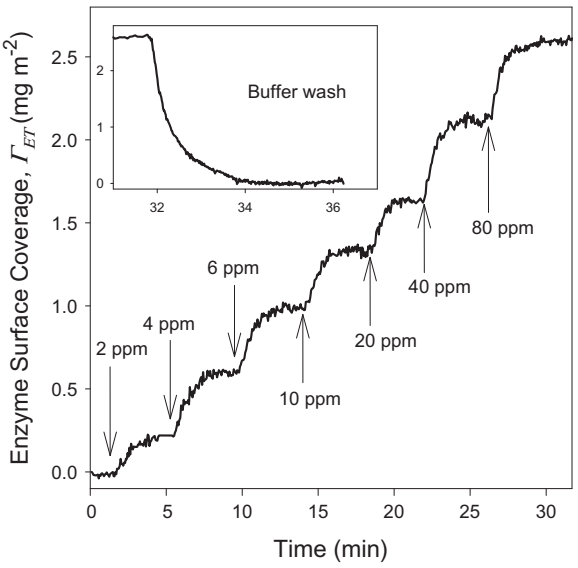

Fig. 4. Adsorption kinetics of partially deactivated subtilisin A onto immobilized and glutaraldehyde cross-linked rubisco on a dyed surface. The enzyme desorption time-course is shown in the inset.

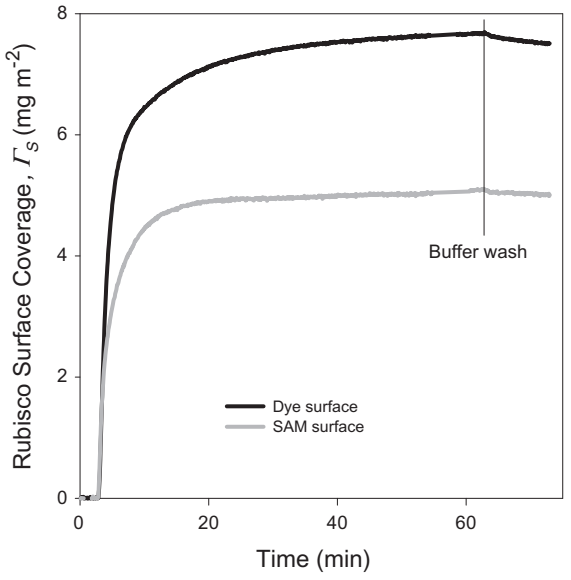

Fig. 3. Rubisco adsorption onto a dyed surface to create a model protein stain. Adsorption was from 0.5 mg mL^{-1} rubisco solution in 20 mM Na^+Phosphate, pH 8.0. Rubisco adsorption from the same solution onto a dye-free SAM is also shown as a control experiment.

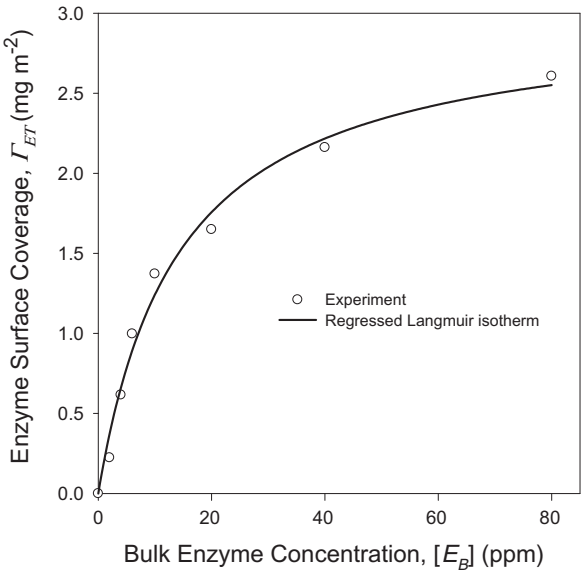

Fig. 5. Adsorption isotherm of partially deactivated subtilisin A onto immobilized and glutaraldehyde cross-linked rubisco on a dyed surface. Solid curve is the Langmuir fit to experimental data using Eq. (4). The estimated values of $\Gamma_{E_{Max}}$ and K_{diss} are 3.0 ± 0.2 mg m^{-2} and 14.2 ± 2.0 ppm, respectively.

The regression also enabled the estimation of two adsorption parameters: maximum surface coverage of the enzyme ($\Gamma_{E_{Max}}$) and the dissociation constant ($K_{diss} = k_d/k_a$). The estimated $\Gamma_{E_{Max}}$ was 3.0 ± 0.2 mg m^{-2}, which is about 90% of the expected close-packed full monolayer coverage of the enzyme (3.3 mg m^{-2}) [51], while K_{diss} was estimated to be 14.2 ± 2.0 ppm. Fig. 4 also shows data for the buffer wash step following enzyme adsorption (see the inset in Fig. 4, reproduced in Fig. 6). The buffer wash data demonstrate the reversible nature of enzyme adsorption onto the stain, and were used to estimate k_d using Eq. (1) with $[E_B] = 0$. Fig. 6 shows the fitting of the buffer wash data, giving an estimated value of k_d as 2.16 ± 0.05 min^{-1}. Fig. 6 also shows that desorption of enzyme from the rubsico film is complete. This observation is in agreement with the complete SA washout from cross-linked BSA, as reported by Foose et al. [51]. With the estimated values of K_{diss} and k_d, the adsorption rate constant (k_a) was estimated to be 0.152 ± 0.025 min^{-1} ppm^{-1}. Analysis of the data in Figs. 4–6 therefore establishes the rates of transfer of protease to and from the dye-linked rubisco stain.

In a previous study [9], we estimated the surface reaction rate constants for SA on rubsico to be $k_c = 1.70 \pm 0.10$ m^2 mg^{-1} min^{-1} and $k_{de} = 0.03 \pm 0.01$ min^{-1}. These reaction parameters were obtained for very different surfaces (SAM layers) without conjugated dye. Here we assume that these reaction constants are fixed for SA catalysing rubsico proteolysis, and will thus apply to the current dyed surface. With the values of the transfer parameters ($\Gamma_{E_{Max}}$, k_a, k_d) estimated by analysis of Figs. 4–6, the model (Eqs. (1)–(3)) is fully parameterised and thus the surface proteolysis time-course can be predicted. Fig. 7 shows the predicted proteolysis time-course and compares this *a priori* prediction with experimental results at five bulk enzyme concentrations (SA at 0.05, 0.1, 0.5, 2 and 5 ppm). In this case the experimental data relate to active SA degrading dye-linked rubisco that has not been cross-linked (i.e., the full system where SA transfer and reaction are occurring in concert). The *priori* predictions are in acceptable agreement with the observed experimental data. Parametric sensitivity analysis confirmed that the assumed values of k_c and k_{de} gave the best-fit of experimental data. This finding confirms our earlier suggestion that the SA proteolysis rate constant, and the deactivation rate constant, are independent of the surface which underlies the rubisco stain. Differences in absolute cleaning rates are therefore attributable to differences in transfer rates, which in turn influence the amount of protease on the surface. The generality of this finding beyond the dye surface examined here, and the three surfaces examined previously [9], requires further confirmation.

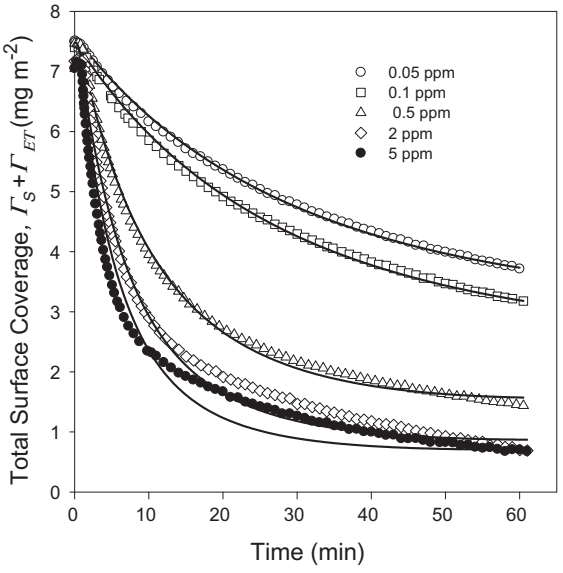

Fig. 7. Proteolysis of rubisco immobilised on a dyed surface as a function of subtilisin A concentration. Protein films were aged for 70 min (including 10 min buffer wash) before enzyme solution was injected. Curves show the predicted surface proteolysis time-course using the model parameters shown in Table 1 for the dye surface.

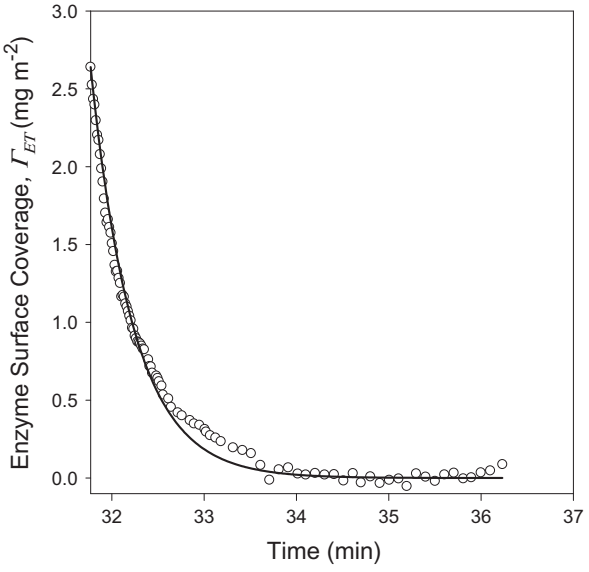

Fig. 6. Desorption of partially deactivated subtilisin A from immobilized and glutaraldehyde cross-linked rubisco on a dyed surface. Solid curve is the fit to experimental data using Eq. (1) with $[E_B] = 0$. The estimated k_d value is 2.16 ± 0.05 min^{-1}.

Current and previous work reveals significant differences in cleaning the same stain from four different surfaces, using the same cleaning agent under the same experimental conditions. These differences likely result from changes in enzyme transfer behaviour due to changes in the stain character caused by changes in the stain during or after adsorption onto the underlying surface. Table 1 shows the adsorption parameters of SA onto rubisco stain immobilized onto four different surfaces. Comparison of the adsorption rate constants (k_a) reveals that the absolute SA adsorption rate onto rubisco stain follows the order dye > negatively-charged > hydrophobic ∼ neutral hydrophilic. Furthermore, the absolute desorption rate (k_d) shows dye > negatively charged ∼ hydrophobic > neutral hydrophilic, while the dissociation constant, K_{diss} (the off/on ratio), is hydrophobic > neutral hydrophilic > negatively charged > dye. Rubisco stain cleanability (Table 2) is highest for the dyed surface, and this result correlates with higher absolute adsorption (k_a) and desorption (k_d) rates for the dyed surface. The negatively charged surface displayed lower cleanability than the dyed surface, but slightly higher cleanability relative to the hydrophobic one (see Table 2). Desorption rates for charged and hydrophobic surfaces were the same within experimental error, but the hydrophobic surface imparted a lower absolute adsorption rate (k_a of 0.016 vs. 0.048 min^{-1} ppm^{-1}), likely causing lower cleaning performance. The neutral hydrophilic surface displayed the lowest cleanability, although it has the same absolute adsorption rate as the hydrophobic surface; however it possesses the lowest absolute desorption rate (see Table 1). Therefore, higher absolute adsorption or desorption rates, alone, do not explain the observed differences in rubisco stain cleanability. Indeed, stain cleanability appears to consistently depend on the absolute values of both k_a and k_d. This finding suggests that enzyme mobility at interfaces might play a crucial role in the enzymatic cleaning of immobilized protein stains (evidence

Table 1
Estimated model parameter values for different surface chemistries underlying an adsorbed rubisco stain.[a]

Parameter	Dye	Negatively charged hydrophilic	Hydrophobic	Neutral hydrophilic
k_c ($m^2\ mg^{-1}\ min^{-1}$)	1.70 ± 0.10	1.70 ± 0.10	1.70 ± 0.10	1.70 ± 0.10
k_{de} (min^{-1})	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
k_a ($min^{-1}\ ppm^{-1}$)	**0.152 ± 0.025**	0.048 ± 0.005	0.016 ± 0.007	0.014 ± 0.004
k_d (min^{-1})	**2.16 ± 0.05**	1.75 ± 0.05	1.60 ± 0.15	1.05 ± 0.02
K_{diss} (ppm)	**14.18 ± 1.98**	36.2 ± 10.7	102.0 ± 22.8	77.1 ± 5.4
$\Gamma_{E_{Max}}$ ($mg\ m^{-2}$)	**3.0 ± 0.2**	2.3 ± 0.3	2.4 ± 0.4	6.5 ± 0.3
MQW contact angle (degree)	**15**	52	93	31

[a] Values in bold were determined in this study. Other parameters were determined for non-dyed surfaces in a previous study [9] except the contact angles, which have not been reported in [9].

Table 2
Rubisco stain cleanability (fraction of adsorbed protein removed) as a function of surface chemistry and cleaning agent concentration.[a]

SA bulk concentration (ppm)	Cleanability (%) after 1 h contact with SA solution			
	Dye	Negatively charged hydrophilic	Hydrophobic	Neutral hydrophilic
0.5	**82**	53	32	40
2	**93**	56	50	34
5	**97**	73	58	33

[a] Parameters in bold were determined in this study. Other parameters were determined for non-dyed surfaces in a previous study [9].

of enzyme mobility at solid–liquid interfaces has been reported in a number of published studies [52–59]). It further suggests that surface treatments such as dyeing, which increase both k_a and k_d, enhance cleanability. This finding potentially explains, at a fundamental level, why the dyeing of fabric has practical as well as aesthetic value.

The proposed model proved capable of providing *a priori* prediction of the experimental data under a wide range of conditions. Reaction rate constants did not change with the variations in the underlying surface chemistry and, thus, regression was used here only to define the adsorption isotherm. This is a useful strength in our approach, minimising the efforts for predicting the proteolysis time-course of rubisco from new surfaces. The model also showed, in a consistent way, how adsorption kinetics can dramatically affect the enzymatic cleaning of immobilized protein stain. Cleanability appears to be consistently correlated with enzyme mobility, suggesting that enzyme transfer processes control overall system performance for a given protease. The results of this study might impact several enzymatic applications and industries, concerning the enzyme behaviour at interfaces, for instance, the design of surfaces (e.g., fabrics) as well as methodologies for designing optimised cleaning formulations. The results might also form a basis for new technologies that will enable fundamentally-based screening of interfacial enzymatic cleaning performance, which will be superior to existing case-specific empirical approaches.

4. Conclusions

A biosensor-linked model dye surface was prepared and used to study the enzymatic cleaning of rubisco protein stain. A textile dye, cibacron blue F3GA (CB), was covalently linked into a SAM-modified SPR biosensor chip to mimic CB-dyed fabrics. Mathematical modelling demonstrated that the process of dyeing significantly altered the transfer processes by which enzymes reach and depart the dye-bound stain, in a way that enhances cleanability. Dyeing appears to have a negligible effect on reaction rate (proteolysis and deactivation) constants. The results of this and previous work show that the physical and chemical properties of the surface underlying a protein stain can significantly alter enzyme adsorption behaviour and thus impact cleanability. This finding suggests that stain cleanability is a function of enzyme mobility, since higher absolute adsorption and desorption rates were consistently found to be associated with better cleaning. This finding might have important implications for both fabric treatment and detergent formulation technologies. The proposed model holds promise as a useful tool for screening detergent-enzyme candidates, and their interaction in formulations and with surfaces.

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