Antibody Combining Sites as Templates for Selective Organic Chemical Reactions

Ayala Balan,[a] B. P. Doctor,[b] Bernard S. Green,*[c] Michael Torten,[a] and Herman Ziffer[d]

[a] *Israel Institute for Biological Research, Ness Ziona, Israel*
[b] *Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D.C., U.S.A.*
[c] *Department of Pharmaceutical Chemistry, The Hebrew University, Haddassah Medical Center, P.O. Box 12065, 91120 Jerusalem, Israel*
[d] *National Institutes of Health, Bethesda, Maryland, U.S.A.*

Antibodies were raised against an analogue of one of the photodimeric products [the cyclobutane (2)] resulting from irradiation of methyl p-nitrocinnamate (1); in the presence of these antibodies, irradiation of (1) led preferentially to the particular stereoisomer (2).

Attempts to imitate enzyme-catalysed reactions continue to be one of the ambitious goals of chemists and biochemists.[1] One approach that is especially interesting involves the use of selectively raised antibodies; this idea, probably first proposed by Pauling[2] was succinctly stated by Jencks.[3] Since antibodies can be raised against essentially any chemical structure one can readily envisage the 'engineering' of complementary antibody combining sites to appropriately chosen haptens which will provide an environment for the selective binding of reactants and promotion of specific chemical reactions. Thus, antibodies raised against structures which mimic the transition states of chemical reactions should selectively catalyse those reactions. Thus far the hydrolysis of labile esters and carbonates has been reported[4] and recently true catalysis using antibodies has been demonstrated.[5]

We report another approach, in which an antibody combining site is designed to serve as a template to bring two reactants together in a selected, pre-reaction geometry. The model reaction chosen,[6] the photodimerization of methyl p-nitro-trans-cinnamate (1), seemed attractive because the cinnamate chromophore often provides information about intermolecular geometry;[7] the nitrophenyl group is strongly immunogenic;(1) absorbs light at wavelengths beyond those absorbed by protein; and the reaction (Scheme 1) had been previously investigated in detail and reported to yield four photodimers on irradiation in concentrated solution and little or no photodimerization in dilute solution.[8]

In view of the close similarity in molecular volume between two monomers and the corresponding photodimer,[9] we reasoned that antibodies raised against a derivative of, for
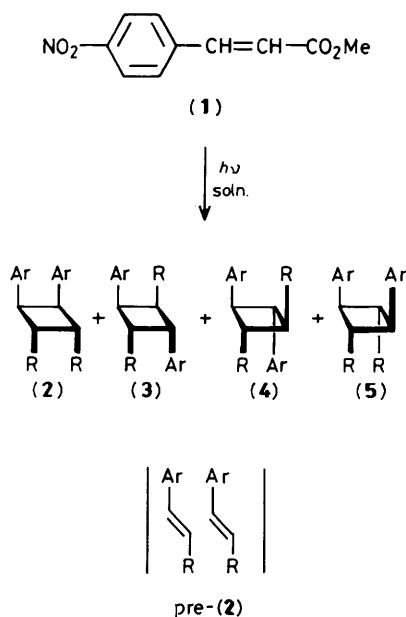
Scheme 1. Ar = $-p$-C$_6$H$_4$NO$_2$; R = $-$CO$_2$Me
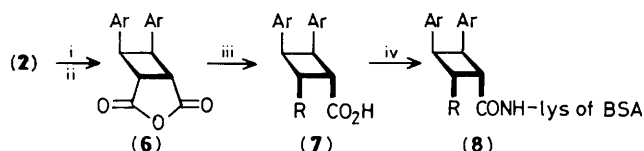
Scheme 2. *Reagents and conditions*: (i) H$_3$O$^+$; (ii) acetyl chloride/reflux; (iii) MeOH; (iv) BSA (H$_2$O)/1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide methotoluene-p-sulphonate (H$_2$O/pyridine). The spectroscopic, analytical, and/or chemical reactivity data obtained for (6) and (7) were in accord with their structures.

Table 1. ^{14}C-Distribution, %, on chromatogram after irradiation of (1)[a]

Component	t.l.c[b] R_f	$h\nu$, no additive	$h\nu$, with BSA	$h\nu$, with antibody
Monomer (1) (*cis* + *trans*)	0.41	56.8	34.4	26.0
(4)	0.28	28.9	41.1	34.8
'Mix-dimer'[c]	0.23	1.3	—	—
(2)	0.16	13.0	24.5	39.2
'Polymer'[d]	0.00	—	—	—

[a] Deaerated, aqueous/acetone solutions (4:1) were irradiated for 2 h in narrow glass tubes using a Rayonet reactor fitted with 3500 Å lamps; (1), 2 mм; antibody, 0.5 mм; BSA, same wt/ml as antibody. Irradiated protein-containing solutions, 50 µl, were incubated with a 100-fold excess of 'cold' irradiated (1) for 60 min before t.l.c. analysis; this procedure effectively released all non-covalently protein bound radioactive monomers and dimers. [b] Baker, channelled glass t.l.c. plates, Silica Gel G, 0.25 mm layer, fluorescent indicator, with preabsorbing area, were developed with benzene/ethyl acetate (9:1). Spots were detected by fluorescence; radiolabelled material was detected and counted with a BIOSCAN position-sensing proportional counter. Components gave a 3—10,000 d.p.m. reading; background was 50 d.p.m. Error limit ± 5%. [c] This spot, assigned earlier to a 1:1 mixture of dimers (3) and (5)[8] was found to be a single compound, formally, the product of *cis*-(1) + *trans*-(1), whose stereochemistry will be reported separately (H. Ziffer, A. Bax, R. J. Highet, and B. S. Green, *J. Org. Chem.*, in the press). This component was neglected in the % distributions of irradiation with protein. [d] The R_f = 0 spot, which may contain polymer, photodegradation products, substances covalently bound to protein, *etc.*, is not included in the % distribution; it typically contained *ca.* 15% of the radioactivity under the conditions used but increased with increasing irradiation time and after extended periods became the dominant spot.

example (2), would preferentially bind and orientate pairs of monomer (1) in the pre-reaction geometry (pre-2).

Compound (2) was converted into the bovine serum albumin (BSA) conjugate (8), as described in Scheme 2. [In the same way a rabbit serum albumin conjugate of (2) was prepared for quantitative immune precipitation.] The resulting analogue (8) was used to raise rabbit *anti*-(8) antibodies by standard methods. The antisera of two rabbits, containing 0.65 mg/ml and 0.70 mg/ml of specific antibody, respectively, were used.

Irradiation of aqueous acetone solutions of [^{14}C]methyl labelled (1) (prepared by the reaction of 4-nitrocinnamoyl chloride with [^{14}C]methanol) and unlabelled (1) gave an identical distribution and t.l.c. location of products, in overall accord with the reported results,[8] Table 1. When solutions of (1) were irradiated in the presence of BSA, which binds organic substances[10] but is not expected to display molecular selectivity for the dimers of (1), a slight preference for the dimer (2) over (4) was observed [(2):(4) ratio *ca.* 0.60:1 as compared to *ca.* 0.45:1 in the absence of protein]. However, a distinct preference for (2) was observed on irradiation in the presence of specific antibody, the ratio being 1.13:1. In addition, the rate of disappearance of (1) was appreciably

enhanced in the presence of antibody. In control experiments it was shown that BSA showed a slight preference for (2) over (4) whereas the *anti*-(8) antibodies displayed a greatly enhanced affinity for (2) over (4).

Antibody combining sites have been analysed in terms of subsites, each of which binds a unit or subgroup of the hapten or antigen.[11] It has long been known that the volume of the antibody combining site can accommodate more than one molecule,[12] but the binding of separate, unlinked moieties in adjacent subsites has not been previously studied.[13] This use of an antibody raised against a molecule A–A (or A–B) as a constraint (or template) for the *simultaneous* binding of A and A (or A and B) in order to promote a selective reaction within an antibody combining site has great potential for use in more significant and useful synthetic organic reactions *e.g.* Diels–Alder reactions, asymmetric photo- and thermal reactions, *etc.*

The reaction of (1) was complicated by the unexpected long wavelength absorption and photoinstability of the dimers and the poor solubility of reactants. Future work, we are confident, will provide improved template effects, including release of product and catalysis. This study represents only one of the many directions that may now be considered for antibody molecules acting as 'tailor-made,' enzyme-like promoters of organic reactions, in novel biotechnology, and even medical applications.

References

1 *E.g.*, 'Chemical Approaches to Understanding Enzyme Catalysis. Biomimetic Chemistry and Transition-State Analogs,' eds., B. S. Green, Y. Ashani, and D. Chipman, Elsevier, Amsterdam, 1982; A. I. Laskin, G. T. Tsao, and L. B. Wingard, Jr., *Ann. N.Y. Acad. Sci.*, 1984, **434**, 302—346; 'Design and Synthesis of Organic Molecules Based on Molecular Recognition,' Solvay Conference on Chemistry, ed., G. Van Binst, Springer–Verlag, Berlin, 1986; and refs. therein.

2 L. Pauling, *Chem. Eng. News,* 1946, **24**, 1375; *Am. Scientist*, 1948, **36**, 51.

3 W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw–Hill, New York, 1969, p. 288.

4 F. Kohen, J. B. Kim, H. R. Lindner, Z. Eshhar, and B. S. Green, *FEBS Lett.*, 1980, **111**, 427; A. Tramontano, K. D. Janda, and R. A. Lerner, *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 6736.

5 A. Tramontano, K. D. Janda, and R. A. Lerner, *Science*, 1986, **234**, 1566; S. J. Pollack, J. W. Jacobs, and P. G. Schultz, *Ibid.*, 1986, **234**, 1570; J. Jacobs, P. G. Schultz, R. Sugaswara, and M. Powell, *J. Am. Chem. Soc.*, 1987, **109**, 2174; A. Balan, I. R. Cohen, B. S. Green, and D. Elias, to be published.

6 This model was first suggested by Prof. W. A. Little of Stanford University (Conversations with M. D. Cohen and B. S. Green, July, 1974).

7 P. L. Egerton, E. M. Hyde, J. Trig, A. Payne, M. V. Mijovic, and A. Reiser, *J. Am. Chem. Soc.*, 1981, **103**, 3859, and refs. therein; B. S. Green, Y. Rabinsohn, and M. Rejto, *Carbohydr. Res.*, 1975, **45**, 115.

8 T. Ishigami, T. Murata, and T. Endo, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 3578.

9 H. Nakanishi, W. Jones, J. M. Thomas, M. Motevalli, and M. B. Hursthouse, *J. Phys. Chem.*, 1981, **85**, 8636.

10 F. Helmer, K. Kiehs, and C. Hansch, *Biochemistry*, 1968, **7**, 2858 and refs. therein.

11 I. Shechter, *Ann. N.Y. Acad. Sci.*, 1971, **190**, 394.

12 V. P. Kreiter and D. Pressman, *Immunochemistry*, 1964, **1**, 151.

13 See, however, V. Raso, and B. D. Stollar, *Biochemistry*, 1975, **14**, 584, 591.