Selection of catalytic antibodies for a biosynthetic reaction from a combinatorial cDNA library by complementation of an auxotrophic *Escherichia coli*: Antibodies for orotate decarboxylation

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ABSTRACT Antibodies capable of decarboxylating orotate were sought by immunization with a hapten designed to elicit antibodies with combining sites that resemble the orotate-binding and catalytic portion of the active site of the enzyme orotidine 5'-monophosphate (OMP) decarboxylase (orotidine-5'-monophosphate carboxy-lyase, EC 4.1.1.23). Active recombinant antibody fragments (Fabs) were selected from a combinatorial cDNA library by complementation of a *pyrF* strain of *Escherichia coli* and growth of the library-expressing cells on pyrimidine-free medium. In this biological screen, a sufficiently active antibody from the library would decarboxylate orotate to produce uracil, a pyrimidine source for the auxotroph, and would provide the cells with a growth advantage compared to cells without an active antibody. Six recombinant Fabs yielded identifiable colonies in a screen of 16,000 transformants. To enhance its stability and expression level, one of the six positive fragments was converted into single-chain form. In this form, the antibody fragment conferred a definite growth advantage to the auxotroph that was eliminated when the hapten was included in the medium. The purified single-chain antibody displayed orotate decarboxylase activity *in vitro*, as determined by a $^{14}\text{CO}_2$ displacement assay. The specific activity of the antibody is $\approx 10^{-7}$ times that of naturally occurring OMP decarboxylase, but this antibody-catalyzed rate is estimated to be 10^8 times the background rate. The results offer the potential to use these methods to obtain catalytic antibodies for other biosynthetic reactions as well as to assess the effectiveness of the hapten transition state or active site analog in eliciting antibody catalysts.

One of the potential applications in the development of catalytic antibodies (1, 2) is to create catalysts that can serve as replacement enzymes in organisms that lack the enzyme of interest (3) due either to genetic mutation or to the lack of the metabolic pathway of which the enzyme of interest is a component. Accompanying the realization of this goal would be the development of methods to select active antibodies, produced in *Escherichia coli* or other microorganisms, by utilizing the antibody's ability to catalyze the missing reaction *in vivo*, thus providing the cell with a necessary metabolite and a growth advantage on restrictive medium. Of the many chemical reactions that catalytic antibodies have been found to accelerate (2), however, few to date involve reactants or products that are found in metabolic pathways.

We have attempted to create antibodies capable of the decarboxylation of orotate, an intermediate in the *de novo* pyrimidine biosynthetic pathway. In the naturally occurring pathway (4), orotate is first converted to the nucleotide orotidine 5'-monophosphate (OMP), which is then decarboxylated to yield UMP, the precursor for all other pyrimidine nucleotides. An auxotroph lacking OMP decarboxylase (oro-

FIG. 1. Pathways for UMP biosynthesis. Upper route involves naturally occurring enzymes orotate phosphoribosyltransferase (OPRTase) and OMP decarboxylase (ODCase). Mutants lacking one or both of these enzymes could complete a route to UMP with an antibody for orotate decarboxylase and the naturally occurring enzyme uracil phosphoribosyltransferase (Uracil PRTase).

tidine-5'-monophosphate carboxy-lyase, EC 4.1.1.23) could synthesize UMP if provided with a replacement enzyme (e.g., a catalytic antibody) with orotate decarboxylase activity, since the product, uracil, could be converted to the nucleotide UMP by the salvage pathway enzyme uracil phosphoribosyltransferase (Fig. 1).

Our method of selection for active catalytic antibodies described here is to induce an immune response to a hapten designed to elicit an antibody with a binding site that may be capable of orotate decarboxylation; isolate antibody cDNA and assemble a combinatorial heavy- and light-chain phagemid library; infect a pyrimidine auxotrophic *E. coli* strain with the antibody library-encoding phage; and evaluate the catalytic activity of antibodies produced in bacterial colonies that arise on pyrimidine-free medium. This method may be generally applicable for selection of antibodies that catalyze other metabolic reactions. The process of determining the number of members in a combinatorial library with catalytic activity also provides a statistically meaningful assay for evaluation of the effectiveness of transition-state analogues or other haptens in eliciting catalytic antibodies.

MATERIALS AND METHODS

Hapten Design. The design of a hapten to be used for generating antibodies capable of orotate decarboxylation is based on the mechanistic studies on naturally occurring OMP

Abbreviations: OMP, orotidine 5'-monophosphate; Cam, chloramphenicol; IPTG, isopropyl β -D-thiogalactopyranoside; SCA, single-chain antibody; LcFv, light-chain antibody variable region; HcFv, heavy-chain antibody variable region; BMP, barbituric acid ribonucleotide (6-hydroxy-UMP).

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decarboxylase. Fig. 2A shows the transition state for decarboxylation of the zwitterion intermediate. This enzyme appears to have two major catalytic functions, as first postulated by Beak and Siegel (5): (i) protonation of the pyrimidine substrate (6) with a cationic side chain [a lysine, invariant in all known sequences (7)], leading to the zwitterion intermediate; and (ii) immersion of the charged carboxylate of the substrate into a hydrophobic portion of the binding site, which catalyzes CO_2 production by disfavoring the increased electron density on the charged carboxylate. A hapten for eliciting an antibody binding (and possibly catalytic) site with these features should be designed to recruit a cationic proton-donating amino acid side chain of the antibody into proximity with the C2-C4 ring segment of the substrate, while simultaneously creating a hydrophobic pocket in the active site into which the carboxylate of the substrate would be situated.

We have chosen an N-substituted 2,4-dihydroxyquinoline as a hapten designed to produce an antibody binding site with the two catalytic features necessary for orotate decarboxylase activity (Fig. 2B). The conjugated enediolate (C2-C4) of this molecule has a pK_a value of ≈ 6.5 and is thus anionic at physiological pH, suitable for inducing a positively charged residue within the antibody combining site. This charge complementarity approach is similar to a hapten design previously used (8). The fused phenyl ring is designed to create a hydrophobic space within the antibody binding site in a way similar to that used for obtaining antibodies for decarboxylation of carboxybenzisoxazoles (9). N-Carboxypropyl-2,4-dihydroxyquinoline (compound 1) was synthesized by a published method (10) and this hapten was conjugated to the carrier protein keyhole limpet hemocyanin for mouse immunization (11).

Generation of Antibody cDNA Libraries. mRNA was isolated from homogenized spleens of immunized mice using oligo(dT) affinity chromatography [Poly(A) Quik kit; Stratagene]. This mRNA was used as a template for cDNA synthesis with reverse transcriptase and an oligo(dT) primer. Antibody cDNA was then amplified by PCR (materials from Perkin-Elmer/Cetus), with primers designed to be complementary to consensus antibody sequences at the 3' ends and containing restriction sites at the 5' ends (12). The 660-bp products of separate heavy- and light-chain amplification reactions encode functional antibody (Fab) portions of mouse antibodies. Restriction digestions with *Xho* I and *Spe* I (for heavy-chain cDNA) and *Sac* I and *Xba* I (for light-chain cDNA) yielded fragments with compatible ends for ligation into a similarly digested plasmid vector, pComb3-C [a chloramphenicol (Cam) resistance-encoding version of pComb3 (13), provided by C. F. Barbas III, Research Institute of Scripps Clinic]. Expression of cDNA inserts in pComb3-C is controlled by the *lac* promoter and is thus inducible with isopropyl β -D-thiogalactopyranoside (IPTG). As with pComb3, Fabs from pComb3-C are directed to the periplasm

by the *pelB* leader sequence and anchored in the inner membrane by the gIII fusion protein (13). Electroporation-competent JS5 cells (Bio-Rad) were transformed with products of the separate heavy- and light-chain ligations, and aliquots of the transformation mixtures were plated on LB agar plus Cam to assess the number of total transformants and thus the number of members in the individual heavy- and light-chain libraries.

The combinatorial library was made by isolation of heavy-chain cDNA from the corresponding plasmid library, and ligation with restriction-digested light-chain library plasmid. Electrocompetent JS5 cells were transformed with products of the combinatorial ligation, and the number of total transformants was determined in order to calculate the number of members in the combinatorial library, as with the individual libraries. The remainder of the transformation mixture was grown in superbroth (SB; ref. 13) liquid medium (with tetracycline and Cam), and plasmid DNA was isolated. For production of intact phage, electrocompetent cells were transformed with an amount of this plasmid library sufficient to yield a number of transformants in 10-fold excess of the library size, and the transformation mixture was incubated as described except that helper phage VCSM13 (Stratagene) was added with kanamycin for selection (14). After overnight incubation, cells were removed by centrifugation and phage were precipitated by the addition of 2% PEG 8000 and 1.5% NaCl. Phage were stored at 4°C and used within 1 week of preparation.

Infection of Pyrimidine Auxotroph with Antibody-Encoding Phage: Biological Screen for Decarboxylase Activity. *E. coli* strain JFS116 (*pyrF*, *F' lacI^Q*; ref. 15) was used to attempt complementation of pyrimidine auxotrophy with the antibody cDNA library. Cells were always maintained in the presence of kanamycin and tetracycline to ensure the pyrimidine requirement of this strain. For phage infection, cells were first grown to saturation at 37°C in LB, and a portion of this culture was used to inoculate medium containing M9 salts (16), 0.2% Casamino acids, 5% glycerol, and uracil (20 $\mu\text{g}/\text{ml}$). After this culture reached saturation at 37°C, the cells were harvested by centrifugation and resuspended in fresh M9/Casamino acids/glycerol medium without uracil. These cells were incubated at 37°C for 2 hr and then infected with the antibody library-encoding phage.

The cells were infected with a dilution of phage sufficient to give ≈ 1000 colonies per plate. The infected bacteria were spread onto 1.5% agar (Agar Noble; Difco) plates of the following composition: LB plus Cam (for quantitation of infection); M9/Casamino acids/glycerol with kanamycin, tetracycline, and Cam/0.3 mM IPTG (selection for pyrimidine autotrophy). Incubation was carried out at 37°C for up to 4 days.

Colonies arising in this selection process were transferred to SB plus Cam, and plasmid DNA was isolated from the resulting cultures. The presence of antibody cDNA in these plasmids was determined by restriction digestion with enzymes used for insertion of heavy- and light-chain fragments in the cloning procedure and agarose gel electrophoresis of the products.

Conversion of Selected Fab Genes into Single-Chain Antibody (SCA) Genes. A plasmid was designed for the purpose of expressing SCA in fusion with the gIII phage protein. This system is similar to that originally described (17) for phage display of antibody variable domains. The parent plasmid, pSCL, was constructed from pComb3-C in two steps: (i) deletion of the light-chain cDNA cloning region and the corresponding *lac* promoter, and (ii) replacement of the heavy-chain cloning region with a synthetic oligonucleotide encoding two cloning regions and a 19-codon linker (similar to that in ref. 18) to fuse the light-chain variable region (LcFv) and the heavy-chain variable region (HcFv) genes (Fig. 3).

FIG. 2. Hapten design for eliciting antibodies for orotate decarboxylation. (A) Transition state for orotate decarboxylation by zwitterion formation. Protonation at O-2 results in a transient positive charge at N-1, leading to catalysis for OMP (enzyme catalyzed; R = ribose 5-phosphate) or orotate (target for antibody catalysis; R = H). (B) Hapten for creating orotate decarboxylating antibody combining site. Compound 1, R = $(\text{CH}_2)_3\text{COOH}$; compound 2, R = H.

SCAs encoded by pSCL will retain the *pelB* leader sequence for transport to the periplasm and the gIII phage coat fusion protein, for anchoring the SCA in the periplasmic space (14), as with Fabs expressed from pComb3-C.

Oligonucleotide primers for PCR amplification of SCA DNA were designed as described (11, 20) except that the restriction enzyme sites incorporated into the primers corresponded to the sites in pSCL: *Sac* I and *Hind*III for the light-chain fragments, and *Xho* I and *Spe* I for heavy-chain fragments. LcFv and HcFv DNA was amplified from Fab-encoding versions of pComb3-C, which arose in the biological screen, and the amplification products were restriction digested and ligated to digested pSCL to create plasmids encoding SCAs (Fig. 3).

The SCA-encoding version of one of the biologically screened Fab plasmids, designated pSC-8 (Fig. 3), was used to attempt complementation of the original uninfected JFS116 strain. Again, cells were maintained on antibiotics to ensure the genetic integrity of the strain. Cells were electroporated with pSC-8 (or plasmid pSC-43C9, a single-chain version of the hydrolytic antibody 43C9; or pSCL) and plated onto SB agar plates to obtain single colonies; overnight cultures of individual colonies were grown in SB plus 2% glucose. Cells with no plasmid were used as an additional control, except that Cam was omitted from the medium. Portions of the saturated overnight cultures were transferred to M9/Casamino acids/glycerol plus uracil and again grown to saturation. Cells were then collected by centrifugation, resuspended to equal cell densities in M9/Casamino acids/

glycerol without pyrimidines, and incubated at 37°C for 2 hr. Cells were then plated onto selective medium (without Cam) and incubated at 37°C for 2 days. Separated colonies arising at the edge of the streak were photographed at low magnification with a Polaroid camera attached to a Nikon microscope for the purpose of determining colony size and thus comparing the pyrimidine autotrophy-conferring ability of the antibody-encoding and control plasmids.

Purification of SCA 8. To obtain higher amounts of SCA, the entire SCA-encoding region was amplified from pSC-8 and inserted into plasmid p4.1 (19), which contains the strong T3 promoter (Fig. 3). Primers for amplification were designed to anneal upstream from the *Sac* I site of the LcFv gene (5') and at the *Spe* I site of the HcFv gene (3'). The latter primer was also designed to introduce new stop codons at the end of the SCA, since stop codons for protein expression in pSC-8 are at the end of the fused gIII gene.

SCA production was induced by the addition of 2 mM IPTG to cultures of BL21 cells carrying plasmids pTG119 (19) and the SCA-encoding p4.1 derivative p4.1.8. The cells and plasmids for this expression system were graciously provided to this laboratory by R. La Polla (R. W. Johnson Pharmaceutical Research Institute, La Jolla, CA). The SCA from this system is excreted into the bacteria culture supernatant. Cultures of SCA-producing cells were grown to $OD_{600} = 10$ with a BioFlo III fermentor (New Brunswick Scientific) and then cooled to 25°C for induction for 16 hr. Cells were removed by centrifugation, the supernatant was concentrated and dialyzed, and the SCA was purified by column chromatography as described (19).

Assays for Decarboxylase Activity. Enzymatic activity was assayed by collection of $^{14}CO_2$ evolved from [*carboxyl*- ^{14}C]orotate (DuPont/NEN), similar to assays for OMP decarboxylase activity using labeled OMP (21). Contaminating $^{14}CO_2$ present in the substrate was removed by acidification with HCl, and the acidified substrate preparation was evaporated to dryness before use. In decarboxylase assays, liberated $^{14}CO_2$ was collected on alkali-moistened filter paper in air-tight reaction vials upon quenching the reactions with perchloric acid.

In preliminary experiments, activity was assayed in cell lysates of JFS116 carrying pSC-8 (or control plasmids) without purification of the SCA. Cells from a 10-ml culture were treated with 0.5 mM IPTG, incubated at 37°C for 4 hr, collected by centrifugation, and resuspended in lysis buffer containing 25 mM Tris-HCl (pH 8.0), 50 mM glucose, 10 mM EDTA, and lysozyme (5 mg/ml). After 30 min of incubation at room temperature, the assay was initiated by addition of [*carboxyl*- ^{14}C]orotate (isotopically undiluted) to aliquots of the lysate, and the reactions were quenched after 1 hr. Subsequent assays were performed on purified SCA. Reaction mixtures contained 5 μ g of SCA and [*carboxyl*- ^{14}C]orotate in 20 mM Mops buffer (pH 6.3). Protein was quantitated by comparison of gel electrophoresis bands with purified, quantitated 43C9 SCA (19).

RESULTS

Antibody Libraries and Biological Screen. Cloning of Fab genes from the immune response to compound 1 yielded individual heavy- and light-chain libraries of $\geq 10^5$ members. Although larger library sizes have been reported (14), the anticipated number of clones to be screened was $1-2 \times 10^4$, so these libraries were considered to be sufficient. Cloning of the combinatorial library yielded 10^6 members, and this library was used to produce phage for the complementation assay.

The original biological screen with the combinatorial Fab phage library yielded 24 identifiable colonies in a screen of 16 plates containing 1000 infected cells per plate. However,

FIG. 3. Construction of plasmids encoding orotate decarboxylating antibody fragments. Plasmid pC3C-8 is an Fab-encoding plasmid selected from the biological screen (see text) and is similar in construction to plasmids in ref. 13, except that the ampicillin-resistance gene in pC3C-8 is replaced with a Cam-resistance gene in pC3C-8. Plasmid pSC-8 encodes the single-chain form of this Fab and was used for attempted complementation of pyrimidine auxotroph JFS116. Plasmid p4.1.8 is derived from p4.1, a vector for high-level production of SCAs from ScFv inserts (19).

upon culturing these 24 colonies, it was found that only six of the cultures produced plasmids that contained intact Fab genes. Deletions from double *lac* plasmid systems for Fab expression have been observed in our laboratory (D. B. Smithrud and I. Lee, personal communication) and by others (22).

Although these six plasmids were able to confer a growth advantage to the original JFS116 strain (data not shown), the frequency of deletions in these plasmids proved to be troublesome. Thus, the ability of a single-chain antibody-encoding plasmid to confer a similar growth advantage and produce an orotate decarboxylating catalyst was examined.

Complementation of Auxotroph with pSC-8. Comparison of colonies of *pyrF* strain JFS116 carrying pSC-8 and control plasmids is shown in Fig. 4. Control colonies with no plasmid, or with pSCL or pSC-43C9, are barely visible and can be clearly observed only with magnification (Fig. 4 A–C). The presence of any colony growth in JFS116 on this medium is likely due to trace amounts of pyrimidines in the medium components, either the Casamino acids or the agar.

Colony growth of the auxotroph carrying pSC-8, the single-chain form of the biologically selected Fab, is enhanced over the controls (Fig. 4D). These colonies arose on a separate portion of the same plate as the control cells and are visible without magnification.

On separate plates containing identical medium with the addition of 0.5 mM hapten 2, cells with pSC-8 produced colonies no larger than those of the control cells (Fig. 4E). Compound 2 at this concentration had no effect on control cells, either on restrictive or complete medium.

Assays of Decarboxylase Activity in Crude Lysates. Orotate decarboxylation is a remarkably slow reaction (see *Discussion*), and $^{14}\text{CO}_2$ displacement is not observed in lysates of cells lacking OMP decarboxylase. [In the normal metabolic pathway (Fig. 1) orotate would first be converted to OMP and then decarboxylated to give UMP and CO_2 .] Attempts to collect $^{14}\text{CO}_2$ from assays of JFS116 cell preparations yielded no counts above orotate incubated in lysate buffer. However, in quadruplicate assays of cells carrying plasmid pSC-8, orotate decarboxylation was clearly observable above background (≈ 100 cpm) and linear up to 15 μM orotate; at this concentration, 500 cpm of $^{14}\text{CO}_2$ (10 pmol) was collected. These preliminary assays indicated the presence of an orotate decarboxylating antibody, and so the SCA gene was inserted into plasmid p4.1 for high-level expression.

Purification of SCA. The single-chain antibody was obtained from the bacterial expression system described (19) in yields much lower than those obtained for the hydrolytic antibody 43C9. Only 50–75 μg of protein, with the same molecular weight and FPLC elution profile as 43C9, was obtained from 5-liter cultures. The protein was not purified to the same high degree as with 43C9; the band of M_r 27,000 from antibody SCA-8 preparations was one of three major bands identified by gel electrophoresis and Coomassie blue staining.

Assays of Decarboxylase Activity of Purified SCA. Partially purified SCA 8 displays orotate decarboxylase activity *in*

vitro. In assays containing 5 μg of SCA and 15 μM labeled orotate, $\approx 2\%$ of the ^{14}C of the substrate was liberated as $^{14}\text{CO}_2$ in 20 hr, yielding a calculated activity of 1.0×10^{-5} nmol·min $^{-1}$ · μg^{-1} at this orotate concentration. The decarboxylase activity was inhibited by $\approx 90\%$ by the addition of 1 mM compound 2 in assays with 10 μM orotate at various time points up to 20 hr.

The presence of contaminating OMP decarboxylase activity was examined by attempted inhibition with barbituric acid ribonucleotide (BMP; 6-hydroxy-UMP), a powerful inhibitor of the naturally occurring enzyme ($K_i < 10^{-11}$ M; ref. 23). With 0.1 μM BMP, an amount that should inhibit all residual OMP decarboxylase activity, the orotate decarboxylase activity (at 10 μM orotate) is reduced by $\approx 10\%$. With 1 μM BMP, the activity is reduced by $\approx 50\%$.

DISCUSSION

Conferring a growth advantage to auxotrophic microorganisms by transfer of a gene for the necessary metabolic enzyme was developed in the 1970s (24) and remains in use as a common genetic engineering tool today. The concept of using such complementation for screening antibodies for catalytic activity was early recognized as a means for evaluating the efficiency of the transition-state analog in the induction of catalytic antibodies as well as for sampling large combinatorial libraries (25). The ability to use a catalytic antibody as a replacement enzyme was accomplished by Hilvert and co-workers (3) with a hybridoma-derived chormate mutase antibody and a yeast strain lacking this enzyme activity. Schultz and co-workers (26) found that an esterolytic antibody would produce biotin (a carboxylic acid) from biotin *p*-nitrophenyl ester, but no growth advantage was detected with biotin auxotrophic bacteria carrying a plasmid encoding this antibody when grown on medium containing the ester. We now report the selection of antibodies for a desired reaction from a combinatorial cDNA library using a biological screen.

We have isolated plasmids encoding antibody fragments, which confer a growth advantage to a pyrimidine auxotrophic bacteria strain. Fab-encoding plasmids derived from colonies of JFS116, which appear on selective medium in the screening process, can be reinserted into the original auxotrophic strain and enhance growth on selective medium. The probability of finding a catalyst in the combinatorial Fab library proved to be 6 in 16,000 plasmids.

One of these Fab genes was converted to a SCA gene, and the plasmid carrying this gene also conferred an observable growth advantage. Colonies of JFS116 cells carrying the plasmid pSC-8, the single-chain version of the biologically screened Fab, show a distinct growth advantage over cells carrying control plasmids or no plasmid (Fig. 4). The growth advantage of JFS116 with pSC-8 in pyrimidine-free medium is nullified when compound 2, the free hapten against which the immune response was directed, is included in the growth medium, indicating that the hapten blocks the pyrimidine-producing activity of the antibody.

FIG. 4. Colonies of pyrimidine auxotrophic *E. coli* strain JFS116, with and without SCA-encoding plasmids, on medium without added pyrimidines. (A) Cells with no plasmid. (B) Cells with cloning vector pSCL (no SCA insert). (C) Cells with pSC-43C9, a version of pSCL encoding the hydrolytic antibody 43C9. (D) Cells with pSC-8, a version of pSCL encoding a SCA from an Fab screened for orotate decarboxylase activity. (E) Same as D, except that compound 2 is included in the growth medium at 0.5 mM.

The detection of orotate decarboxylase activity in lysates of the auxotrophic *E. coli* producing the selected SCA provides a direct biochemical explanation for the enhanced growth of cells producing this antibody. An advantage of the orotate decarboxylation assay is that it can be used successfully on crude preparations because, in the absence of OMP decarboxylase, orotate is not converted to any other products, and product $^{14}\text{CO}_2$ is easily removed from the reaction.

The *in vitro* orotate decarboxylase activity of partially purified SCA 8 is linear with increasing orotate concentration up to 15 μM ; at this concentration, the specific activity is $1.0 \times 10^{-5} \text{ nmol} \cdot \text{min}^{-1} \cdot \mu\text{g}^{-1}$ with a rate constant of $2.7 \times 10^{-4} \text{ min}^{-1}$. This activity is about 10^{-7} times that of the corresponding reaction catalyzed by wild-type yeast OMP decarboxylase ($70\text{--}80 \text{ nmol} \cdot \text{min}^{-1} \cdot \mu\text{g}^{-1}$; ref. 27). However, the exceptionally slow rate of uncatalyzed orotate decarboxylation, which is estimated from model studies to be $3 \times 10^{-12} \text{ min}^{-1}$ (5), means that the rate acceleration by the antibody is on the order of 10^8 . Although precise kinetics have yet to be determined and the uncatalyzed rate is somewhat of an approximation, the estimated rate enhancement is remarkable.

The orotate decarboxylase activity is inhibited by $\approx 90\%$ by 1 mM compound 2. The elimination of growth advantage of the auxotroph by 0.5 mM compound 2 is in agreement with the inhibition of decarboxylase activity *in vitro*. The correlation between the differences in hapten and substrate affinities and a measure of catalytic efficiency (K_m/K_i vs. $k_{\text{cat}}/k_{\text{uncat}}$; ref. 2) may not hold strictly, since compound 2 is not a proper transition-state analog; establishment of this correlation for this decarboxylating antibody awaits further kinetic characterization.

Although OMP decarboxylase would not be expected to use the pyrimidine base orotate effectively as a substrate, trace activity by contaminating enzyme toward orotate was considered to be a potential source of misleading results. The possibility of contamination of the antibody preparation with *E. coli* OMP decarboxylase from the pyrimidine autotrophic BL21 strain was ruled out by the observance of only limited inhibition ($\approx 10\%$) in assays containing 0.1 μM barbituric acid ribonucleotide, an amount 10^4 -fold greater than the K_i for this inhibitor.

The proteins encoded by the bacterial plasmids have been shown to be antibodies by partial DNA sequencing and comparison of the deduced protein sequence to known antibodies (data to be presented elsewhere). Thus, the catalytic activity is apparently the function of the antibody protein responsible for growth enhancement in the pyrimidine auxotroph. Despite its reduced activity relative to the enzyme, the antibody is apparently capable of producing uracil in amounts sufficient to enhance growth of the host on restrictive medium.

Selection of catalytic antibodies for metabolic reactions using a bacterial auxotroph may be generally applicable to many different reactions, but successful complementation will probably depend on several experimental criteria. (i) The metabolite produced by the catalyst should be one that is necessary only in small amounts in the growth medium. (ii) The reaction to be mimicked by the antibody should probably be of a general type that is not common among metabolic reactions—e.g., esterases and amidases are abundant in metabolism, and naturally occurring enzymes (or mutated forms of such enzymes) may replace the missing activity in an auxotroph. (iii) The substrate for the reaction must be found in the cellular milieu where the antibody is assembled. With OMP decarboxylase auxotrophs, orotate accumulates and can cross the *E. coli* membrane, where it can be decarboxylated by periplasmic Fab or SCA and the product uracil can return to the cytoplasm.

The ability to screen activity for a metabolic reaction by growth enhancement of a bacterial auxotroph should prove to be very valuable for studies of catalytic antibodies. The number of members in a combinatorial library that can be screened by this method is a vast increase over that which can be screened practically by standard monoclonal antibody technology. Alteration of catalytic activity (and perhaps protein stability) by random or site-directed mutagenesis can be initially determined by observation of the growth rates of antibody-producing auxotrophs on restrictive medium. Modifications in hapten design, and the ability of different haptens to produce more and better catalysts, might be more easily evaluated by assessing the number of transformants obtained from a biological screen of a combinatorial library.

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