# Interaction of lysozyme with synthetic anti-lysozyme D1.3 antibody fragments studied by affinity chromatography and surface plasmon resonance

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## Abstract

Synthetic antibody fragments of monoclonal anti-lysozyme antibody D1.3 have been tested on binding with hen egg white lysozyme using immunoaffinity chromatography and surface plasmon resonance. Upon immunoaffinity chromatography, peptides containing one or two complementarity determining regions (CDRs) of D1.3 show interaction with lysozyme. Surface plasmon resonance with immobilized CDR peptides showed that this interaction is not based on the antigen–antibody interaction. Nevertheless, these peptides could be useful as ligands for the purification of lysozyme from a mixture of proteins.

## 1. Introduction

The use of immobilized antibodies in immunoaffinity chromatography for purifications of antigens or the application in biosensors for detection of proteins is limited by the stability of the antibody. Size reduction of the antibody may improve the stability of an immobilized antibody. Antibodies ($M_r = 150\,000$) can easily be reduced to a fragment variable (Fv) ($M_r = 25\,000$) region without losing much of the specificity of the antibodies and in some cases to variable regions of the heavy chain ($V_H$) or light chain ($V_L$) ($M_r = 12\,500$) [1]. The specificity of antibody–antigen interactions is determined by three complementarity determining regions (CDRs) located at the variable region of the heavy chain and three CDRs at the variable

region of the light chain. The Fv region of an antibody is the antigen binding site which consists of $V_H$ and $V_L$ regions. Further reduction in size results in peptides which contain one or two CDRs ($M = 1000$–$4000$) which may be able to bind the antigen. Synthetic CDR peptides have been found to bind an antigen or to inhibit binding of an antibody [2–8]. The advantage of small synthetic antibody fragments compared to an antibody is their stability and the possibility for large-scale production.

Selection of a CDR-containing fragment of an antibody for peptide synthesis is facilitated by tertiary structure information of well studied antibody–antigen complexes. For this reason we have chosen the monoclonal antibody (MAb) D1.3–hen egg white lysozyme (HEL) interaction as a model system to select antibody fragments for peptide synthesis and test them as immobilized ligands in immunoaffinity chromatography

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and in surface plasmon resonance on binding lysozyme. X-Ray diffraction studies of the complex between Fab D1.3 and lysozyme revealed the contact residues of lysozyme and D1.3 [9,10]. The epitope of lysozyme which interacts with D1.3 consists of peptide 18–27 and peptide 116–129 of the linear sequence of lysozyme. The binding of D1.3 to lysozyme is based on hydrogen bonds, Van der Waals interactions and hydrophobic interactions but not on charge interactions (salt bridges). Upon binding, no changes in the conformation of D1.3 occur, except for a slight change in quaternary structure by a change of the "elbow bending" angle, the angle between V and C domain of Fab D1.3. The interacting surfaces of lysozyme and D1.3 are complementary; only a few amino acids residues of the contact area contribute directly to the binding energy. The contribution of the contact residues of Fv D1.3 to the binding of lysozyme has been calculated by Novotny et al. [11]. A much larger contribution to complex formation of the $V_H$ domain ($-11.4$ kcal; 1 cal $= 4.1868$ J) than of

the $V_L$ domain ($-2.5$ kcal) was found. For this reason only peptides of the $V_H$ domain were selected for synthesis (Table 1). In general, small synthetic peptides do not possess one stable preference conformation in solution. The CDR peptides of Table 1 will probably not have the same solution conformation as the conformation in D1.3. Some of these peptides (peptides 3 and 6) were cyclized to mimic the reverse turns present in the MAb D1.3. Another possibility to mimic the conformation of a peptide in the protein conformation is to enlarge the peptide to a "small protein" (peptide 7). H3D1.3 CDR peptides (peptides 1, 2 and 3 in Table 1) are potentially the most suitable synthetic antibody fragments, based on the number and the contribution to the binding of the contact residues.

First, the binding of the CDR peptides of D1.3 studied by immunoaffinity chromatography will be discussed. In previous studies it appeared that two CDR peptides of MAb Gloop2 (H2Gloop2 and L3Gloop2) directed against lysozyme could bind lysozyme in immunoaffinity chromatog-

Table 1
Selected peptides for synthesis based on structure information of the D1.3–lysozyme complex [9,10]

| Peptide | CDR in D1.3 | $M$ | $\Delta G$ (kcal) | Amino acid sequence |
|---|---|---|---|---|
| 1 | H3D1.3 92–101 | 1356 | $-6.8$ | xxxx<br>H-<u>ARERDYRLDY</u>-NH$_2$ |
| 2 | H3D1.3 92–105 | 1784 | $-6.8$ | xxxx<br>H-<u>ARERDYRLDY</u>WGQG-NH$_2$ |
| 3 | cH3D1.3 98–103 | 1224 | $-6.8$ | xxxx<br>Ac-C<u>ERDYRL</u>CK-NH$_2$ |
| 4 | H2D1.3 50–63 | 1708 | $-2.0$ | xxx<br>Ac-(Nle)<u>IWGDGNTDYNSAL</u>K-NH$_2$ |
| 5 | H2D1.3 50–62 | 1538 | $-2.0$ | xxx<br>H-(Nle)<u>IWGDGNTDYNSAL</u>-NH$_2$ |
| 6 | cH2D1.3 51–57 | 1135 | $-2.0$ | xxx<br>Ac-C<u>IWGDGNT</u>CK-NH$_2$ |
| 7 | H1H2D1.3 28–63 | 3977 | $-3.4$ | xxx    xxx<br>Ac-SLT<u>GYGVNW</u>VRQPPGKGLEWLG(Nle)<u>IWGDGNTDYNSAL</u>-NH$_2$ |

The CDR regions are underlined and the contact residues of the CDR are marked by an $\times$ sign. The contribution of the peptides to complex formation is given in Gibbs free energy $\Delta G$ [11]. Some of the peptides were acetylated at the N-terminus to prevent N-terminal coupling of the activated Sepharose in immunoaffinity chromatography or the activated dextran-coated sensorchip in a biosensor. Methionine was substituted by isosteric norleucine (Nle) to prevent oxidation of Met-containing peptides. A prefix c in column 2 indicates a cyclized peptide.

raphy [2,3]. Immunoaffinity chromatography of lysozyme on columns with D1.3 antibody fragments (Fv D1.3 and $V_H$D1.3 as ligands) was performed by Berry and co-workers [12–14]. A reduced specificity of the $V_H$ column towards HEL was found. Separation of HEL from turkey lysozyme that could be accomplished on a Fv column was no longer possible on a $V_H$ column.

Second, the results of the study of biospecific interaction of the synthetic antibody fragments by surface plasmon resonance (SPR) [15,16] will be given. Binding constants ($K_a$) of D1.3, Fv D1.3 and $V_H$D1.3 with immobilized HEL were determined with SPR by Borrebaeck et al. [17]. The published values of $K_a$ were respectively $> 10^{11}$, $5.9 \cdot 10^9$ and $0.1 \cdot 10^9 M^{-1}$.

The aim of this study is to test the hypothesis whether it is possible to predict potential synthetic antibody fragments which may bind the antigen based on tertiary structure information of a antibody–antigen complex. The D1.3–lysozyme interaction is used as a model system for the selection and synthesis of such fragments.

## 2. Materials and methods

### 2.1. Peptide synthesis

The peptides were synthesized according to the solid-phase method with 9-fluorenylmethoxycarbonyl (Fmoc) amino acids [18]. The in situ activation of the Fmoc-amino acids was carried out by means of the benzotriazol-1-yloxytris-(dimethylamino) phosphonium hexafluorophosphate (BOP) reagent of Castro et al. [19]. BOP was purchased from Richelieu Biotechnologies (St. Hyacinthe, Canada). The peptides were synthesized as amides by the use of 4-(α-Fmoc-amino-2′,4′-dimethoxybenzyl) phenoxyacetic acid as a linkage agent [20], obtained from Novabiochem (Bubendorf, Switzerland). The linker was attached to Pepsyn K, purchased from MilliGen Biosearch (Etten-Leur, Netherlands). The Fmoc-amino acids were purchased from MilliGen Biosearch and Senn Chemicals (Dielsdorf, Switzerland). N,N-Dimethylacetamide (DMA), diisopropylethylamine (DIPEA),

piperidine, trifluoroacetic acid (TFA), 1,2-ethanedithiol (EDT), thioanisole and phenol were obtained from Janssen (Geel, Belgium). Sidechain protection groups of the Fmoc-amino acids were: tert.-butyl for Asp, Glu, Ser, Thr and Tyr; tert.-butoxycarbonyl for Lys; Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl) for Arg; Trt (trityl) for Gln and His. DMA was distilled under reduced nitrogen pressure, DIPEA was distilled from ninhydrin and piperidin was distilled from KOH before use. The peptides were synthesized with a laboratory-built automated peptide synthesizer [21]. The continuous flow synthesis was monitored at 304 nm. Each coupling was performed in DMA with 4 equivalents of in situ activated Fmoc-amino acids for 45 min. The Fmoc protecting group was deprotected by piperidine–DMA (20:80) for 9 min. Finally, the N-terminus was blocked by coupling with DMA–acetic anhydride (50:50) for 15 min. The peptides were cleaved from the resin with reagent K [22] (TFA–phenol–water–thioanisole–EDT, 82.5:5:5:5:2.5), precipitated in diethyl ether and washed with ether for five times and finally the peptides were lyophilized. The purity of the peptides was confirmed by RP-HPLC and by amino acid analysis performed by Eurosequence (Groningen, Netherlands). Cysteine-containing peptides were oxidized by stirring a solution of 0.1 mg/ml peptide pH 8 for at least 12 h at room temperature. Cyclisation of the peptides was followed with RP-HPLC. The peptides were lyophilized from a diluted acetic acid solution, followed by desalting on Sephadex G-25.

### 2.2. Immunoaffinity chromatography

The CDR peptides were tested on binding lysozyme using two different column materials, activated CH-Sepharose 4B obtained from Pharmacia (Uppsala, Sweden) and Affigel-10 obtained from Bio-Rad Labs. (Richmond, CA, USA). Coupling of the peptides was performed basically according to the protocols of the manufacturers. Briefly, 2 mg peptide (peptides 1, 2 and 4) in 2 ml coupling buffer (0.1 $M$ NaHCO$_3$–Na$_2$CO$_3$, pH 8.2) were coupled to 0.5 g acti-

vated CH-Sepharose 4B during 2 h. The excess of unreacted active groups was blocked by 1 $M$ ethanolamine in coupling buffer. Peptides were coupled to 2 ml Affigel-10 (4.5 mg peptide 7) and 1 ml Affigel-10 (7.5 mg peptide 4) in 2 ml coupling buffer (peptide 7 in coupling buffer with 40% ethanol) for 16 h at 4°C. The excess of reactive groups was blocked by 1 $M$ ethanolamine in coupling buffer. Coupling percentages of the immobilized peptides were determined by comparison of peak heights in RP-HPLC of the coupling samples before and after coupling. The coupling percentages of the peptides were: peptide 1, >99%; peptide 2, 97%; peptide 4 to activated CH-Sepharose 4B 97%; peptide 4 to Affigel-10 75%; and peptide 7, 93%. Affinity chromatography was performed in columns of 1 cm diameter and a length of approximately 2 cm using an elution buffer of 0.05 $M$ NaSCN (Fluka, Buchs, Switzerland) in 0.02 $M$ Tris–HCl pH 7.4. A 1-ml volume of 1 mg/ml HEL (Boehringer Mannheim, Germany) was applied to the column at a flow-rate of 9 ml/h at room temperature. The flow-rate during chromatography was 18 ml/h. Regeneration of the columns was performed with 1 $M$ NaSCN in 0.02 $M$ Tris–HCl pH 7.4. The absorbance was measured at 278 nm. Chromatography was performed with a UV detection system from LKB (Bromma, Sweden), Model 2238 Uvicord SII and a Varioperpex II pump from LKB, Model 2120 and a recorder from LKB, Model 2210. Chemicals were obtained from Merck (Darmstadt, Germany) unless mentioned otherwise.

### 2.3. Surface plasmon resonance

SPR studies were carried out using the BIA-core system of Pharmacia Biosensor (Uppsala, Sweden). In all measurements a solution of 0.15 $M$ NaCl, 50 m$M$ Tris–HCl and 0.05% P20 (surfactant Tween 20 of Pharmacia) pH 8.0 was used as buffer solution with a flow-rate of 5 $\mu$l/min and a temperature of 25°C. Ligands were immobilized using standard procedures: (1) activation of the dextran layer by 35 $\mu$l 0.2 $M$ N-ethyl-N'-(dimethylaminopropyl) carbodiimide- –0.05 $M$ N-hydroxysuccinimide mixture (cou-

pling kit from manufacturer); (2) coupling of 35 $\mu$l 10 m$M$ sodium acetate (NaAc) ligand solution; (3) 50 $\mu$l 1 $M$ ethanolamine pH 8.5 (stock solution from manufacturer) and finally (4) regeneration with 15 $\mu$l 100 m$M$ phosphoric acid.

### Direct binding study

The CDR peptides of D1.3 and hLys6 (a humanized D1.3 antibody [23]) were immobilized at different dextran coated flow cells. The ligand solution of hLys6 was $2 \cdot 10^{-7}$ $M$, in 10 m$M$ NaAc pH 5.0 resulting in 13 023 resonance units (RU) coupling. The ligand solution of the peptides and the amount of immobilization in RU were: peptide 1 in 3 mg/ml water, 174 RU; peptide 2 in 0.1 mg/ml 10 m$M$ NaAc pH 4.0, 228 RU; peptide 3 in 1 mg/ml water, 234 RU; peptide 4 in 0.1 mg/ml 10 m$M$ NaAc pH 6.0, 412 RU; peptide 5 in 1 mg/ml dimethyl sulphoxide (DMSO)–water (5:95), 283 RU; peptide 6 in 1.6 mg/ml DMSO–10 m$M$ NaAc pH 6.0 (10:90), 160 RU and peptide 7 in 0.1 mg/ml DMSO–10 m$M$ NaAc pH 6.0 (1:99), 192 RU. Binding of the peptides was tested by injecting 20 $\mu$l 1 mg/ml HEL into the flowcells.

### Inhibition assay

HEL (10 ng/ml, $7.0 \cdot 10^{-10}$ $M$) was preincubated with a 0.1 mg/ml ($2.5 \cdot 10^{-5}$–$8.8 \cdot 10^{-5}$ $M$) peptide solution for 6 h. This lysozyme–peptide mixture was then injected into a 13 023 RU immobilized hLys6 channel. The same experiment was performed with peptide mixtures with a concentration of 0.1 mg/ml per peptide. Mixtures of H1H2D1.3 (peptide 6) with cyclic H3D1.3 (peptide 3), H1H2D1.3 (peptide 6) with H3D1.3 (peptide 2) and cyclic H2D1.3 (peptide 5) with H3D1.3 (peptide 2) were tested by this procedure. Chemicals were obtained from Merck unless mentioned otherwise.

## 3. Results and discussion

### 3.1. Affinity chromatography

Binding of lysozyme on immobilized CDR peptides of D1.3 was tested using two different

column materials, activated CH-Sepharose 4B and Affigel-10. The main difference between these two materials is the spacer attached to the solid phase. Activated CH-Sepharose 4B possesses a hydrophobic spacer and Affigel-10 a hydrophilic spacer. A blank column of Affigel-10 showed the least interaction with lysozyme (a very basic protein, p$I$ 11) of these two materials. The results obtained from affinity chromatography of HEL with the tested peptides which showed interaction, are given in Fig. 1. Lysozyme was most retarded on columns with peptide 4 (H2D1.3) and peptide 7 (H1H2D1.3) compared to a blank ethanolamine column. Peptides of CDR 3 (peptides 1, 2 and 3) of the heavy chain of D1.3 showed much less interaction with lysozyme, although the contribution in binding energy of the Fv D1.3–lysozyme complex is about three times larger than that of peptide 7 and about two times larger than that of peptide 4 (see Table 1). This can only be explained by another interaction than that present in the tertiary structure of lysozyme and D1.3. Peptide 1 H3D1.3 92–101 has also been
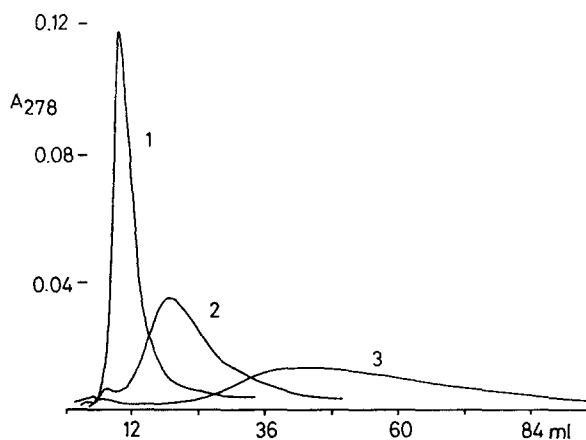


Fig. 1. Immunoaffinity chromatography of 1 ml 1 mg/ml hen egg white lysozyme. Lysozyme is eluted with 0.05 $M$ NaSCN, 0.02 $M$ Tris-HCl, pH 7.4 over peptides attached to Affigel-10 columns, flow-rate is 18 ml/h during elution. Column 1 is a 2-ml blank column (activated groups blocked by ethanolamine); column 2 is a 1-ml column of 5.6 mg/ml (3.3 m$M$) peptide 4 (H2D1.3 50–63); column 3 is a 2-ml column of 2.1 mg/ml (0.53 m$M$) peptide 7 (H1H2D1.3 28–63). The absorbance of lysozyme was measured at 278 nm at room temperature.

tested with cyanogen bromide-activated Sepharose 4B in immunoaffinity chromatography by Berry and Davies [13]. No binding of HEL could be detected.

## 3.2. Surface plasmon resonance

To prevent aspecific binding at a concentration of 1 mg/ml HEL with the dextran matrix the standard buffer in SPR studies with the BIAcore [10 m$M$ 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 0.15 $M$ NaCl, 3.4 m$M$ EDTA and 0.05% P20] had to be changed to a buffer solution containing 0.15 $M$ NaCl, 50 m$M$ Tris, 0.05% P20 pH 8.0. Direct binding studies of immobilized ligands with lysozyme were performed with 13 023 immobilized RU hLys6 as a positive control antibody (containing all 6 CDRs [23]), 174 RU peptide 1, 234 RU peptide 2, 228 RU peptide 3, 412 RU peptide 4, 283 RU peptide 5, 160 RU peptide 6 and 192 RU peptide 7. No binding curve was obtained after the injection of 20 $\mu$l 1 mg/ml lysozyme, therefore the immobilized CDR D1.3 peptides did not bind lysozyme. The humanized D1.3 antibody hLys6 did bind lysozyme as can be seen in Fig. 2. Kinetic parameters of the 13 023 RU immobilized hLys6 with lysozyme were determined from the binding curves: $k_{ass} = (4.7 \pm 0.2) \cdot 10^4$ $M^{-1}$ s$^{-1}$, $k_{diss} = (3.71 \pm 0.07) \cdot 10^{-3}$ s$^{-1}$ and $K_a = (1.26 \pm 0.05) \cdot 10^7$ $M^{-1}$.

Indirect binding of small ligands to proteins can be studied with the BIAcore instrument in an inhibition assay [24]. The analyte (lysozyme) is preincubated with a large excess of small ligand (synthetic peptide) before injecting the sample into the flowcell containing the immobilized antibody (hLys6). Only free, non-complexed analyte (lysozyme) will bind to immobilized hLys6, which can be seen as a binding curve. Disappearance of the binding curve at a large excess of small ligand (synthetic peptide) indicates interaction of peptide and lysozyme. The inhibition assays were performed on the 13 023 RU hLys6 channel. Preincubation of the CDR peptides (0.1 mg/ml, $2.5 \cdot 10^{-5}$–$8.8 \cdot 10^{-5}$ $M$) with lysozyme (10 ng/ml, $7.0 \cdot 10^{-10}$ $M$) and mixtures of the peptides (0.1 mg/ml) with
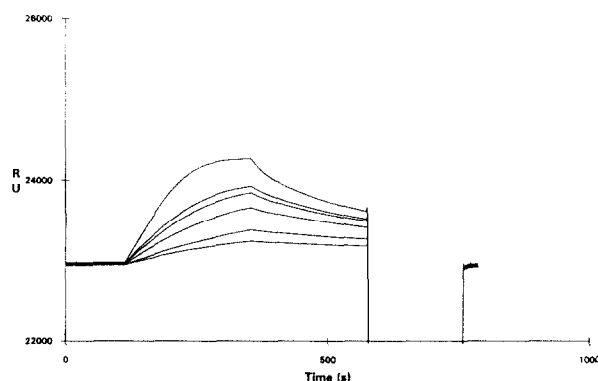
Fig. 2. Overlay plot of six different sensorgrams of different concentrations hen egg white lysozyme (0.2–2.0 μg/ml) with 13 023 RU immobilized hLys6 (a humanized D1.3 antibody [23]). Samples of 20 μl lysozyme solution in 50 mM Tris–HCl, 0.15 M NaCl, 0.05% surfactant P20 pH 8.0 were injected into the micro flow system with a flow-rate of 5 μl/min. The temperature was kept constant at 25°C during the measurements. The hLys6 flowcell was regenerated by injecting 15 μl 100 mM phosphoric acid. The association rate constant $k_{ass}$ was determined from the six binding curves of lysozyme with immobilized hLys6 using the BIAlogue software as described [15,16,23]. Briefly, $dR/dt$ versus $R$ was plotted for each concentration. The obtained slopes were plotted against the lysozyme concentration. The association rate constant, $k_{ass} = (4.7 \pm 0.6) \cdot 10^4$ $M^{-1}$ $s^{-1}$ ($r^2 = 0.995$), was obtained from the slope of this straight line. The dissociation rate constant $k_{diss}$ was obtained from datapoints of the dissociation (during the first 100 s) of bound lysozyme resulting from the injection of 20 μl lysozyme (2 μg/ml). A plot of ln $(R_0/R_t)$ versus $t_t - t_0$ resulted in a straight line, which was used to determine $k_{diss}$ [$R_0$ is signal in RU at the beginning ($t_0$) of dissociation; $R_t$ is signal in RU at time $t_t$]. The dissociation rate constant value $k_{diss} = (3.7 \pm 0.7) \cdot 10^{-3}$ $s^{-1}$ ($r^2 = 0.993$). The equilibrium constant $K_a$ was calculated from the rate constants according to $K_a = k_{ass}/k_{diss}$. The determined $K_a$ of the interaction of immobilized hLys6 with lysozyme is $(1.26 \pm 0.05) \cdot 10^7$ $M^{-1}$.

lysozyme for 6 h did not change the binding curve of lysozyme to hLys6. This is illustrated by one example of a mixture of cyclic H3D1.3 98–103 (peptide 3) and H1H2D1.3 28–63 (peptide 7) in Fig. 3. If the CDR peptides would have bound lysozyme specifically, then they should have blocked the antigen (lysozyme) at the contact residues, thereby disturbing the binding of lysozyme to immobilized hLys6, which should be seen as at least a less steeper binding curve or as no binding curve at all. Also, peptide mixtures of CDR peptides involving the three most im-
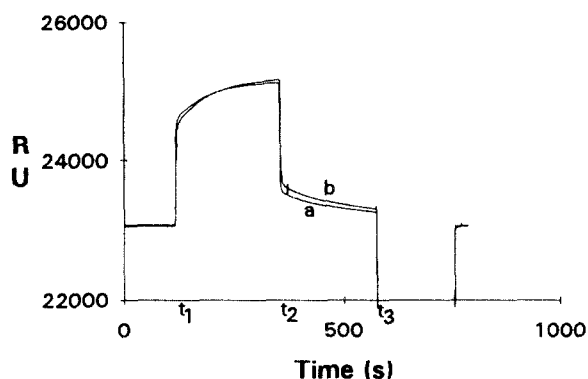


Fig. 3. Overlay plot of two sensorgrams of an inhibition assay of lysozyme with immobilized hLys6. Sensorgram a shows the binding curve of 20 μl 10 ng/ml ($0.7 \cdot 10^{-9}$ $M$) hen egg white lysozyme with 13 023 RU immobilized hLys6. Sensorgram b shows the binding curve obtained after incubation of 10 ng/ml ($7.0 \cdot 10^{-10}$ $M$) lysozyme with 0.1 mg/ml ($8.2 \cdot 10^{-5}$ $M$) peptide 3 (cyclic H3D1.3 98–103) and 0.1 mg/ml ($2.5 \cdot 10^{-5}$ $M$) peptide 7 (H1H2D1.3 28–63) for 6 h. The lysozyme samples of both sensorgrams contained 50 mM Tris–HCl, 0.15 M NaCl, 0.05% P20 pH 8.0 and 1% DMSO. The flow-rate during the measurements was 5 μl/min. Lysozyme (20 μl) was injected into the flowcell at $t_1$, dissociation of bound lysozyme started at $t_2$ followed by regeneration of the flowcell at $t_3$ with 15 μl 100 mM phosphoric acid. The large increase in signal at $t_1$ and large decrease in signal at $t_2$ was caused by the presence of 1% DMSO in the lysozyme sample, due to difference in refractive index of buffer and sample.

portant CDRs from the H chain should have inhibited binding of lysozyme to hLys6.

In BIAcore measurements it is very easy to reduce aspecific interaction of lysozyme to the dextran-coated gold layer. Raising the pH to 8 was sufficient to eliminate interaction of lysozyme with a blank channel. The solid phase in affinity chromatography mainly differs from the dextran layer of the sensorchip in the BIAcore instrument by the 10-atom spacer, which may partly cause aspecific interaction with lysozyme. The inhibition assay of lysozyme (measured with SPR) shows that the retardation of lysozyme shown in Fig. 1 is caused by other interaction than specific antibody–antigen interaction. However, such peptide columns might still be useful in the purification of lysozyme from a mixture of proteins.

The CDR peptides of the heavy chain of D1.3

do contain the most important contact residues for binding lysozyme. Nevertheless such peptides, even those containing several residues important for binding, did not bind lysozyme. Apparently it is not possible to reduce the $V_H$ D1.3 fragment (3 CDRs) to smaller fragments without losing specificity towards hen egg white lysozyme. The large surface complementarity of D1.3 with lysozyme is also a major factor in the specific interaction of lysozyme with D1.3. In general, this suggests that Fv fragments are capable of binding antigens and that $V_H$ or $V_L$ fragments may bind antigens, but that these fragments will lose specificity. In some cases CDR peptides [2–8] may bind antigens, probably with much less specificity than the antibodies. Some of the reported CDR peptides [4–7] are derived from anti-idiotype antibodies and those CDR peptides are homologous with the linear epitope of the antigen. Inhibition of the antibody by the CDR peptide can be accomplished by competition of the antibody between the antigen and the CDR peptide, and not by binding of the CDR peptide with the antigen [7,25].

## 4. Conclusions

So far, the minimal lysozyme-binding part of monoclonal antibody D1.3 is the $V_H$ region. Small synthetic fragments ($M = 1135$–3977) of the $V_H$ region are not able to bind lysozyme specifically. This shows the difficulty to predict from tertiary structure information of antibody–antigen complexes whether CDR peptides may bind antigens. It also shows the importance of surface complementarity in antibody–antigen complexes besides the direct contact area in antibody–antigen interaction.

## 5. Acknowledgements

## 6. References

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