Antigen recognition by conformational selection

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Received 11 February 1999; received in revised form 26 March 1999

Abstract Conformational adaptation between antigen and antibody can modulate the antibody specificity. The phenomenon has often been proposed to result from an 'induced fit', which implies that the binding reaction induces a conformational change in the antigen and the antibody. Thus, an 'induced fit' requires initial complex formation followed by a conformational change in the complex. However, an antibody may select those antigen molecules that happen to be in a fitting conformational state. This leads to the same end result as an induced fit. Here, we demonstrate conformational selection by a single chain antibody fragment, raised against a random coil variant of the leucine zipper domain of transcription factor GCN4, when it cross-reacts with the wild-type dimeric leucine zipper. Kinetic and equilibrium data show that the single chain antibody fragment fragment selects monomeric peptides from the population in equilibrium with the leucine zipper dimer.

Key words: Antigen-antibody recognition; Antibody specificity; Conformational selection; Induced fit; Binding kinetic; Transcription factor GCN4

1. Introduction

Structural differences observed between liganded and unliganded antibodies and antigens indicate that spatial adaptation occurs within an antigen-antibody complex [1,2]. As a result, antibodies often react with several related and sometimes even unrelated antigens, extending the more limited cross-reactivity that might occur between rigid epitopes and paratopes. The reaction of a protein reactive antibody with a peptide or of a peptide reactive antibody with a whole protein are examples thought to typify induced fit reactions. The term 'induced fit' was coined by Koshland to describe the induction of an active state in an enzyme by a substrate molecule [3]. In the context of antigen-antibody recognition, the term refers to improved shape complementarity based on flexible protein structures. An induced fit implies that the binding reaction itself induces conformational changes leading to a better complementarity between epitope and paratope, that is, an initial complex formation is followed by a conformational change in the complex.

Conformational selection is an alternative mechanism producing the same end result without the need for conformational changes during the binding reaction [4–6]. The antibody simply selects those antigen molecules whose epitope is already in a fitting conformational state [6] or, in the reciprocal case, the antigen selects the antibody which happens to be in the complementary conformation [4]. In general, an induced fit and conformational selection will both contribute to antigen recognition. This is most obvious in the binding of a random coil peptide to an antibody combining site. Of the many conformational states the peptide can assume, some may be similar to the bound state and be selected with a little requirement for structural adaptation if we ignore minor adjustments probably inherent in all protein-ligand complex formations. Other molecules may be bound while they are still in a 'wrong' conformation and may adapt to the antibody combining site by an induced fit. Here, we present an example of antigen recognition in which conformational selection dominates.

2. Materials and methods

2.1. Peptides and single chain antibody fragment (scFv) fragment

Sequences of peptides are shown in Table 1. Peptides general control of amino acid synthesis non-derepressible mutant 4 (GCN4)(7P14P) and GCN4-p1 [7] were chemically synthesized by the Fmoc strategy as in [6]. Peptides C62GCN4, $(CGCN4)_2$ and AAGCN4 were expressed in *Escherichia coli* as in [8,9]. The scFv fragment c11L34Ser was obtained by the ribosome display method [10,11] from a murine library and expressed in *E. coli* as in [11]. Peptide and scFv fragment concentrations were determined from the absorption at 280 nm in 6 M guanidine hydrochloride [12] using $\varepsilon_{280} = 1.280\ M^{-1}/cm$ for peptides and $\varepsilon_{280} = 51.590\ M^{-1}/cm$ for the scFv fragment. Peptide concentrations always refer to the total concentration of the peptide monomer, i.e. 1 μM GCN4-p1 is equivalent to 0.5 μM leucine zipper dimer. All experiments were performed in phosphate-buffered saline (PBS) (8.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.2).

2.2. Fluorescence and circular dichroism (CD) spectra

Fluorescence spectra were recorded at 25°C in 1 nm steps with a step time of 1 s (Spex Fluorolog spectrofluorimeter). The scFv fragment (0.2 or 0.5 μM in PBS) was placed in the cuvette and titrated with concentrated solutions of peptide antigen added in small aliquots. After each addition, the solution was stirred for 5 min before the emission spectrum from 310 to 400 nm (excitation at 295 nm) was measured and the buffer spectrum was subtracted. The fluorescence intensity change at the respective emission maximum, ΔF, was normalized to calculate the fractional degree of saturation $Y = \Delta F/\Delta F_{max}$, where ΔF_{max} is the fluorescence intensity change at saturation. CD spectra were recorded on a JASCO J-715 spectropolarimeter. Spectra were measured at 25°C at a scan rate of 2 nm/min, a step size of 0.2 nm and an optical pathlength of 0.2 mm.

2.3. Stopped-flow measurements

A SF-61 stopped-flow spectrofluorimeter (High Tech Scientific, Salisbury, UK) was used and the experiments were performed as in [13]. Equal volumes of scFv fragment (1 μM) and antigen (variable con-

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Abbreviations: AP-1, activation protein 1; CRE, cyclic AMP response element ATGACGTCAT; CD, circular dichroism; GCN4, general control of amino acid synthesis non-derepressible mutant 4; PBS, phosphate-buffered saline; scFv, single chain antibody fragment

PII: S0014-5793(99)00458-5

centrations) were mixed and the emission of the intrinsic Trp fluorescence of the scFv fragment was measured at 25°C (excitation at 295 nm, emission above 320 nm). Kinetic traces could be fitted to a mono-exponential decay characterized by the apparent rate constant k_{obs}. The second order rate constant of the reaction of GCN4(7P14P) with the scFv fragment was determined under pseudo-first order conditions (excess of antigen) from the slope of a plot of k_{obs} versus the total peptide concentration. Initial velocities of the reaction with GCN4-p1 and GCN4(7P14P), respectively, were calculated from the slopes of the first 10–20 ms of the kinetic traces and were expressed in arbitrary fluorescence units per unit of time. Since the dissociation of GCN4-p1 into monomers has a time constant of several seconds [14], the concentration of monomers did not change appreciably during the first few seconds of the stopped-flow experiment. Therefore, the monomer concentration in the stopped-flow cuvette immediately after mixing of antigen and antibody could be calculated from the initial concentration of GCN4-p1 using a dimer-monomer dissociation constant of 5×10^{-7} M [15].

3. Results

The scFv fragment c11L34Ser was selected from a ribosome display library originating from three mice that were immunized with peptide GCN4(7P14P) (Table 1, [11]). This 33 residue peptide corresponds to the C-terminal residues 249–281 of GCN4 except for an Asp/Pro substitution in position 255 and a Ser/Pro substitution in position 262 (Table 1). Because of the two prolines, the peptide is a random coil with a mass of 4030 Da ([6] and data not shown). In contrast, peptides C62GCN4 and GCN4-p1 (Table 1) form dimeric leucine zippers in the micromolar concentration range [16,17].

The intrinsic Trp fluorescence of the scFv fragment was quenched by binding to the antigen. Fig. 1 shows the titration curves for GCN4(7P14P), C62GCN4 and GCN4-p1. The curves were obtained at a scFv concentration that was far above the K_d value of 4×10^{-11} M for the original antigen GCN4(7P14P) [11] and thus indicate the stoichiometry of binding. The three curves are very similar, even though GCN4(7P14P) was monomeric and C62GCN4 and GCN4-p1 were mainly dimeric at the concentrations of the titration experiment. The binding stoichiometry was approximately one peptide chain per scFv fragment for all three antigens (intersection of dashed asymptotes in Fig. 1). Thus, in the case of GCN4-p1 and C62GCN4, either two scFv fragments were bound to the dimeric leucine zipper or one monomer was bound to one scFv fragment. In the first model, the antibody would have to react with an epitope that is accessible in both the dimeric leucine zippers and the monomeric random coil peptide GCN4(7P14P). In the second model, the antibody would have to induce the dissociation of the leucine zippers either during the binding reaction by an induced fit or by conformational selection of the small amount of unfolded monomer in equilibrium with the dimeric leucine zipper. In support for monomer-binding, we found that the disulfide-linked dimer $(CGCN4)_2$ (Table 1) was not recognized (Fig.

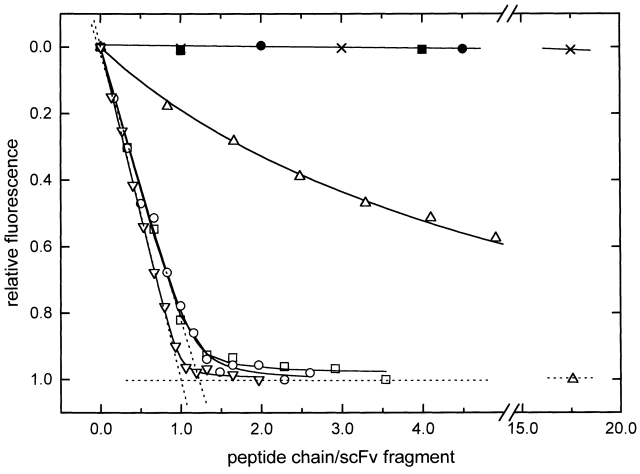

Fig. 1. Fluorescence titration of scFv fragment c11L34Ser with GCN4(7P14P) (○), GCN4-p1 (▽), C62GCN4 (□), AAGCN4 (△), $(CGCN4)_2$ (■), C62GCN4:DNA complex (●) and buffer (X). The point of intersection of the dotted asymptotes indicates a stoichiometry of binding of one monomeric peptide per scFv fragment. A 19 bp DNA duplex containing the CRE consensus sequence was used to prepare the C62GCN4:DNA complex [25].

Table 1
The sequence of peptide antigens, apparent dissociation constants of antibody complexes (K_d) and apparent rate constants (k_{obs}) of complex formation

Abbreviation	Sequence	K_d (M)	k_{obs} (s^{-1}) [c]
	220 240 260 280		
C62GCN4 [a]	MIVPESSDPAALKRARNTEAARRSRARKLQRMKQLEDKVEELLSKNYHLENEVARLKKLVGER	$<10^{-9}$ [b]	0.3
$(CGCN4)_2$	(----------GSGC)$_2$	no binding	no reaction
AAGCN4	----------A--A-------	$(8.2\pm1.1)\times10^{-7}$ [e]	16
GCN4-p1	Ac----------amide	$<10^{-9}$ [b]	1.8
GCN4(7P14P)	Ac------P------P----------amide	$<10^{-9}$ [b,d]	26

[a] N-terminal Met was introduced in the cloning procedure and does not belong to the GCN4 sequence [19,25].
[b] Upper estimate from fluorescence titration (Fig. 1).
[c] Complex formation from reaction of 0.5 μM scFv fragment with 4 μM peptide (Fig. 4). S.E.M.s are circa 5%.
[d] $K_d = 4\times10^{-11}$ M from competition BIAcore [11].
[e] Fitted from data in Fig. 1 as described in [9].

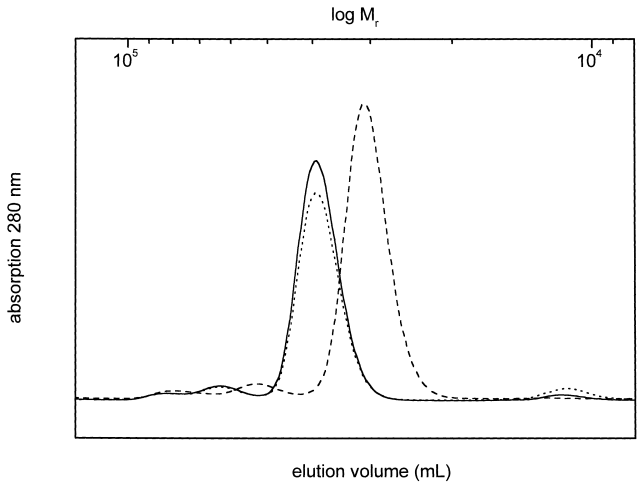

Fig. 2. Gel chromatography of the complex of scFv fragment c11L34Ser with GCN4(7P14P) (solid line) and with GCN4-p1 (dotted line) and of the scFv fragment alone (dashed line). Samples (50 μl) containing 9 μM each of the scFv fragment and the peptide antigen in PBS were chromatographed on a Superdex 75 PC 3.2/30 column (Pharmacia FPLC) at 25°C.

1). The coiled coil conformation of this peptide is very strongly stabilized through the disulfide bridge at the end of the helices (not shown).

C62GCN4, which encompasses the C-terminal residues 220–281 of GCN4, contains the basic DNA-binding region (residues 220–248) and the leucine zipper domain (residues 249–281). It binds to the activation protein 1 (AP-1) and cyclic AMP response element ATGACGTCAT (CRE) target DNA sites to form protein:DNA complexes whose crystal structures have been solved [18,19]. In the complex with DNA, the leucine zipper dimer is very strongly stabilized even though the leucine zipper domain is well separated from bound DNA [8]. Indeed, when the scFv fragment was titrated with the C62GCN4:CRE complex, no binding was observed, which again is in line with monomer-binding (Fig. 1).

Mutant peptide AAGCN4 has a destabilized leucine zipper domain because of Val/Ala and Leu/Ala substitutions in the hydrophobic core [8]. The peptide was $>50\%$ monomeric in the fluorescence titration experiment of Fig. 1 (K_d of the monomer-dimer equilibrium $>3\times10^{-5}$ M [8]). AAGCN4 bound less tightly than the original antigen or the cross-reacting dimeric peptides C62GCN4 and GCN4-p1 (Fig. 1). From this, we infer that the epitope for c11L34Ser may be located in the region of the Ala substitutions in positions 271 and 274 where the tight binding peptides GCN4(7P14P), C62GCN4 and GCN4-p1 have the same sequence (Table 1).

The gel chromatography experiment of Fig. 2 confirmed binding of the monomeric form of the leucine zipper peptide GCN4-p1. Antibody-bound GCN4-p1 eluted at the very same position as antibody-bound GCN4(7P14P). A complex of the scFv fragment with dimeric GCN4-p1 would have eluted with a 4 kDa higher mass.

Dissociation of a leucine zipper changes the far-UV CD spectrum from helical to random coil [17]. If GCN4-p1 is monomeric and non-helical when bound to the scFv fragment, its CD spectrum should be very similar to the CD spectrum of scFv-bound GCN4(7P14P). The spectrum of the bound peptide antigen could be calculated, to a first approximation, from the spectrum of the complex minus the spectrum of the free scFv fragment (Fig. 3). The difference spectra thus calculated for scFv-bound GCN4-p1 (spectrum 4) and for scFv-bound GCN4(7P14P) (spectrum 5, dotted) were identical within the error. In particular spectrum 4 of scFv-bound GCN4-p1 was devoid of minima at 208 and 222 nm, characteristic of a helical leucine zipper [17]. Furthermore, the spectrum of the complex with the original antigen, scFv:GCN4(7P14P) (spectrum 3, dotted), was identical within

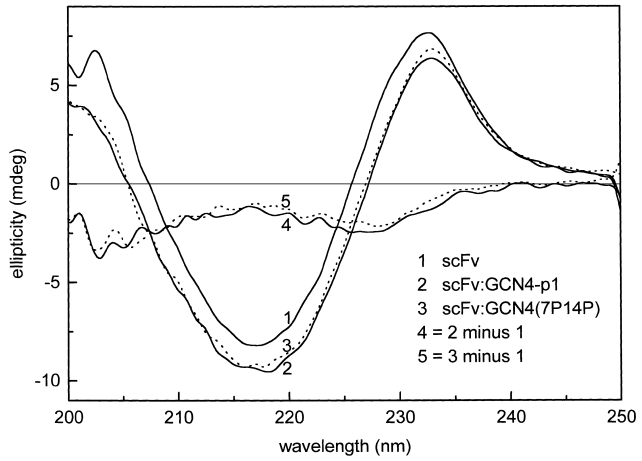

Fig. 3. Antibody-induced dissociation of leucine zipper demonstrated by CD spectroscopy. Spectra of scFv fragment c11L34Ser (spectrum 1) and of the complex scFv:GCN4-p1 (spectrum 2, solid line) were recorded and the spectrum of scFv-bound GCN4-p1 (spectrum 4, solid line) was calculated, as a first approximation, as spectrum 4 = spectrum 2−spectrum 1. In the same way, the spectrum of the scFv-bound original antigen GCN4(7P14P) (spectrum 5, dotted line) was calculated as spectrum 5 = spectrum 3−spectrum 1, where spectrum 3 (dotted line) belongs to the complex scFv:GCN4(7P14P). The very close similarity of spectra 4 and 5 and of spectra 2 and 3, respectively, indicates that GCN4-p1 was bound in the monomeric conformation. Concentrations of scFv fragment and peptides were 25 μM in PBS.

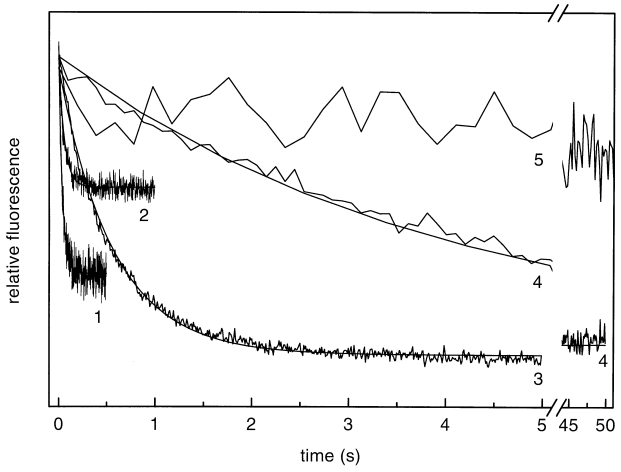

Fig. 4. Kinetic traces of the reaction of scFv fragment c11L34Ser with GCN4(7P14P) (trace 1), AAGCN4 (trace 2), GCN4-p1 (trace 3), C62GCN4 (trace 4) and $(CGCN4)_2$ (trace 5). Reactions were performed with 0.5 μM scFv fragment and 4 μM peptide at 25°C in PBS. Solid lines are best fits for a single exponential fluorescence decay. Apparent first order rate constants are given in Table 1.

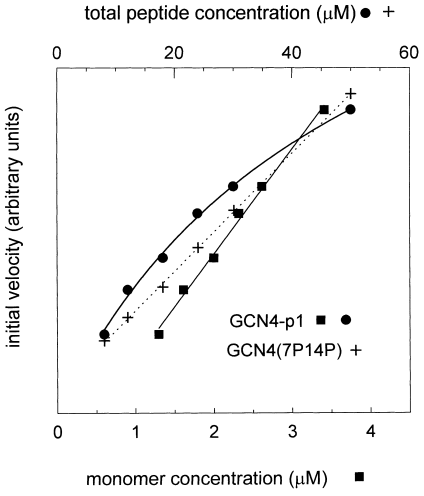

Fig. 5. The change of the initial velocity of the reaction of scFv fragment c11L34Ser with the total peptide concentration is non-linear (●, upper x-axis) for the cross-reacting antigen GCN4-p1. In contrast, the rate changes linearly with the monomer concentration calculated from $K_d = 5 \times 10^{-7}$ M for the monomer-dimer equilibrium [15] (■, lower x-axis). In the control reaction with GCN4(7P14P), the rate changes linearly with the total peptide concentration, which is the same as the monomer concentration since GCN4(7P14P) does not dimerize (+, upper axis). Conditions: 0.5 μM scFv fragment and varying concentrations of antigen in PBS, 25°C [15].

the error to the spectrum of the complex with the cross-reacting antigen, scFv:GCN4-p1 (spectrum 2).

The experiments described so far clearly show that the scFv fragment c11L34Ser binds the monomeric peptide. To demonstrate that the cross-reaction with the dimeric leucine zipper resulted from conformational selection of the monomer rather than from an induced fit within a complex between the antibody and the leucine zipper dimer, we performed the kinetic experiments of Fig. 4 and 5. Antigen recognition by conformational selection will appear to be slow if the antibody selects a minor conformer in equilibrium with a large excess of a major conformer of the antigen because only a fraction of the total antigen will react at any one time [6,20]. The apparent rate constant will thus be the true rate constant of the reactive conformer times the fraction of the reactive conformer. Therefore, the reaction with the cross-reacting leucine zipper dimer will appear to be slower than with the original monomeric antigen GCN4(7P14P), even if the underlying true rate constants are the same. Put differently, the rate of reaction with a predominantly dimeric peptide will show a non-linear dependence on the total peptide concentration, yet a linear dependence on the monomer concentration at equilibrium with the dimer. This would not be the case for a true induced fit mechanism, which should be biphasic with the first phase corresponding to a bimolecular association reaction whose rate depends linearly on the total peptide concentration and the second phase, a concentration-independent conformational rearrangement.

We first compared the rates for the original and the cross-reacting antigens (Fig. 4). Fluorescence changes could be described by single exponential decays. Apparent first order rate constants, k_{obs}, are given in Table 1. The second order rate constant for the original antigen GCN4(7P14P) was $(4.3 \pm 0.4) \times 10^6$ M^{-1}/s, a typical value for an antigen-antibody reaction with a small antigen [21,22]. Predominantly monomeric peptide AAGCN4 reacted almost as fast as monomeric GCN4(7P14P). In contrast, predominantly dimeric peptides GCN4-p1 and C62GCN4 reacted considerably slower.

Fig. 5 shows the change of the initial reaction rate for GCN4-p1 as a function of the concentration of total peptide, which is non-linear and levels off (circles). In contrast, when the initial velocities are plotted against the monomer concentration at equilibrium with the dimer (calculated from the known K_d for the leucine zipper), the plot is linear (squares). This is the behavior predicted for conformational selection of monomeric peptides, against which the antibody was raised. In the control experiment with GCN4(7P14P), rates increased linearly with the total peptide concentration (crosses), which in this case was equivalent to the monomer concentration, as GCN4(7P14P) does not dimerize.

4. Discussion

Antibody-induced conformational changes have long been known (e.g. [1,23,24]). As an explanation, the induced fit paragon is ready at hand but very difficult to demonstrate by an actual experiment because one would have to sample structural information over the course of the binding reaction. A conformational selection pathway (or an 'induced fit by conformational selection' [6]) is more easily detected and it is surprising that only very few studies have addressed this alternative mechanism [4,6,20].

To adjust their conformation in the complex, antigen and antibody have to overcome a free energy difference irrespective of whether the conformational change results from an induced fit or conformational selection. The difference separates the conformation of the antigen and the antibody in the complex from the conformation of the free molecules. For the present discussion, we need only to consider the free energy difference between bound and free antigen. It is the energy to dissociate the leucine zipper dimer which has to be traded against the energy gained by binding of the monomer to the antibody. The free energy of dissociation, $\Delta G°(25°C)$, is approximately 40 kJ/mol for C62GCN4 (T. Hitz, C.B. and H.R.B., unpublished) and about 36 kJ/mol for GCN4-p1 [15]. The complex of scFv fragment c11L34Ser with GCN4(7P14P) has $\Delta G°(25°C) = -59$ kJ/mol [11]. Assuming that $\Delta G°$ of interaction of the scFv fragment is the same for the original antigen GCN4(7P14P) and the cross-reacting monomeric GCN4-p1 peptide, the antigen-antibody complex will be favored by −23 kJ/mol over the leucine zipper dimer. This large free energy difference accounts for strong binding of the cross-reacting peptides and explains why the three titration curves of Fig. 1 are very similar.

The present work shows how a conformational selection mechanism can lead to a seemingly very large 'deformation' of the antigen. Why is conformational selection favored over an induced fit? In the simplest case, monomer-binding by an induced fit consists of two steps:

$$\mathrm{Lz} + \mathrm{scFv} \overset{1}{\rightleftarrows} \mathrm{Lz:scFv} \overset{2}{\longrightarrow} \mathrm{M:scFv} + \mathrm{M}$$

In step 1, the antibody transiently binds to the dimeric leucine zipper (Lz). The induced fit takes place afterwards in the Lz:scFv complex during step 2 in which one monomer (M) is released and the other adapts from its helical state to the tightly-bound conformation. Since the scFv fragment's

epitope is formed only in the monomeric peptide chain, complex Lz:scFv will be very weak and short-lived, that is, equilibrium 1 will be far on the left side. Experimentally, an interaction of the antibody with the coiled coil state is not detectable (disulfide-linked $(CGCN4)_2$ in Fig. 1) and must therefore be several orders of magnitude worse than with the monomeric random coil peptide. We believe this is the main reason why the reaction does not follow an induced fit pathway in which dissociation of the leucine zipper dimer takes place within the initial complex Lz:scFv.

Since the selected monomeric peptide is a random coil that exists in a vast number of conformations, binding of the monomer may involve both induced fit and conformational selection and the relative contribution of each will be kinetically controlled. Conformational selection will dominate if the initial complexes between 'wrong' conformations of the monomer are weak and short-lived and if the monomer easily adopts the bound conformation while it is free in solution. In contrast, an induced fit will be favored if the initial complexes with the 'wrong' conformations are relatively stable and long-lived and if the bound conformation is negligibly populated in the free monomer.

In conclusion, the present experiments demonstrate that the scFv antibody c11L34Ser selects among the monomeric and dimeric states of the cross-reacting leucine zipper antigen. After selection, an induced fit may contribute to the adaptation of the monomer to the antibody combining site.

Since the scFv fragment c11L34Ser forces the transcription factor GCN4 to dissociate into monomers, it will be interesting to see whether this antibody can interfere with the regulatory function of intact GCN4.

Acknowledgements: We thank Antonio Baici for letting us use his stopped-flow instrument and Stefan Klauser for the help with chemical peptide synthesis. This work was supported in part by the Swiss National Science Foundation (Grants 3100-45556.95 to H.R.B. and 3100-056624.96 to A.P.).

References

[1] Davies, D.R. and Cohen, G.H. (1996) Proc. Natl. Acad. Sci. USA 93, 7–12.
[2] Wilson, I.A. and Stanfield, R.L. (1994) Curr. Opin. Struct. Biol. 4, 857–867.
[3] Koshland Jr., D.E. (1958) Proc. Natl. Acad. Sci. USA 44, 98–104.
[4] Foote, J. and Milstein, C. (1994) Proc. Natl. Acad. Sci. USA 91, 10370–10374.
[5] Van Regenmortel, M.H.V. (1995) Biomed. Pept. Prot. Nucleic Acids 1, 109–116.
[6] Leder, L., Berger, C., Bornhauser, S., Wendt, H., Ackermann, F., Jelesarov, I. and Bosshard, H.R. (1995) Biochemistry 34, 16509–16518.
[7] O'Shea, E.K., Klemm, J.D., Kim, P.S. and Alber, T. (1991) Science 254, 539–544.
[8] Berger, C., Piubelli, L., Haditsch, U. and Bosshard, H.R. (1998) FEBS Lett. 425, 14–18.
[9] Weber-Bornhauser, S., Eggenberger, J., Jelesarov, I., Bernard, A., Berger, C. and Bosshard, H.R. (1998) Biochemistry 37, 13011–13020.
[10] Hanes, J. and Plückthun, A. (1997) Proc. Natl. Acad. Sci. USA 94, 4937–4942.
[11] Hanes, J., Jermutus, L., Weber-Bornhauser, S., Bosshard, H.R. and Plückthun, A. (1998) Proc. Natl. Acad. Sci. USA 95, 14130–14135.
[12] Gill, S.C. and von Hippel, P. (1989) Anal. Biochem. 182, 319–326.
[13] Wendt, H., Leder, L., Härmä, H., Jelesarov, I., Baici, A. and Bosshard, H.R. (1997) Biochemistry 36, 204–213.
[14] Zitzewitz, J.A., Bilsel, O., Luo, J.B., Jones, B.E. and Matthews, C.R. (1995) Biochemistry 34, 12812–12819.
[15] Wendt, H., Baici, A. and Bosshard, H.R. (1994) J. Am. Chem. Soc. 116, 6973–6974.
[16] Weiss, M.A. (1990) Biochemistry 29, 8020–8024.
[17] O'Shea, E.K., Rutkowski, R. and Kim, P.S. (1989) Science 243, 538–542.
[18] Ellenberger, T.E., Brandl, C.J., Struhl, K. and Harrison, S.C. (1992) Cell 71, 1223–1237.
[19] König, P. and Richmond, T.J. (1993) J. Mol. Biol. 233, 139–154.
[20] Zeder-Lutz, G., Van Regenmortel, M.H.V., Wenger, R. and Altschuh, D. (1994) J. Chromatogr. B. Biomed. Appl. 662, 301–306.
[21] Mason, D.W. and Williams, A.F. (1986) in: Handbook of Experimental Immunology (Weir, D.M., Herzenberg, L.A., Blackwell, C. and Herzenberg, L.A., Eds.), Vol. 1, pp. 38.1–38.17, Blackwell Scientific, Oxford.
[22] Raman, C.S., Jemmerson, R., Nall, B.T. and Allen, M.J. (1992) Biochemistry 31, 10370–10379.
[23] Crumpton, M.J. (1966) Biochem. J. 100, 223–232.
[24] Schechter, B., Conway-Jacobs, A. and Sela, M. (1971) Eur. J. Biochem. 20, 321–324.
[25] Berger, C., Jelesarov, I. and Bosshard, H.R. (1996) Biochemistry 35, 14984–14991.