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Volume 2

Samples and Reagents

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4.5.2 Catalytic Antibodies

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The idea of generating an enzyme for any desired chemical reaction is certainly intriguing. The use of antibodies for this purpose is especially tempting, since antibodies can be made against almost any compound. This chapter summarizes some of the key techniques and strategies. While impressive progress is being made in this field, the chapter should also give an impression of the magnitude of the problems, and some guidance to areas where such an approach may be worthwhile. It should become clear that this field is still in its infancy and thus a realm of basic research. Thus, concepts are stressed at the expense of detailed methodology, which is bound to change rapidly.

This chapter first summarizes some of the physical chemistry behind the idea of using antibodies for catalysis. Next, the question of whether the antibody structure is really suitable to function as an enzyme is discussed. Today, the best approach to catalytic antibodies still leads through "classic" hybridoma technology. Thus, the most important of all questions is the design of the immunogen. Immunization and antibody production do not differ significantly from the production of other monoclonal anti-hapten antibodies. However, since few catalytic antibodies have reached rate accelerations typical of enzymes, there is great interest in modifying the initial antibody further, and recent progress in antibody engineering is summarized. In this context, some techniques allowing screening and also the circumvention of hybridomas are discussed. The interdisciplinary approach necessary in this field will become apparent throughout.

4.5.2.1 The Catalytic Antibody Concept

To understand the concept of using antibodies for catalysis requires first some consideration of enzymatic reaction mechanisms. The active site of enzymes, much like antibodies, specifically accommodates the substrate. One of the key differences from antibodies is that the structural complementarity of an enzyme is not directed against the substrate but against the transition state of the reaction. This concept was first introduced by *Haldane* [1] and elaborated by *Pauling* [2], who pointed out that part of the intrinsic binding energy of the substrate can be converted to bringing the substrate closer to the transition state, thereby lowering the activation barrier. A detailed discussion of these problems can be found in *Jencks* [3].

There are three important consequences to this theory. First, it predicts that there are compounds that should bind better to enzymes than the substrate itself, by being structural analogues of the transition state. This has been borne out by many experiments for a large variety of reactions [4]. Second, the three-dimensional structure of enzymes should show this structural complementarity directly. However, only in a few cases has it been possible to demonstrate this by crystallography [5]. The third

consequence of this idea is that it might be possible to turn it around. It was *Jencks* [6], who first proposed that one might generate antibodies against analogues of the transition state for a particular reaction, and that these antibodies might then be catalytic. The main problem is therefore reduced to designing the proper immunogen. This is the basis of the catalytic antibody concept.

However, a closer examination of enzymatic reactions reveals a multitude of factors contributing to enzymatic rate enhancements. Their relative contributions obviously depend on the reaction to be catalysed. Table 1 lists a number of these mechanistic factors. Enzymes combine these effects, as each one may by itself contribute only a moderate rate enhancement. This point may be illustrated in the protease subtilisin (EC 3.4.21.14), a serine protease [7, 8]. If the catalytic triad His-64, Ser-221 and Asp-32 are all replaced by Ala residues, the reaction catalyzed by this mutant enzyme is almost 6 orders of magnitude slower than that catalysed by the wild-type enzyme, but is still 2700-fold faster than the reaction in solution, a rate acceleration in the range typical of catalytic antibodies. The remaining contribution of the mutant enzyme to catalysis probably comes from the enzyme's structural complementarity to the transition state. The relevant transition state can be expected to be structurally similar to the first tetrahedral intermediate, produced after nucleophilic attack of the amide carbonyl by the serine oxygen.

Table 1. Catalytic factors contributing to enzyme catalysis

– Desolvation of substrates, increase of nucleophilicity
– Binding of two substrates in close proximity
– Binding of two substrates at productive angles
– Use of cofactors
– Use of nucleophiles (change of reaction mechanism)
– Use of general acid/base catalysts
– Selective polarization of reactive bonds
– Use of intrinsic binding energy to stabilize reactive conformations

4.5.2.2 Antibody Structure

The antibody protein has, as such, none of the desirable features of a potential enzyme other than a binding site. However, it also has no particularly undesirable features. The entire reason for using antibodies in catalysis is that they represent a system of very large diversity. Nature has apparently found a way to use one particular folding topology to bind almost all antigens with relatively few alterations in a limited part of the structure. Currently, there is no other machinery that provides a specific binding site on demand, and for such a wide range of molecules. In the evolution of enzymes, many different frameworks have been used for catalysis, probably generated entirely by random

selection. The antibodies are a modular design: by selecting appropriate V-, (D-) and J-segments in both chains, followed by somatic mutation in the animal, they provide a *rapid* engineering kit for binding foreign molecules tightly.

The combining site of the antibody is made up from six hypervariable complementarity-determining regions (CDR) arranged in loops, three on each variable domain, connecting the framework of a β-barrel [9, 10]. The conformations of the loops have been tabulated and used for the prediction of loops of antibodies with unknown structures [11]. Certain key residues have been identified [12], and the length of the loops has been recognized as the most important single variable in determining the conformation. However, there are definitely many poorly understood determinants of the final conformation. Tabulations [13, 14] have pointed out an unusual concentration of exposed aromatic residues provided by the antigen-binding loops. These aromatic groups (especially tyrosine) can make hydrogen bonds *and* be used in hydrophobic or stacking interactions or typical aromatic-aromatic interactions. Probably for this reason they were found to be useful by nature in binding to a wide variety of antigens. The researcher interested in catalytic antibodies should remember the compositional bias of the antibody's combining site, caused by the sequences encoded in the animal's genome. Nucleophiles and acid/base catalysts will therefore occur merely by accident. However, recent advances in antibody engineering (see below) now provide the tools to introduce other types of residues where they are needed. The problem is then transformed to one of structural prediction: how does the substrate bind to the antibody, and where should catalytic groups be positioned?

The question has often been posed as to whether the antibody has the molecular properties required of an efficient enzyme. There is probably no general answer to this question because there are no general structural requirements for "an enzyme". The particular arrangements of active sites vary widely. The β-barrel architecture of antibodies is used in other proteins as well, exemplified by the enzyme superoxide dismutase [15]. Clearly, many enzymes have evolved to accommodate conformational changes, and multistep reactions will possibly require this ability. While the combining sites of antibodies may also undergo conformational changes [16] upon antigen binding or in catalysis, this cannot yet be predetermined by a particular design of the antigen or the antibody, as the structural understanding of these phenomena is lacking.

4.5.2.3 Design of the Immunogen

Currently, the design of the immunogen is the key consideration in the field of catalytic antibodies. While protein engineering and random mutagenesis are going to play an ever-increasing role, the first access to the "lead structure" of a catalytic antibody is given by the immunogen.

Some general considerations will first be discussed. Usually, the substrate will be a small molecule. The immunogen itself will therefore not be immunogenic, but must be coupled to an immunogenic protein via a linker, just like any hapten. The common linkers and techniques for immunogen preparation are discussed 3.1. Obviously, the

attachment point of the linker on the substrate determines the orientation of the substrate molecule within the binding site; the linker will always point away from the antibody in the immunogenic complex.

The immunogen must not kill the animal, nor should it decompose too rapidly. It is useful first to determine the stability of the immunogen in serum. In the usual process of monoclonal antibody production, cell fusions are carried out 2 weeks after immunization, and the immunogen probably should largely survive this prolonged incubation at the animal's body temperature, although the exact lifetime requirements are not known.

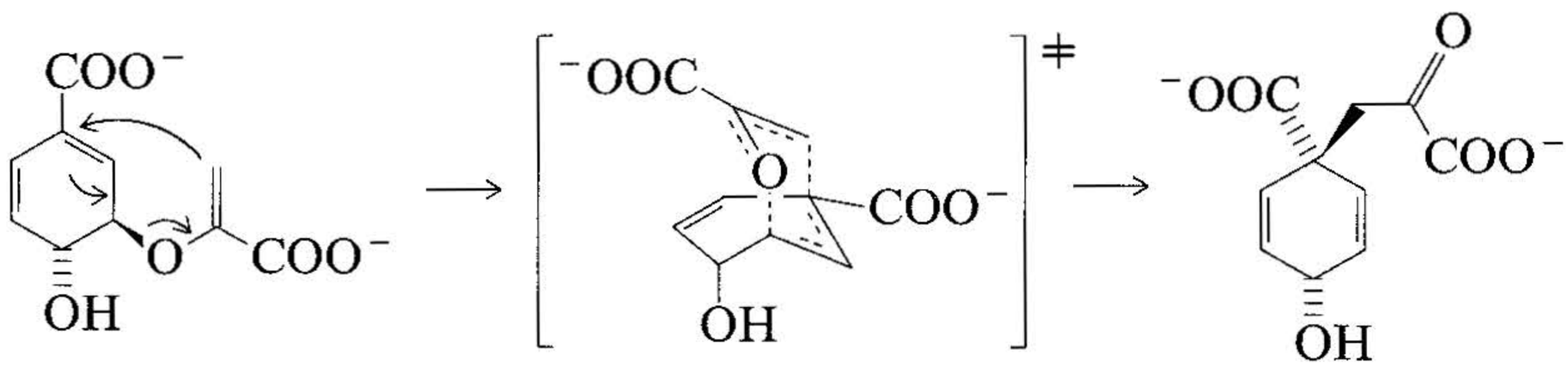
The design of the immunogen should not demand too ambitious an organic synthesis; it is often the synthetic accessibility which limits the types of molecules and reactions that can be investigated with reasonable effort. While not much of the immunogen is needed to elicit an immune response, the analogue must be available for screening of binding, usually in an ELISA format, since the initial screening for catalytic antibodies is usually carried out by assaying merely for binding of the immunogen.

Instead of compiling an extensive list of reported reactions and immunogens (which is available e.g. in [17–19]), a few strategies for the design of immunogens will be discussed in some detail to illustrate the principles.

Structural complementarity to the transition state

The reaction catalysed was that of the enzyme chorismate mutase, EC 5.4.99.5 (Fig. 1a). The immunogen was a stable analogue of the presumed chair-like transition state of this *Claisen* rearrangement [20, 21]. In this case, the antibody probably does not

A



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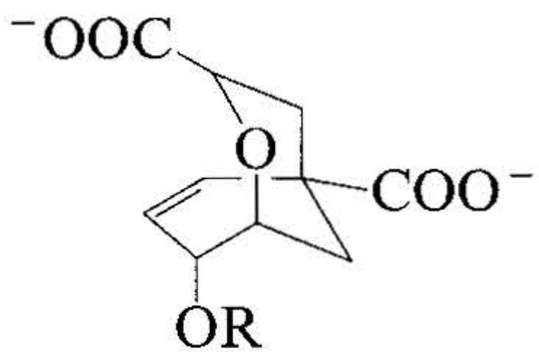

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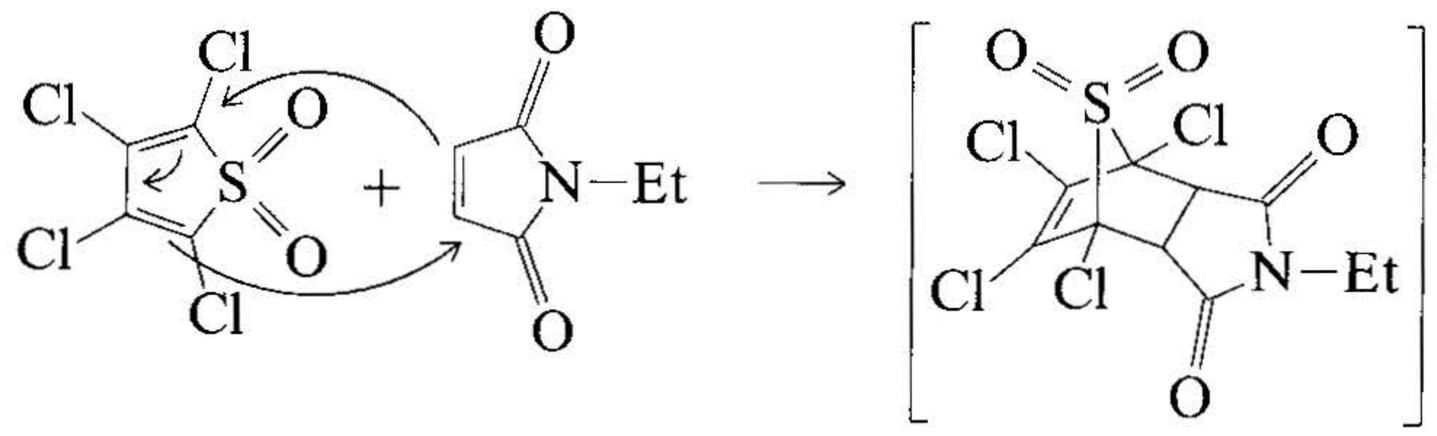
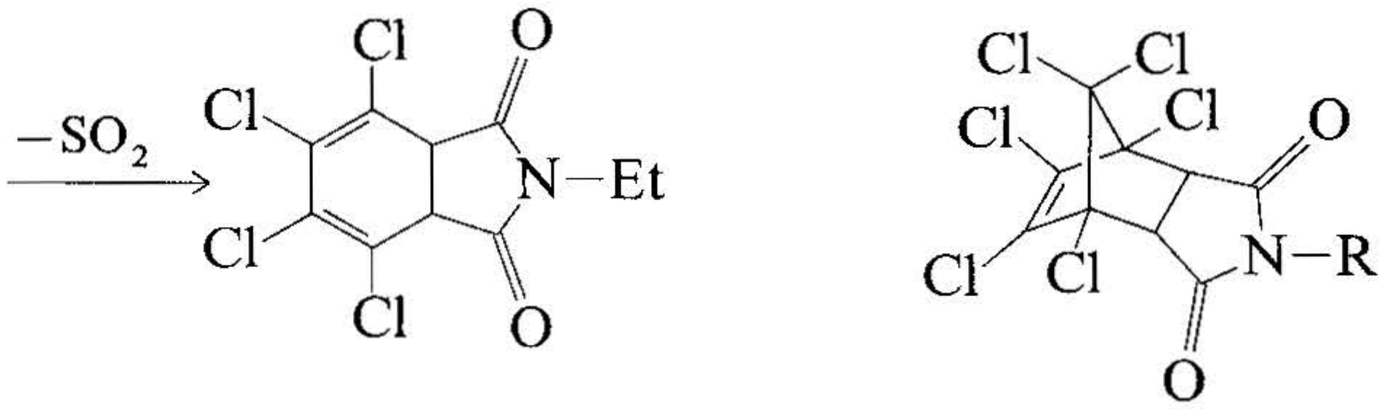
Fig. 1. A) Chorismate mutase reaction (*Claisen* rearrangement), Chorismate (1) is converted to prephenate (3) *via* a chair-like transition state (2). The transition state analogue (4) was used as the immunogen.

B



5 **6** **7**

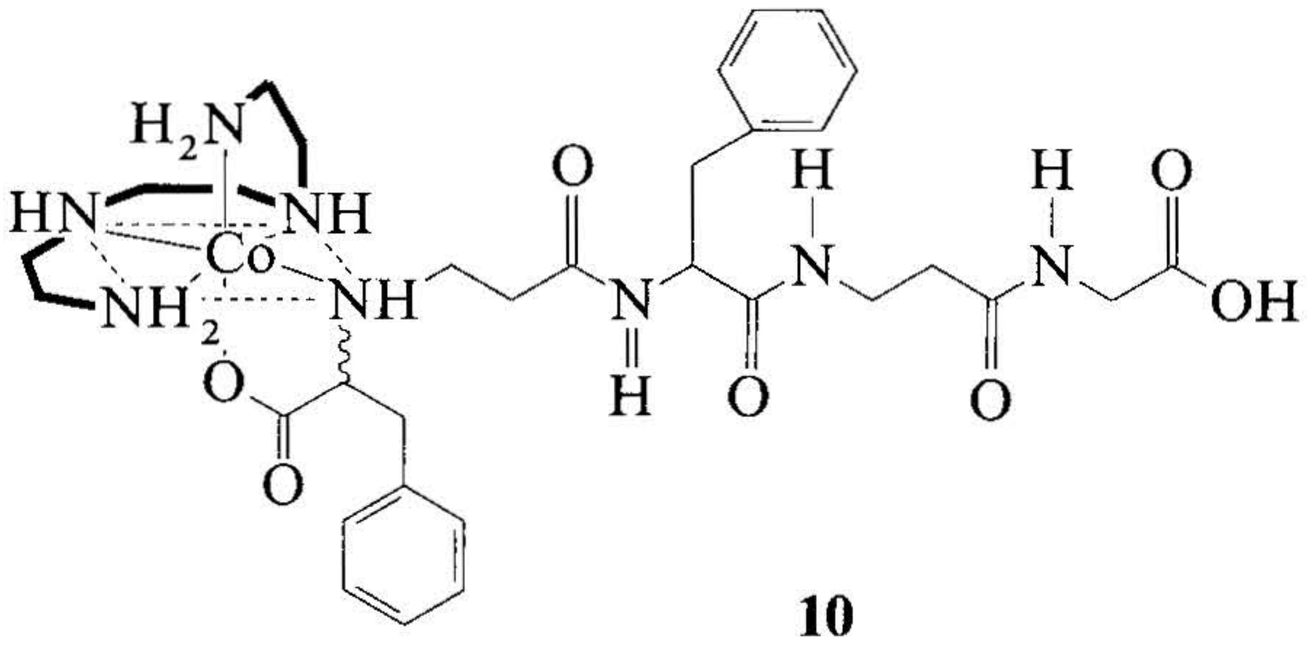


8 **9**

B) *Diels-Alder* reaction. The diene (5) and the dienophile (6) react to form the adduct (7), which decomposes to (8) under SO_2 loss. The immunogen (9) is structurally analogous to the first adduct (7).

C phenylpyruvate β-ala phe β-ala gly

a



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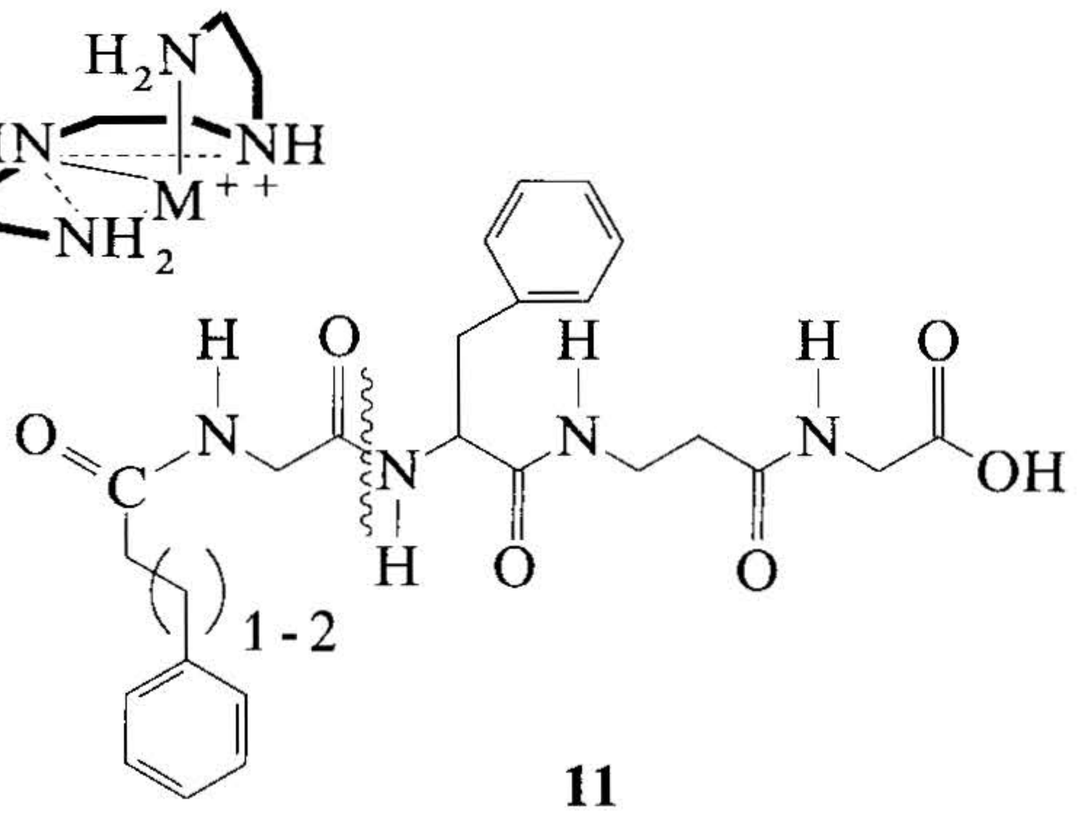
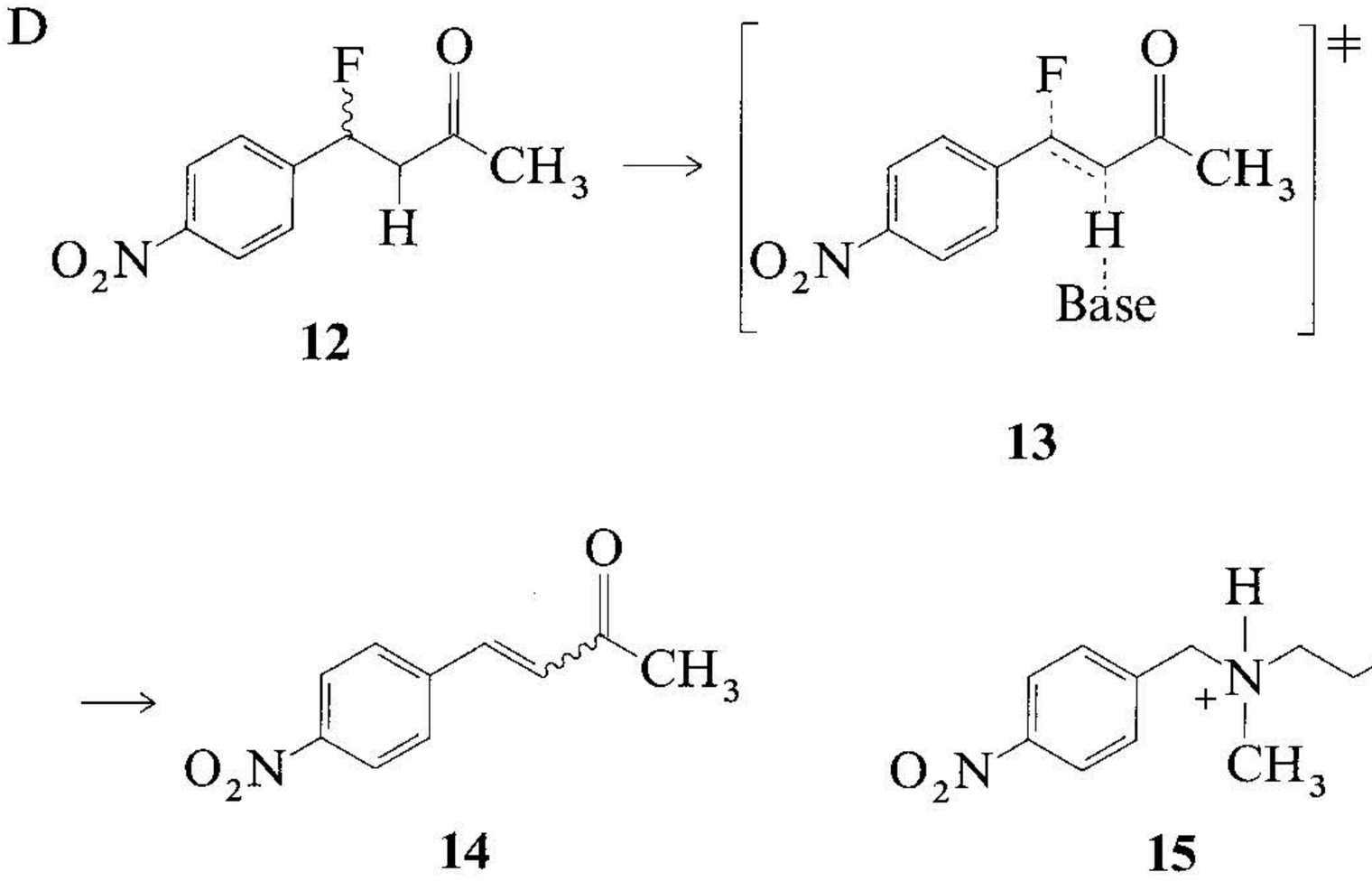
b



11

Active:	Inactive:
Mg	Sc
Mn	La
Fe	Co
Ni	Pd
Cu	Cd
Zn (best)	Hg
Ga	Tb
In	Yb



D) Elimination reaction. The ketone (12) undergoes an HF elimination to give (14), facilitated by a base in the antibody (13). The immunogen (15) contains a positive charge at the position where a base is to be selected for in the antibody.

use acid/base catalysis or nucleophilic catalysis, but works by bringing the reactant into the conformation required for the rearrangement, at the expense of intrinsic binding energy.

Avoiding product inhibition

Any bimolecular synthetic reaction suffers from the intrinsic problem of product inhibition, as the larger product may have far more binding interactions with the antibody than either of the reactants. This severely constrains the type of biosynthetic reactions that are accessible. One solution is exemplified in a *Diels-Alder* reaction (Fig. 1b) [22], in which the initial product is designed to undergo a further elimination to lower the energy of binding to the antibody, since the molecular shape of the secondary product changes significantly.

Recruiting a cofactor

The energetically demanding hydrolysis of a peptide bond was achieved by designing an immunogen containing a complex of a transition metal (Fig. 1c) [23]. The reactants then

C) Peptide cleavage reaction. a) The oligopeptide (10), containing a reduced *Schiff*'s base to phenylpyruvate, serves, together with triethylenetetramine, as the ligand for a cobalt ion. This complex is very stable and was used as the immunogen. b) The actual substrate (11) was cleaved where indicated, when incubated with the antibody and triethylenetetramine and any of the metal ions indicated as active. c) Possible mechanisms of the metal ion in hydrolysis. On the left, it functions as a *Lewis* acid, on the right as a *Bronstedt* base.

used for the kinetic measurements were the peptide substrate and the metal-chelate as separate molecules, which should both be bound by the antibody with relative orientations such that the reaction can take place. Interestingly, the peptide bond next to the one expected from the design was cleaved.

Eliciting a functional group

In an antibody-catalysed elimination reaction, the participation of a basic residue in the antibody combining site was elicited by introducing a positive charge in the immunogen next to the place where the base was desired (Fig. 1d) [24]. The functional group thus elicited in the antibody is probably a carboxylate.

These examples are meant only to be illustrative and may be a guide in the design of immunogens for the particular reaction desired by clarifying some of the design principles available.

4.5.2.4 Generating the Monoclonal Antibody: Classical Approaches and Possible Alternatives

Currently, the "lead structure", e.g., the first antibody showing potential for catalysis or even only the correct binding specificity, must be obtained after immunization of an animal. Exploitation of the powers of the immune system is still, by far, the most efficient first step of screening, i.e. selecting from an enormous repertoire a small number of antibody molecules potentially able to interact tightly with the transition state analogue and the substrate. No particular deviation from established procedures for the generation of monoclonal antibodies (cf. 4.3) is necessary, just because the antibodies might prove to be catalytic.

There are many reasons why, after having successfully elicited a catalytic antibody, genetic manipulations of the antibody itself might still be needed. One reason is that the catalytic rate enhancement may be unsatisfactory. Two principal strategies for cloning the antibody genes are available. The well established procedures for the production of monoclonal antibodies may be followed by amplification of the genes by the polymerase chain reaction (PCR) and cloning. Alternatively, the steps of the procedure may be reversed (Fig. 2), and a PCR amplification of the total antibody mRNA carried out [25, 26] and this mixture screened by a variety of methods after expression in bacteria or phages (see below). It should be stressed that this screening can be successful because the newly elicited antibody mRNA is a very significant fraction of all antibody mRNA (probably a few percent) *after the immunization:* thus, the desired antibody can be found by screening of a manageable number of clones. There is currently no strategy that avoids the immunization of an animal, although eventually such approaches may be developed.

This screening for binding can be done in phage plaques, in colonies, or on the surface of phages, and the strategies are not unique to catalytic antibodies. The basis for these

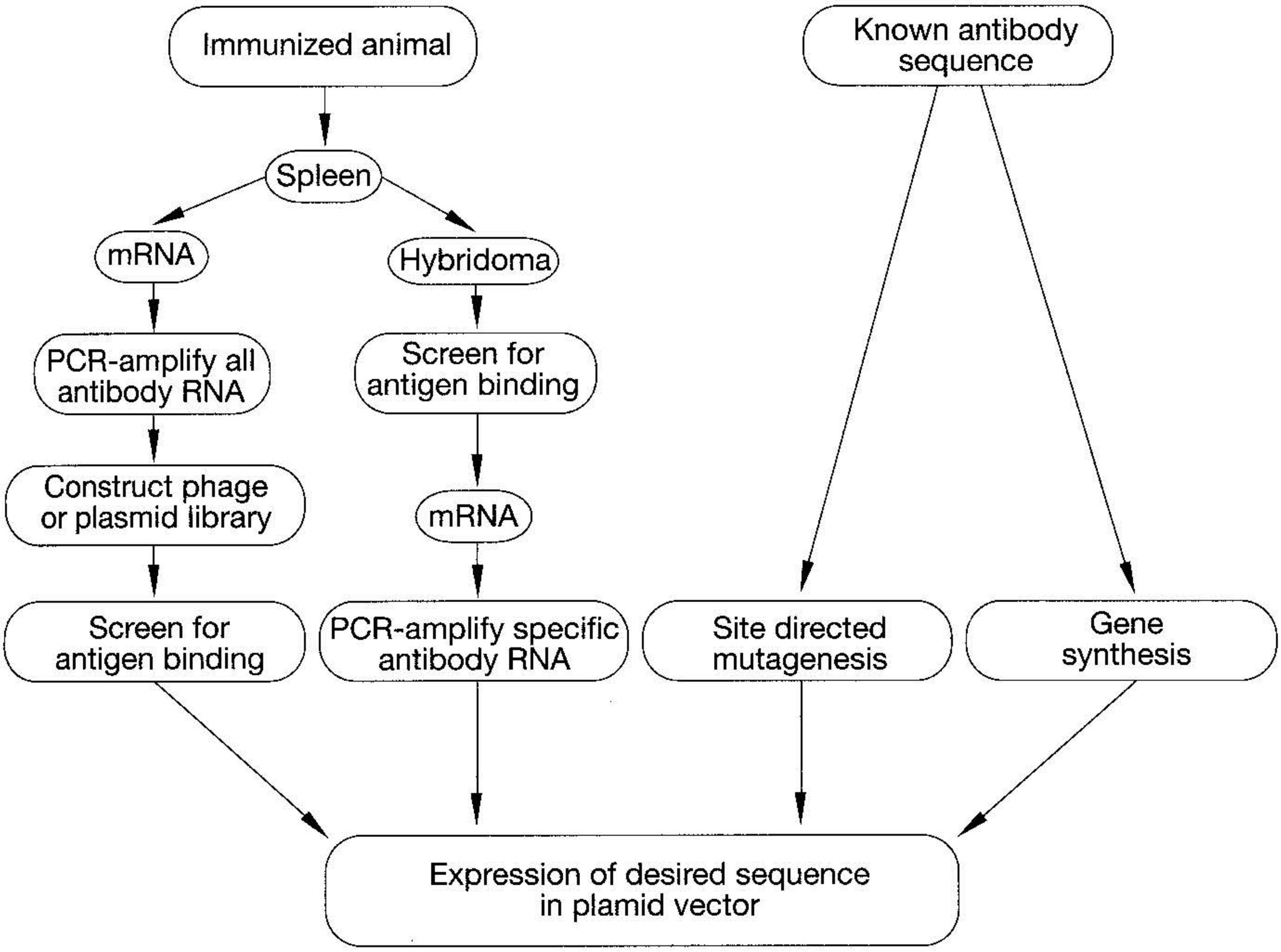


Fig. 2. Different pathways to recombinant antibodies.
If the antibody sequence is known, gene synthesis provides a rapid access. If a similar sequence is already available, site-directed mutagenesis can be used (pathways on the right). If the antibody genes are not available, they may be obtained either by PCR of the mRNA of a particular hybridoma or by PCR amplification of the total spleen mRNA. In the first case, screening is carried out at the stage of hybridomas, in the second, at the stage of bacterial cells (pathways on the left).

techniques is the expression technology in *E. coli* (see below) and it is discussed in more detail in 4.5.2.5 on modification and mutagenesis of the catalytic antibody, since the techniques of screening a library from the mutagenesis of a single antibody or an immunoglobulin library obtained after an immunization are analogous.

4.5.2.5 Altering the Catalytic Antibody: Genetic Manipulation and Expression

The use of *E. coli* expression systems [27] is particularly advantageous for the genetic manipulation of catalytic antibodies (Fig. 3). The genetic engineering procedures are very streamlined, the protein production is fairly simple, and the scale-up can be carried out with simpler fermentation equipment than for any other type of host organisms. The bacterial expression systems are suitable for F_v-, single-chain F_v- and F_{ab}-fragments of the antibody (Fig. 4, 5). These fragments contain the complete antigen-binding site.

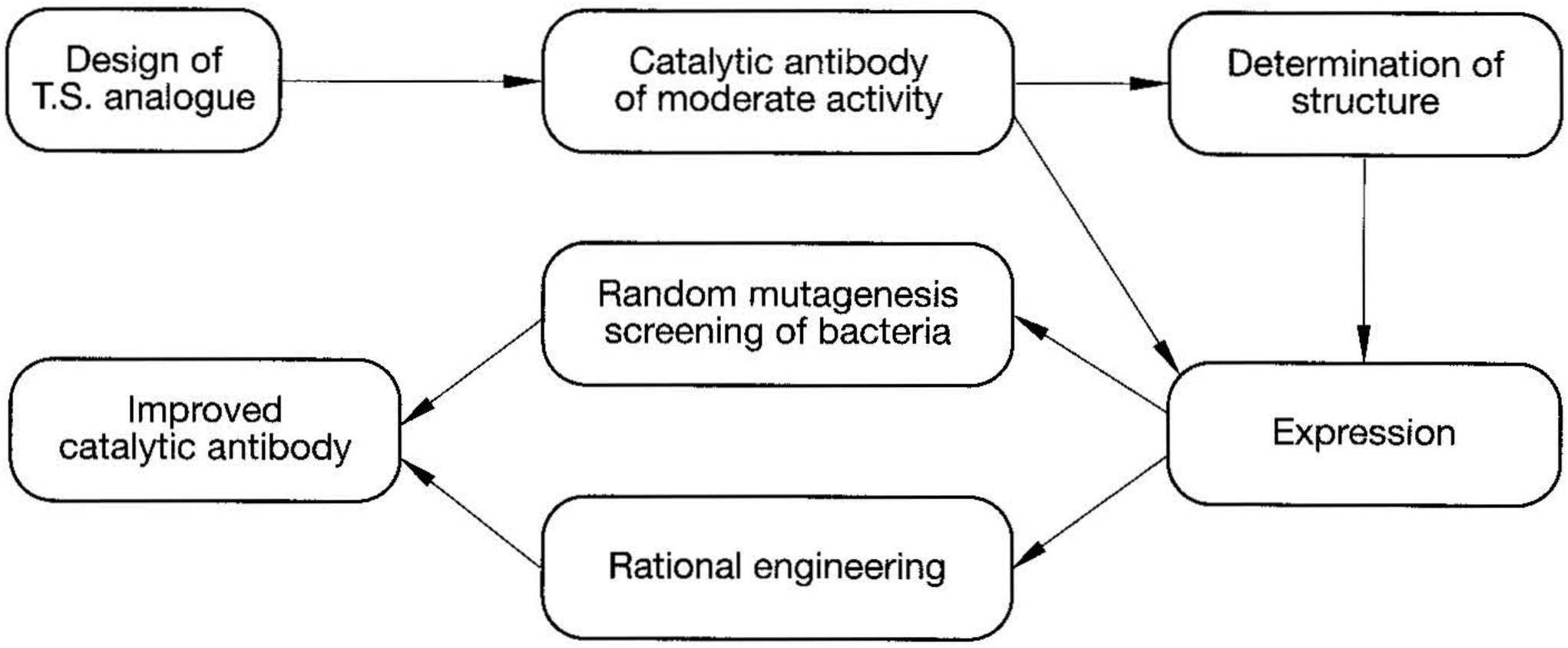


Fig. 3. Strategic routes to catalytic antibodies. The generation of a first antibody by immunization can be followed by rational engineering or random mutagenesis.

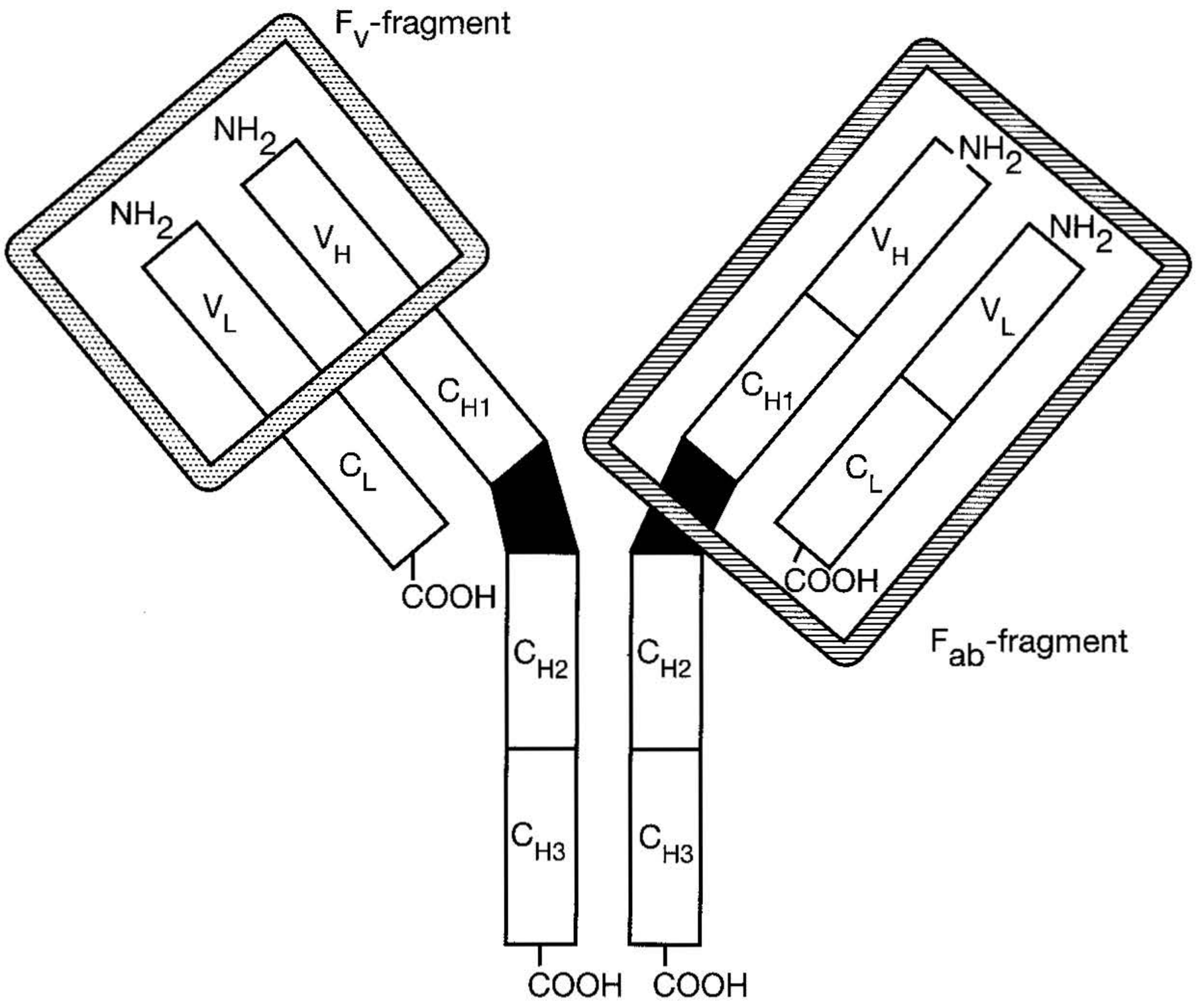


Fig. 4. Antigen binding fragments of an antibody.
The F_{ab}-fragment can be prepared by limited proteolysis around the hinge region, whereas F_v-fragments are usually not accessible by proteolysis. Both can be obtained by expression in *E. coli*. The F_v-fragment, the heterodimer of the V_H and V_L domain, is the smallest fragment still containing the whole antigen binding site.

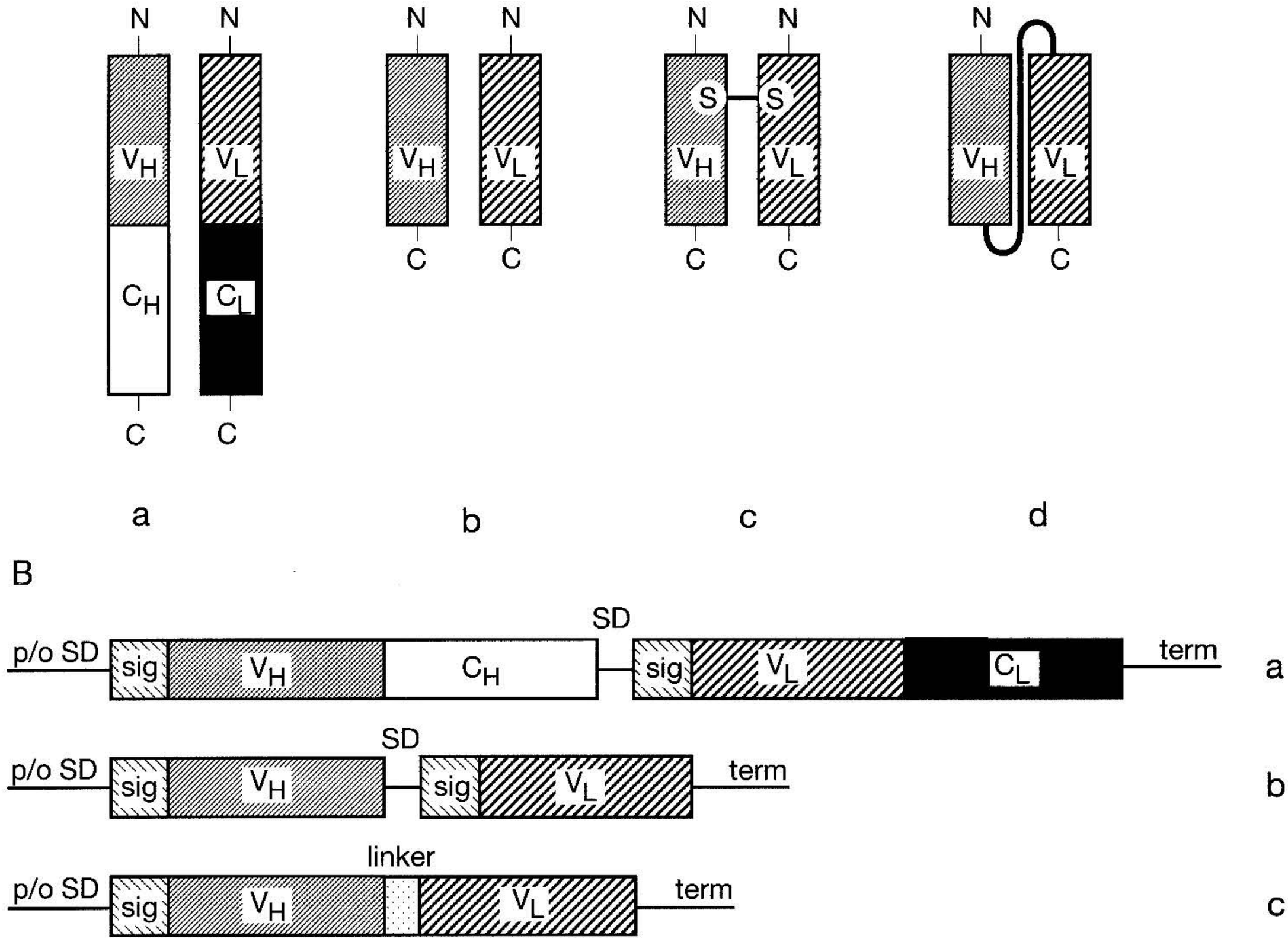


Fig. 5. Antibody fragments functionally expressed in *E. coli*.
On the top A), the proteins are schematically diagrammed, on the bottom B) the corresponding genes. A) The F_{ab}-fragment is shown in a), the F_v-fragment in b), the engineered disulphide-linked F_v-fragment in c) and the single-chain F_v-fragment in d). For details, cf. [28]. B) Co-secretion of both chains of the F_{ab}-fragment a) or the F_v-fragment b) can be achieved by designing an artificial operon, in which both genes are transcribed on one mRNA and controlled under one promotor. c) Alternatively, both domains can be linked by a peptide linker to give the single-chain F_v-fragment. *p/o* denotes a promoter/operator structure, *SD* a *Shine/Dalgarno* sequence, and *sig* a signal sequence. For details, cf. [28, 31].

Experiments have shown that even the smallest fragments (the F_v-fragment and its covalent derivatives) show the same binding affinity to a monomeric hapten as the whole antibody. Because of their small size, the F_v-fragment or the single-chain F_v-fragment are suitable for structural investigations.

For the investigation of catalysis, the F_v-fragment, its derivatives or the F_{ab}-fragment can all be used. It may be useful to discuss some of their properties. The F_v-fragments of various antibodies differ in stability. They may dissociate reversibly into V_H and V_L [28]. The dissociation constant depends on the particular antibody under study, since the hypervariable loops contribute to the domain interaction energy. This problem can be counteracted in several ways [28] (Fig. 5). First, the two domains can be covalently linked by chemical means. Second, a disulphide bond can be designed *between* the two

variable domains. Third, a single-chain F_v-fragment can be used, in which a peptide linker connects the two domains [28–30]. Fourth, the F_{ab}-fragment can be used, in which the constant domains C_L and C_H1 contribute additional association energy between the heavy and light chains.

The current strategies of producing catalytic antibody fragments in *E. coli* [27] may be divided into those leading directly to folded fragments and those requiring *in vitro* refolding. The former are based on the secretion of the protein to the periplasm of *E. coli* [31, 32], and can be applied to the F_v-fragment, the disulphide-linked F_v-fragment, the single-chain F_v-fragment and the F_{ab}-fragment (Fig. 5). The advantage of this methodology is that the fragments are directly obtained in functional form, as they assemble by themselves, and all disulphide bonds can form *in vivo*. Any strategy aiming to screen binding or catalytic activity must therefore be based on secretion, as it obviously requires the presence of correctly folded molecules.

Depending on the magnitude of the catalytic activity of the antibody, the kinetic investigation of recombinant catalytic antibody fragments may require fairly large amounts of protein. The yields in the secretory system are determined by several main factors, including the proteolytic sensitivity of the protein, which may be degraded either before or after transport to the periplasm of *E. coli*, and the folding yield of the protein. Not all of the transported protein is assembled correctly, but up to 50 % of the antibody fragment can be obtained in folded form. *E. coli* usually stops growing upon induction of expression and its outer membrane becomes leaky in response of the induction of antibody production. The factors responsible for this are largely unknown, but this leakage phenomenon can be useful for screening. This phenomenon, whose physiological basis remains unknown, must be taken into account in developing fermentation strategies.

An alternative expression strategy is to refold the recombinant antibody protein from inclusion bodies [27, 29, 30]. While, in many instances, more total antibody protein per cell can be produced than in the secretory system, the overall success depends largely on the yield of refolding of the recombinant protein. The details of the procedure will probably have to be optimized for every fragment.

The main advantages of *E. coli* production methods, i.e. speed and convenience, may be less crucial in special applications where whole glycosylated antibodies might be desired; e.g. for medical use, or for applications in transgenic animals or plants. In many cases, it may still be useful to engineer the binding site using a bacterial expression system and then switch to other eukaryotic expression hosts with the final version. Mammalian cells of myeloma origin [33] or non-myeloma origin [34] are established production hosts, and new eukaryotic hosts such as yeast [35], baculovirus-infected insect cells [36, 37] and plants [38] are being tested.

4.5.2.6 Engineering: the Rational Approach

Currently, a prediction of a structure from the sequence alone is not possible, although some trends have emerged from the large number of known antibody sequences and a

few crystal structures. The problems relevant for catalytic antibodies can be divided into three parts:

- How does an antibody loop with a given sequence fold?
- How does a given antigen bind to the antibody?
- What is the most desirable environment for the substrate to ensure maximal turnover?

None of these questions can be answered today.

However, some progress has been achieved in all of these areas. The analysis of the published antibody structures has led to the interesting hypothesis that only few of the residues in the antibody loops are important for determining the final conformation [11, 12]. Thus, it has been proposed that loops can be clustered to a few conformations only, the so called "canonical structures". However, caution is necessary, as the number of experimentally determined structures is still far too low to test this hypothesis. Likewise, the evaluation of the potential energy of random conformations, or of systematically generated conformations, has been investigated [39–41]. In all cases, useful predictions are possible but they are at present not accurate enough to predict the precise location for the binding of small molecules. The prediction of the interaction with large molecular surfaces is much less critical since far more interactions contribute to the binding of a protein antigen, allowing weak interactions to be compensated by others, and progress in this area is likely to be faster.

The binding of the substrate can be investigated with potential energy functions [42], provided there is a good experimental structure of the antibody. A variety of alternative energy functions combined with *Monte-Carlo* simulated amealing are currently under development.

Perhaps the most difficult question is the last one: what is the optimal environment for the substrate in the particular reaction? The input comes mostly from the mechanistic and crystallographic study of analogous enzymes. The difficulty does not lie in the difficulty to propose catalytic devices (e.g. nucleophiles, acids, bases), but is rather based in the fact that their usefulness depends very strictly on the precise location of the catalytic groups [43]. It is this problem that hampers enzyme engineering, and thus catalytic antibody engineering most of all. Thus, in many instances, a given geometry of substrate binding to an antibody may not easily allow the introduction of a metal or other catalytic device *at a position useful for catalysis*, as no amino acid might reach the exact point in space required. Any uncertainty about the structural consequences of a mutation must of course be added to the designer's problems.

Random mutagenesis may be a complementary approach as it may introduce additional mutations *not* at the binding site, thus slightly shifting the orientation of some groups. Small positional effects of this kind are almost impossible to achieve by design, given the current state of knowledge.

4.5.2.7 Random Mutagenesis: a Perspective

The availability of bacterial expression systems is the prerequisite for advancement to the next step: screening and selection. In this context, it is important to distinguish

screening for binding (e.g. of the transition state analogue) and screening for catalysis directly.

The high transformation frequency of *E. coli* is the decisive feature of using this organism as expression host for screening applications. It can be maximized by using electroporation or infective phages [26]. In this case, the phage has no other function than to package the DNA and to deliver it efficiently to the cells. Phage λ has been used for this purpose, and an expression plasmid was integrated into the phage genome. The actual protein production is carried out by phage-infected cells, which apparently still produce some secreted protein while being lysed. Antibody binding to the transition state analogue can be screened for directly on phage plaques on a nitrocellulose filter.

Alternatively, the expression of antibody fragments on the surface of the filamentous bacteriophage M13 has been investigated [44, 45]. Fusions of one chain of an F_{ab}-fragment, or of a whole single-chain F_v-fragment to either the major coat protein (product of gene 8) or one of the minor coat proteins (product of gene 3) have been reported. The phages carrying antibody fragments on this surface can be enriched for binding properties by enrichment cycles of antigen affinity purification and infection, since the phages carry the genetic information for the antibody they display [44, 45].

A number of different hybrid proteins suitable for expression on the surface of *E. coli* are also currently under investigation in a number of laboratories. While fluorescence-activated cell sorting of bacteria may be feasible, it is likely to be too slow to handle the very large numbers of cells involved in screening very diverse libraries.

In summary, there are now several new methods available for random mutagenesis and screening for binding in *E. coli*. The next step is therefore the design of a screen for catalysis.

The screening for enzymatic activity in any system, be it hybridoma supernatants or in bacterial colonies or phage plaques, must overcome two problems. First, the amount of antibody protein present in any of those systems is very low. Therefore, the assay has to be extremely sensitive as the activities usually obtained in catalytic antibodies are rather low. Second, the cells themselves are full of all kinds of enzymes of very high activity. Thus, if an activity already present in the cells is being screened for, extreme care must be taken that the trace contaminations of such cellular enzymes do not mislead the investigator. Many enzymes have some range of "specificity"; therefore, even if unnatural substrates are used, there is no guarantee that a cellular enzyme is not active on the assay substrate.

The direct screening for activity is clearly one of the main challenges for catalytic antibody technology. It is not at all clear, for instance, whether tight binding of the transition state analogues is even a desirable first screen, or whether some of the best catalysts might actually fail this test.

In random mutagenesis experiments, the substrate used for screening should obviously allow an easy handling of extremely large numbers of clones. Therefore, a colour reaction, a characteristic flourescence change or light production are particularly advantageous. Clearly, one cannot rely on a chromatographic separation of reaction products, or an NMR analysis, if thousands or even millions of clones are to be screened in bacteria.

How likely is random mutagenesis to be successful in boosting activity? The

alteration in the antibody sequence might have to occur at a remote place in the structure to effect the desired subtle consequences at the binding site. The likelihood of success depends on the number of changes required, and on whether there is a path of consecutive single changes leading to activity, or whether only a multiple-change molecule will be active. No screening system is yet efficient enough, however, to evaluate the number of variants generated by completely randomizing very long stretches of amino acids. The number of clones generated if N amino acids are being totally randomized, 20^N ($= 10^{1.3N}$), quickly becomes prohibitive. If the length of the chain is also varied, and not only its sequence, this number has to be multiplied with each length allowed. Therefore, the screening system must be very simple, or better yet, a positive metabolic selection of the catalytic activity must be attempted.

Bacterial metabolism allows a huge number of reactions to be selected for, as specific mutants can be made by classical bacterial genetics or modern reverse genetics. In bacteria, only secreted antibody fragments appear to be functional. There have been reports to the contrary [46] but, at the time of writing, it was still unclear whether the cytoplasmic antibody protein reported to be functional might have folded and its disulphide bonds been formed *after* opening of the cells for purification. In yeast, there have been reports about functional cytoplasmic expression of antibodies [47], but here also the problem of changes brought about by the work-up has not been completely solved.

If the antibody can be expressed cytoplasmically, it must replace a missing function in the microbial metabolism or supply a detoxifying reaction. If it is expressed outside of the cell, it must convert a "masked nutrient" to a real nutrient, or again have a detoxifying action, e.g. by eliminating an antibiotic. While this may limit the scope of reactions to be selected for, it still allows an enormous variety of reaction types and pathways to be scrutinized, and research in this area is just beginning. The most difficult problem is obtaining the first mutant with sufficient activity to sustain growth, since many catalytic antibodies reported so far give rise to only fairly moderate rate accelerations.

4.5.2.8 Are there Uses for Catalytic Antibodies?

In what areas might catalytic antibodies become important? In organic synthesis, it is rather unlikely that this technology will have a great impact in the very near future. The economic value of most organic chemicals is not high enough to justify the substantial effort in developing a very special catalysis for a particular reaction. Exceptions may prove the rule. Rapid progress in the use of natural enzymes for catalysis, the synthetic methodology itself (as in stereospecific reactions, or chiral auxiliaries), as well as in the engineering of existing enzymes to adapt them to new targets, are competing technologies. Furthermore, the very screening techniques developed for antibodies may of course also be applied to existing enzymes or other binding molecules.

The particular niche of applications of catalytic antibodies will most likely lie in reactions where the absolute rate itself is of no importance (as enzyme-like rates will in

general not easily be accessible), but where exquisite selectivity counts and no existing enzyme can easily be adapted to the new substrate. Examples might be specific prodrug-drug conversions in human medicine, or detoxifying reactions in plants. An exciting application might also lie in selective degradation reactions of harmful substances in human therapy. Specific diagnostic assays may also be an interesting target.

Why has nature not developed catalytic antibodies? Probably because it has found better solutions: for chemical warfare against a foreign invader, the armada of cytotoxic T-cells is available, and for the many reactions required in metabolism, uniquely designed enzymes have arisen by evolution along many pathways. Nevertheless, catalytic antibodies provide an exciting approach to enzymatic activities never designed by nature, requiring an interdisciplinary approach in science.

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