rows, exhibits a stronger correlation between molecules in corrals and molecules on terraces. When the molecular rows in a corral have the same chirality as the rows on the nearby terrace, they can either align in the same direction or form intersection angles of ±120°. The threefold symmetry of graphite prohibits any other angles (*14*). Measurements of the intersection angle reveal a strong tendency for molecular rows in corrals to align in the same direction as those on the terrace. Intersection angles of ±120° are observed in less than 20% of all corrals studied. In the absence of orientational interactions, the expected purely statistical value would be 67%. Also in contrast to the case for the chirality ratio, the tendency of molecular rows in corrals to align in the same direction as those on the terrace depends strongly on corral size. In small corrals (~500 Å in radius), the proportion aligned at ±120° approaches 0% but increases steadily to 18% for corrals 1347 Å in radius, the largest size studied. This trend appears to reflect an unexpectedly long-range orientational interaction, acting over tens or hundreds of angstroms. It may perhaps be communicated through second and higher layers of 8CB not adsorbed directly to the substrate (*15*).

These findings demonstrate that molecule corrals permit the analysis of a large number of semi-independent, nanometer-sized sample ensembles, each with an identical thermal history. A statistical approach can be taken to STM data analysis that may open new avenues for observing and modeling surface phenomena by scanning probe microscopy. Corrals may also be used in studies of confinement effects on monolayer structure, in the determination of activation energies for nucleation through variable-temperature STM, in studies of the strength and distance dependence of molecular interactions between nearby crystalline domains, and in the calculation of the relative configurational free energies of different molecular structures from their measured occurrence probabilities.

REFERENCES AND NOTES

1. A. Ulman, *An Introduction to Ultrathin Organic Films* (Academic Press, San Diego, CA, 1991).
2. G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **254**, 1312 (1991).
3. M. F. Crommie, C. P. Lutz, D. M. Eigler, *ibid.* **262**, 218 (1993).
4. H. Chang and A. J. Bard, *J. Am. Chem. Soc.* **112**, 4598 (1990).
5. ———, *ibid.* **113**, 5588 (1991).
6. X. Chu and L. D. Schmidt, *Carbon* **29**, 1251 (1991); *Surf. Sci.* **268**, 325 (1992); F. Atamny, R. Schlogl, W. J. Wirth, J. Stephan, *Ultramicroscopy* **42**, 660 (1992); L. M. Siperko, *J. Vac. Sci. Technol. B* **9**, 1061 (1991).
7. J. S. Foster and J. E. Frommer, *Nature* **333**, 542 (1988).
8. D. L. Patrick and T. P. Beebe Jr., *Langmuir* **10**, 298 (1994), and references therein.
9. R. Hentschke, B. L. Schürmann, J. P. Rabe, *J. Chem. Phys.* **96**, 6213 (1992).
10. The effect of tip-induced ordering and nucleation was considered and rejected, because the rate constants did not depend on the amount of time the sample was scanned.
11. For those rare times in which molecules in a corral order during imaging, the process is completed within the time required to collect a few points along a scan line (tens of milliseconds). Furthermore, partially ordered corrals are never observed: Corrals are either completely ordered or disordered.
12. L. Kuipers, M. S. Hoogeman, J. W. Frenken, *Phys. Rev. Lett.* **71**, 3517 (1993).
13. The helicity (handedness) of an 8CB monolayer describes whether the periodic dislocation that occurs every fourth molecule is toward the left or toward the right when viewed along a row (see Fig. 1B). Domains with opposite helicities are mirror images of one another, not superimposable by rotation alone [see D. P. E. Smith, *J. Vac. Sci. Technol. B* **9**, 1119 (1991)].
14. When the chirality of molecular rows is different, other intersection angles are possible. For simplicity, we discuss here only the subset of pits containing molecular rows with the same chirality as the terrace; however, pits with molecular rows of different chirality than the terrace follow a similar trend.
15. At the temperatures used in this study, bulk 8CB is a layered smectic-A phase with an interlayer distance similar to the interrow spacing of the molecules adsorbed to graphite (29 to 32 Å versus ~38 Å) (*7*). If the smectic planes are oriented approximately normal to the surface in the region of the substrate, they could provide an efficient route for promoting long-range alignment.

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Peptide Synthesis Catalyzed by an Antibody Containing a Binding Site for Variable Amino Acids

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Monoclonal antibodies, induced with a phosphonate diester hapten, catalyzed the coupling of *p*-nitrophenyl esters of *N*-acetyl valine, leucine, and phenylalanine with tryptophan amide to form the corresponding dipeptides. All possible stereoisomeric combinations of the ester and amide substrates were coupled at comparable rates. The antibodies did not catalyze the hydrolysis of the dipeptide product nor hydrolysis or racemization of the activated esters. The yields of the dipeptides ranged from 44 to 94 percent. The antibodies were capable of multiple turnovers at rates that exceeded the rate of spontaneous ester hydrolysis. This achievement suggests routes toward creating a small number of antibody catalysts for polypeptide syntheses.

The chemical synthesis of large peptides has been accomplished by two distinct but not mutually exclusive approaches. The first, involving the stepwise addition of single amino acids from the COOH- to the NH_2-terminus (*1*), was revolutionized by the advent of the Merrifield solid-phase technique (*2*), in combination with reversed-phase, high-performance liquid chromatography (HPLC) purification techniques (*3*, *4*). Fragment condensation, the second approach, permitted the first total synthesis of an enzyme in solution in 1969 (*5*). The fragment condensation strategy is particularly attractive for the synthesis of chemically related proteins that differ in one or more of the fragments. Solubility problems, a result of multiple hydrophobic protecting groups, have limited the usefulness of this approach. In an effort to overcome this problem, proteolytic enzymes have been used in peptide bond formation (*6*). This variation of the fragment condensation method is also beset by limitations, principally product inhibition and the susceptibility of the products to cleavage at positions corresponding to the natural hydrolytic sites.

The advent of antibodies as catalysts (*7*) for organic transformations suggested another approach to the coupling of unprotected amino acids and peptide fragments that potentially would exceed the scope and specificity attainable with proteolytic enzymes as catalysts. We report here the antibody-catalyzed formation of a dipeptide (*8*).

The traditional approach to generating catalytic antibodies involves the design of haptens that are topologically and structurally nearly identical to the transition state of the reaction. For peptide synthesis (Fig. 1), this strategy has two shortcomings: first, a large

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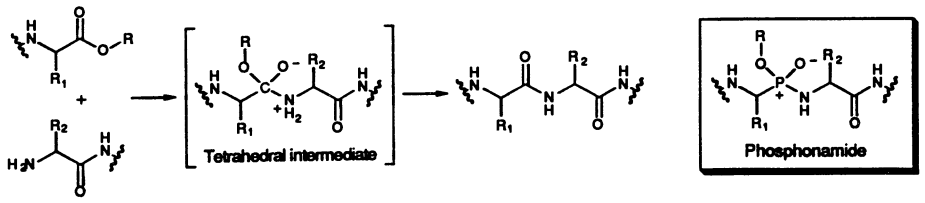

Fig. 1. The condensation of an NH_2-terminus of a peptide or amino acid with the COOH-terminus of a peptide or amino acid and the presumed tetrahedral intermediate formed along the reaction pathway. The phosphonamide is a transition-state analog for such coupling reactions. R = H, *p*-NO_2-(C_6H_4)CH_2-, Cl-(C_6H_4)-, or *p*-NO_2-(C_6H_4)-; R_1 = -$CHMe_2$, -CH_2CHMe_2, or -CH_2Ph; R_2 = -CH_2-(3-indolyl). Ph, phenyl; Me, methyl.

number of antibody catalysts would be required to accommodate diverse acylating agents and nucleophiles; second, product inhibition, previously encountered in nonsolvolytic bimolecular coupling reactions, would limit the turnover number of the catalyst. These design requirements led to transition-state analogs **1** and **2**. Analog **2a** (*9*) was used to generate antibodies for coupling *N*-acetylated, *p*-nitrophenyl esters of amino acids **5a** to **5c** to tryptophan amide (**6**) to form dipeptides **7a** to **7c** (Fig. 2). We reasoned that the cyclohexyl group of the transition-state analog would create a binding site in the antibody that would accommodate diverse hydrophobic α side chains of the ester substrates. Further, the transition state analog possessed an additional structural element (*p*-nitrobenzyl) that was neither present in the dipeptide product nor structurally or electronically congruent to the leaving group (*p*-nitrophenyl). These structural differences should facilitate dissociation of the leaving group and dipeptide from the antibody.

Twenty-four monoclonal antibodies were screened for their ability to catalyze the coupling of the L-valine derivative **5a** with D-tryptophan amide (**6**) to form dipeptide **7a** (*10*, *11*). Two antibodies (16G3 and 18C10) accelerated the reaction approximately 220-fold above the control (without antibody) (*12*). However, these two antibodies did not significantly accelerate the coupling of the *p*-chlorophenyl (**3**) or *p*-nitrobenzyl esters (**4**) of *N*-acetyl-L-valine. This result demonstrated that the reaction involved a specific antibody interaction with the *p*-nitrophenyl leaving group and did not simply arise from a higher intrinsic reactivity of the leaving group in aqueous aminolysis reactions (*13*). Moreover, the increase in rates was proportional to the antibody concentration, and peptide bond formation was completely inhibited by the addition of an amount of hapten **2b** equal to twice the antibody concentration. Antibodies 16G3 and 18C10 catalyzed dipeptide formation with activated *N*-acetyl-L-leucine **5b** at rate enhancements of 383- and 323-fold, respectively. Further studies with 16G3 and activated *N*-acetyl-L-phenylalanine **5c** yielded dipeptide **7c** at rates >1000-fold over background (*14*). The relative rates consequently increased as the α side chain on the ester substrate more extensively occupied the cavity created by the cyclohexyl group of the hapten.

Steady-state kinetic analysis of the 18C10-catalyzed condensation of L-**5a** with L-**6** provided Michaelis constant (K_m) values of 4 mM and 1.6 mM, respectively, and a catalytic rate constant (k_{cat}) of 12.6 min^{-1}. The intersecting graphical pattern was in accord with an equilibrium bireactant system (*15*). The effective molarity for the antibody condensation was 194 M. With L-**5c**, the effective molarity was about 1000 M, although kinetic measurements were constrained by low solubility. In contrast, the effective molarities for nonsolvolytic, bimolecular condensation reactions catalyzed by antibodies are 16 M for the formation of an amide from the condensation of a lactone with 1,4-phenylenediamine (*16*), a calculated value of 0.21 M for bimolecular imine formation (*17*), and a calculated value of 50,000 M for the aminoacylation of the 3′-hydroxyl group of thymidine with an alanyl ester [although this reaction was seriously inhibited by the product (*18*)].

It is well known that *N*-acylated *p*-nitrophenyl esters are susceptible to racemization (*19*), and as expected the amount of the second diastereomeric dipeptide (attributable to the racemization of esters L-**5a** and L-**5b** when coupled to L-**6**) reached 25% of the total product formed within 6 min without antibody added (it was undetectable for L-**5c** in this time span). However, in the antibody-catalyzed reactions this value was only 4% for L-**5a**, 0.5% for L-**5b**, and undetectable for L-**5c**, which suggests that the antibody does not catalyze the intramolecular cyclization of the *N*-acetyl

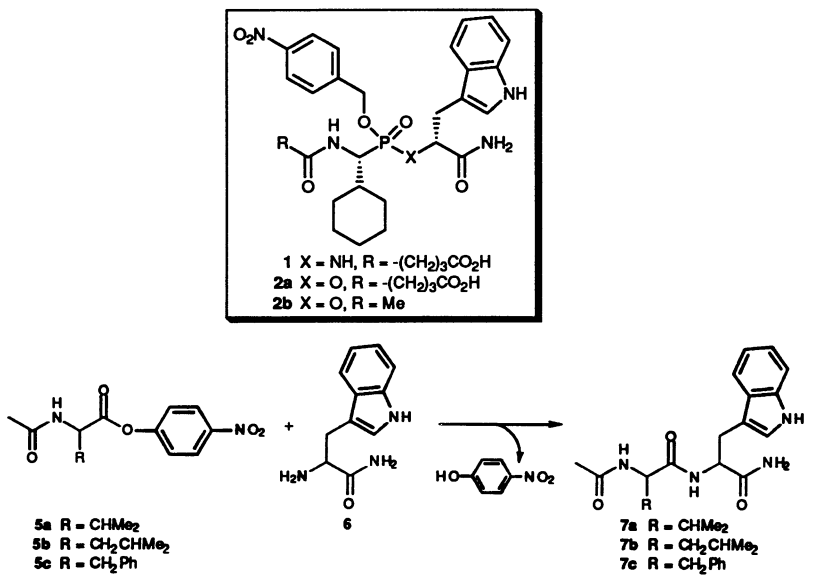

Fig. 2. Phosphonamide and phosphonate haptens, with substrates and products for the antibody-catalyzed reaction. Both the levorotatory and dextrorotatory forms of the substrates were used.

Table 1. Relative rates for the formation of the four stereoisomeric dipeptides from the D and L stereoisomers of **5a**, **5b**, **5c**, and **6** with monoclonal antibody 16G3. The reactions were performed as described (*11*). The rates of the reaction between the L form of each ester (**5a**, **5b**, and **5c**) and the L form of the amide **6** are set to 1.0.

Substrates		Rate
5	**6**	
L (**5a**)	D	0.2
L	L	1*
D	D	0.1
D	L	1.5
L (**5b**)	D	0.5
L	L	1*
D	D	0.4
D	L	1.2
L (**5c**)	D	0.5
L	L	1*
D	D	0.07
D	L	0.4

*__5a:5b:5c__ are in the relative rates of 1:1.7:4.7.

esters to produce the oxazolones that are prone to racemization (*19*). Moreover, under these conditions the antibody did not catalyze the hydrolysis of the ester substrates or amide product.

Antibody 16G3 was also examined for its ability to couple various combinations of stereoisomeric forms of the ester and amine substrates (Table 1). Regardless of the configuration of the ester, the reaction with the L-tryptophan amide was slightly favored throughout the series **5a** to **5c** by rate factors of 2- to 15-fold. Conversely, there was a smaller preference (up to approximately eightfold for L-**5c** with D-**6** versus that for D-**5c** with D-**6**) for the stereochemistry of the ester. The rate differences observed amounted to a change in $\Delta G°$ (change in the Gibbs free energy) of <1.6 kcal mol^{-1}. Although the antibodies were induced to a hapten containing the levorotatory and dextrorotatory forms of the carboxyl and amine components, respectively, these studies revealed no strong stereochemical preferences.

The lack of strong preference for substrate stereochemistry is not surprising because the antibody is programmed to provide binding pockets for the nitroacyl group and for the α side chains of the reactants. In contrast, the small size of the NH_2-terminal acetamide, COOH-terminal carboxamide, and α protons may be accommodated by nonspecific binding. One can imagine that the antibody binding site may be a shallow channel, as is revealed by x-ray crystallographic analysis of the antibodies that bind lysozyme and peptides (*20*). The general lack of a synergistic influence by various substrate pairs on the substrate selectivity of the antibody is also consistent with little or no cooperativity between the antibody binding sites, in accord with our kinetic analysis. This cross-reactivity with the various stereoisomers resembles the limited complementarity to the steroid in the x-ray crystallographic structure of the progesterone antibody (*21*). The binding site, nevertheless, is limited; 16G3 does not catalyze the condensation of **5a** with either the desired α or undesired ϵ amino groups of D-tryptophan-L-lysine amide.

We determined the total yield of dipeptide (relative to ester) produced in the presence of antibody and the turnover number after which all of the ester substrate had been consumed by condensation or hydrolysis (Table 2). The antibody produces dipeptide with yields ranging from 44 to 94%. Moreover, for the leucine and phenylalanine-derived substrates (**5b** and **5c**), product formation in the presence of antibody competes with ester racemization so that the amount of product formed by means of racemized oxazolone over the time of synthesis is suppressed (Table 2). With higher concentrations of antibody, this percentage would be further decreased. The turnover number for 16G3 suggests that the antibody does not undergo autocatalytic deactivation (*22*) and, more importantly, is not subject to extensive product inhibition. The eventual onset of product inhibition reduces the correlation between initial rate and observed yields (compare Table 1 to Table 2).

Table 2. Yield of dipeptide formed from the coupling of **5a**, **5b**, **5c**, and **6** in the presence of monoclonal antibody 16G3. The reactions were performed as described (*11*), except the reaction was monitored until all of the ester substrate was consumed by condensation or hydrolysis. The yield of dipeptide formed is based on the starting ester, which was the limiting substrate. Turnover number is based on the amount of dipeptide product formed by antibody-catalyzed coupling of the substrates. Racemization is defined as the ratio of undesired dipeptide to desired dipeptide. ND, not determined.

Substrates		Yield of dipeptide (%)	Turnover	Racemization (%)	
5	6			With 16G3	Without 16G3
L (**5a**)	D	ND	ND	ND	ND
L	L	75	30	12	90
D	D	44	13	27	90
D	L	ND	ND	ND	ND
L (**5b**)	D	94	38	10	100
L	L	56	21	10	66
D	D	53	24	10	66
D	L	73	33	10	100
L (**5c**)*	D	79	3.7	1	ND
L	L	52	2.3	1	43
D	D	61	2.1	6	43
D	L	61	2.7	4	ND

*Because of limited solubility, the concentration of **5c** was 100 μM.

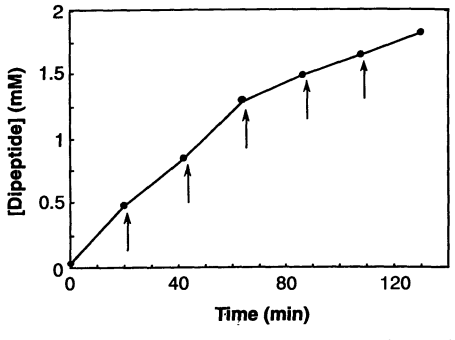

Fig. 3. Amount of dipeptide product formed from the reaction of L-**5b** and D-**6** in the presence of 20 μM 16G3. The reaction was performed as described (*11*). Aliquots were withdrawn at 20-min intervals, and the amount of dipeptide product was determined with HPLC. Approximately 2 min after removal of an aliquot, **5b** and **6** were added (indicated by arrows) to restore their concentrations to 1.0 to 1.3 mM and 2.0 to 2.3 mM, respectively. The products were isolated, and their structures confirmed by mass spectrometry.

To illustrate the low level of product inhibition, we carried out a scale-up synthesis of the L-leucine-D-tryptophan amide dipeptide by multiple additions of substrates (Fig. 3). After approximately 1 hour, dipeptide with a concentration of 1.3 mM was formed in the presence of 20 μM antibody and, most importantly, the D,D-dipeptide accounted for only 4.0% of the total amount of dipeptide formed. In contrast, in the absence of antibody the reaction produced a dipeptide product with a concentration of only 35 μM, 47% of which was the D,D-dipeptide. The amount of product formed with time slowed noticeably at product concentrations greater than 1.2 mM, suggesting that the dissociation constant for dipeptide-bound 16G3 is >1.2 mM^{-1} with a turnover number for the antibody >60. All of the kinetic parameters discussed herein meet previous kinetic criteria (*23*) and are commensurate with the synthetic potential of this catalytic antibody. These results represent a first step toward generating antibodies capable of coupling unprotected amino acids and peptide fragments.

REFERENCES AND NOTES

1. R. Schwyzer and P. Sieber, *Helv. Chim. Acta* **49**, 134 (1966).
2. R. B. Merrifield, *J. Am. Chem. Soc.* **85**, 2149 (1963); B. Gutte and R. B. Merrifield, *ibid.* **91**, 501 (1969).
3. G. Siepke, H. Mullner, U. Grau, *Angew. Chem. Int. Ed. Engl.* **25**, 535 (1986).
4. E. T. Kaiser *et al.*, *Science* **243**, 187 (1989); J. Rivier, *J. Chromatogr.* **1**, 343 (1978); C. Hoeger, J. Porter, J. Boublik, J. Rivier, *ibid.* **404**, 307 (1989); D. A. Kirby, C. L. Miller, J. E. Rivier, *ibid.* **648**, 257 (1993).
5. R. Hirschmann *et al.*, *J. Am. Chem. Soc.* **91**, 509 (1969).
6. H.-D. Jakubke, in *The Peptides*, S. Udenfriend and J. Meienhofer, Eds. (Academic Press, San Diego, CA, 1987), vol. 9, chap. 3, pp. 103–159. An attractive molecular biology–based approach has recently been described [L. Arahmsér *et al.*, *Biochemistry* **30**, 4151 (1991)].
7. R. A. Lerner, S. J. Benkovic, P. G. Schultz, *Science* **252**, 659 (1991); S. J. Benkovic, *Annu. Rev. Biochem.* **61**, 29 (1992).
8. Although ours is the first documented, P. G. Schultz [*Angew. Chem. Int. Ed. Engl.* **28**, 1283 (1989)] has referred to unpublished results for peptide bond formation.
9. Although our initial target hapten was **1**, incorporating a phosphonamide unit as the mimic of the tetrahedral intermediate, phosphonate **2** proved to be more synthetically accessible. An immunogenic conjugate was then prepared from **2a** and keyhole lympet hemocyanin. Monoclonal antibodies were prepared by standard protocols [G. Kohler and C. Milstein, *Nature* **256**, 495 (1975)].
10. The 24 monoclonal antibodies were selected on the basis of their ability to bind a bovine serum albumin conjugate with **2** and were purified to >90% homogeneity, as judged by SDS-gel electrophoresis. All 24 antibodies were screened for their ability to catalyze the coupling of the *p*-chlorophenyl, *p*-nitrobenzyl (2.0 mM), or *p*-nitrophenyl (1.0 mM) esters of *N*-acetyl-L-valine (**3**, **4**, and **5a**, respectively) with 2.0 mM D-tryptophan amide **6** at pH 8.3 (**3** and **4**) or pH 7.0 (**5a**) to form dipeptide **7a**.
11. All measurements were carried out at 25°C in 0.1

M 3-[*N*-morpholino]propanesulfonic acid [ionic strength = 0.10 (NaCl) (pH 7.0)] and 5% dimethyl sulfoxide containing 20 μM antibody. The concentration of esters **5a** and **5b** was 1 mM, amine **6** was 2 mM, and, because of limited solubility, **5c** was 100 μM. After 20 s and 6 min, an aliquot was withdrawn and quenched with perchloric acid to a final pH of approximately 2.5. The quenched sample was injected into an HPLC column (Waters 600E) equipped with an analytical C_{18} reversed-phase column (VYDAC, Hesperia, CA) and eluted with an acetonitrile-water (0.1% trifluoroacetic acid) gradient with the detector set at 280 nM. We identified the product by comparing the retention time of the product formed during the reaction with that of authentic samples. The relative rates were determined by dividing the amount of **7** formed by the antibody with that formed in the control reaction.

12. The control experiments were carried out under the same conditions as described (*11*), except antibody was not added to the reaction mixture.
13. A. R. Fersht and W. P. Jencks, *J. Am. Chem. Soc.* **92**, 5442 (1970).
14. This is the lower limit with an estimated spontaneous rate of 0.06 μM min^{-1}.
15. R. Hirschmann *et al.*, unpublished results.
16. A. D. Napper, S. J. Benkovic, A. Tramontano, R. A. Lerner, *Science* **237**, 1041 (1987).
17. A. G. Cochran, T. Pham, R. Sugasawara, P. G. Schultz, *J. Am. Chem. Soc.* **113**, 6670 (1991).
18. J. R. Jacobsen, J. R. Prudent, L. Kochersperger, S. Yonkovich, P. G. Schultz, *Science* **256**, 365 (1992). In this case, the dissociation constant of 18 nM for the product relative to a value of 0.77 mM for the alcohol substrate effectively inhibited the reaction after one turnover.
19. A. L. Heard and G. T. Young, *J. Chem. Soc.* **1963**, 5807 (1963); N. L. Benoiton, in *The Peptides*, S. Udenfriend and J. Meienhofer, Eds. (Academic Press, San Diego, 1983), vol. 5, chap. 4.
20. R. Stanfield, M. Takimoto-Kamimura, J. Rini, A. T. Profy, I. Wilson, *Structure* **1**, 83 (1993); J. Moult *et al.*, *J. Mol. Biol.* **74**, 137 (1976).
21. J. H. Arevalo, M. J. Tausig, I. A. Wilson, *Nature* **365**, 859 (1993).
22. B. S. Green, in *Catalytic Antibodies* (CIBA Foundation Symposium 159, Wiley, West Sussex, United Kingdom, 1991), p. 139.
23. J. D. Stewart and S. J. Benkovic, *Chem. Soc. Rev.* **22**, 213 (1993).
24. R.H. acknowledges J. R. Huff, E. Thornton, and T. Widlanski for substantive discussions. We thank D. Schloeder for assistance in the preparation of the monoclonal antibodies and B. Jiang for assisting S.D.T. and P.A.B. Supported by NIH (Institute of General Medical Sciences) through grant GM-45611 (R.H. and A.B.S.), Bachem (R.H.), and Merck Research Laboratories (R.H.). C.M.T. is a recipient of a William Georgetti Scholarship. S.D.T. is a recipient of a Natural Sciences and Engineering Research Council of Canada postdoctoral fellowship. K.M.Y. is a recipient of an NIH (National Cancer Institute) postdoctoral fellowship.

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Homozygous Human TAP Peptide Transporter Mutation in HLA Class I Deficiency

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Human lymphocyte antigen (HLA) class I proteins of the major histocompatibility complex are largely dependent for expression on small peptides supplied to them by transporter associated with antigen processing (TAP) protein. An inherited human deficiency in the TAP transporter was identified in two siblings suffering from recurrent respiratory bacterial infections. The expression on the cell surface of class I proteins was very low, whereas that of CD1a was normal, and the cytotoxicity of natural killer cells was affected. In addition, $CD8^+$ αβ T cells were present in low but significant numbers and were cytotoxic in the most severely affected sibling, who also showed an increase in $CD4^+CD8^+$ T cells and γδ T cells.

Class I molecules of the major histocompatibility complex (MHC) present peptides to $CD8^+$ T cells. These peptides derive from proteolytic degradation in the cytosol. They are subsequently imported by a peptide transporter into the lumen of the endoplasmic reticulum where they associate with class I molecules (*1*). The peptide transporter is a heterodimeric protein formed of two homologous polypeptides encoded by the *TAP1* and *TAP2* genes located in the MHC class II region (*2–4*). In mutant cell lines that do not express this transporter, most β_2-microglobulin (β_2M)–class I heavy chain complexes do not acquire peptides. Such "empty" complexes are unstable at physiological temperature and are inefficiently transported through the Golgi compartment. Consequently, most of the class I heavy chains remain unsialylated. Relatively few peptide-free class I molecules reach the cell surface where they can be stabilized by exogenous class I–specific peptides (*5*). Mice in which the *TAP1* gene has been disrupted by homologous recombination have almost no detectable $CD8^+$ T cells and no alloreactive cells when bred in germ-free conditions (*6*). In this report we describe a human peptide transporter (TAP) genetic defect.

A TAP deficiency was observed in family E which is from Morocco and includes the parents, who are first cousins, and five children. The first case, EFA, was a 15-year-old female who had chronic bacterial sinobronchial infections but no history of viral infections. Human lymphocyte antigen (HLA) serotyping did not detect HLA class I molecules on her peripheral blood mononuclear cells (PBMCs). A deficiency in β_2M was ruled out. A complete HLA typing of the family (Fig. 1A) showed that two children in the family, EFA and a 6-year-old brother, EMO, are HLA-homozygous and express class II but not class I molecules. The other members of the family, who are heterozygous or HLA different (EAH) express both class I and class II antigens. These results suggest that the defect is genetically linked to the MHC.

Because "absence" of class I molecules as determined by serological typing methods does not mean complete absence of these molecules from the cell surface, we labeled PBMCs with the W6/32 class I monomorphic monoclonal antibody (mAb) and analyzed them by flow cytometry (Fig. 1B). The fluorescence intensity was 1% (T cells and monocytes) to 3% (B cells) that of normal PBMCs. Epstein-Barr virus (EBV)–transformed B cell lines (named ST-XXX) were generated from B cells from EMO and EAH. Flow cytometry showed a 99% reduction of class I molecules on the ST-EMO cells compared with the ST-EAH cell line. Because both cell lines expressed equivalent amounts of class I mRNA, detected by Northern (RNA) blot analysis, we looked for defects in processing of class I molecules. The time course of polysaccharide side chain maturation in ST-EMO (class I^-) and SCH10W9013, a control HLA-A3–homozygous cell line, showed that HLA-A3 molecules became resistant to endoglycosidase H (endo H) (Fig. 1C); thus, they were transported to the *cis*-Golgi compartment. However, whereas endo H–resistant molecules remained stable after a 2-hour chase in SCH10W9013, those of ST-EMO decreased with time. In the extracts of ST-EMO, β_2M was poorly detected, showing that it was loosely associated with the class I heavy

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