An Antibody-Catalyzed Nucleophilic Substitution Reaction at a Primary Carbon That Appears To Proceed by an Ionization Mechanism

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Received November 8, 1994

Nucleophilic substitutions are arguably the most thoroughly studied and best understood reactions in organic chemistry. Both S_N1 and S_N2 mechanisms are operative for reactions occurring at an aliphatic carbon atom. While an S_N2 mechanism is usually displayed when substitution takes place on a primary carbon, an S_N1 mechanism predominates in reactions involving a tertiary carbon center. In contrast to chemical reactions, the mechanisms by which nucleophilic substitution occurs in biological systems are poorly understood. Antibody catalysis allows a fresh look at the problem because one can utilize highly specific binding energy to isolate reaction routes from an otherwise complex spectrum.[1] Herein we report antibody catalysis of a nucleophilic substitution reaction with interesting mechanistic parameters involving development of a high degree of carbocation character at a primary carbon (Scheme 1).

The *N*–oxide functionality of hapten **2** was designed to induce antibodies that catalyze ionization of sulfonate **1** (Figure 1) by induction of complementary charges in the binding pocket which interact with the developing carbocation and oxyanion such that the ionization event at the primary carbon center is not prohibitively high in energy.[2] Several antibodies to this hapten catalyzed the cationic cyclization of olefinic substrates.[3] One antibody, 16B5, was interesting because, while it was not a cyclization catalyst, it was alkylated during the reaction, suggesting the formation of a reactive substrate carbocation in the antibody binding site. In contrast to its alkylation with olefinic substrates, this antibody catalyzes nucleophilic substitution of the 4-acetamidobenzene sulfonate group in substrate **1** by sodium iodide.

The mass balance of the antibody-catalyzed release of 4-acetamidobenzenesulfonic acid matches the production of alkyl iodide **4**.[4] Under saturating conditions (**1**, 750 μM), a double reciprocal relationship between the rate of the antibody-catalyzed reaction and the NaI concentration was observed (Figure 2). These results are consistent with a model in which NaI binds to the antibody rather than simply acting as an external nucleophile. The catalyst was efficient, exhibiting multiple turnovers and no product inhibition (k_{cat} = 0.028 min^{-1}; k_{cat}/k_{uncat} = 580 M; K_m for NaI at saturating substrate **1** concentration = 150 mM; K_m for substrate **1** at 150 mM NaI concentration = 130 μM). The catalyzed reaction is inhibited by hapten **2**, with a K_i of approximately 10 μM).

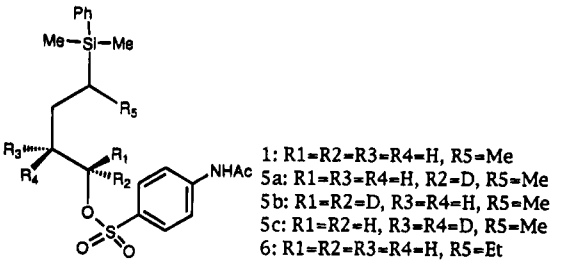
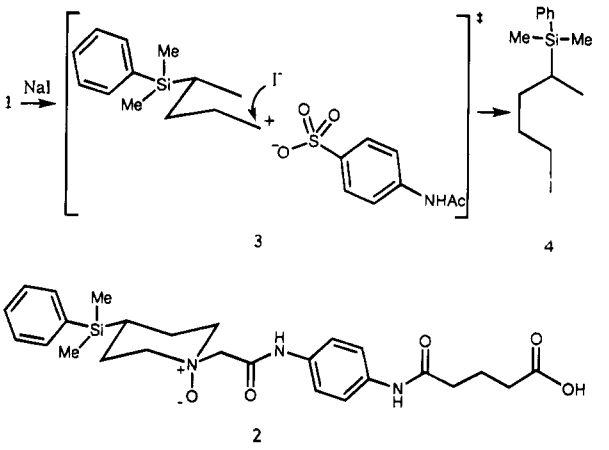
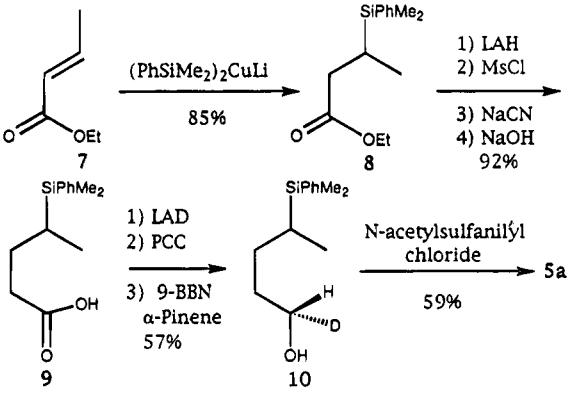


Figure 1. Substrates for the nucleophilic substitution reaction.

Scheme 1. Reaction Mechanism and Hapten Design for Nucleophilic Substitution Reaction



Scheme 2. Synthesis of Substrate **5a**



To determine the stereochemical outcome at the primary carbon where the substitution reaction takes place, one of the methylene hydrogens was replaced with deuterium to introduce chirality. Compound **5a** bearing the chiral methylene group was synthesized according to Scheme 2. The enantiomeric purity of the S-enriched **5a** was determined[5] by NMR of the Mosher's ester of alcohol **10** to be 92%. Incubation of compound **5a** with IgG16B5 and NaI overnight led to the desired alkyl iodide, which was then converted to the Mosher's ester with Mosher's acid and Cs_2CO_3 in DMF. The NMR spectrum of the resulting Mosher's ester indicated a very high stereospecificity of the *S* configuration (89% ee) for the chiral methylene group. Since the reaction of the primary alkyl iodide with the cesium salt of Mosher's acid in DMF should occur with inversion at the chiral methylene group, the observed enantioselectivity shows that the reaction proceeds by inversion of the configuration at the primary carbon in the antibody-catalyzed reaction.[6]

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(1) Janda, K. D. *Biochem. Soc. Trans.* **1993**, *21*, 1090.

(2) Janda, K. D.; Chen, J. Y.-C. The pharmacology of monoclonal antibodies. *Handbook of Experimental Pharmacology*; Springer-Verlag: Berlin, 1994; Vol. 113, pp 209–242.

(3) Li, T.; Janda, K. D.; Ashley, J. A.; Lerner, R. A. *Science* **1994**, *264*, 1289.

(4) A biphasic reaction (Ashley, J. A.; Janda, K. D. *J. Org. Chem.* **1992**, *57*, 6691) was set up containing 5 μM 16B5 antibody, 200 μM substrate **1**, and 15 mM NaI. The reaction proceeded at 37 °C for 48 h and was halted by the separation of the two phases. Analysis of both phases allowed determination of the product ratio, which was found to be 28 μM 4-acetamidobenzenesulfonic acid and 30 μM alkyl iodide **4**. All the concentrations reported in this paper were calibrated on the basis of the total volume of the reaction.

(5) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

(6) The slight amount of epimerization seen may be the result of an uncatalyzed reaction between **4** and sodium iodide.

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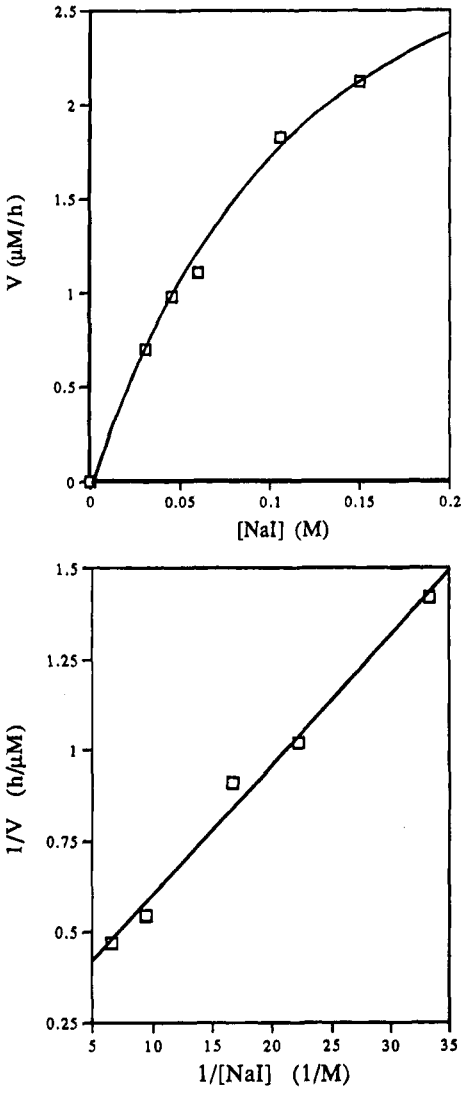

Figure 2. Rate dependence of the antibody-catalyzed reaction on NaI concentration.

The hapten design calls for an ionization mechanism in which the primary carbon has considerable cationic character. To investigate this possibility, we first determined the dependence of the rate of the catalyzed reaction on the nature of the nucleophile. NaSCN, NaN_3, and NaI were substrates, while NaBr, NaCl, and NaCN failed. The relative k_{cat} values at fixed substrate **1** concentration (200 μM) are 1.0 for NaSCN, 1.4 for NaI, and 1.7 for NaN_3. In contrast, the relative rates of the uncatalyzed reaction between substrate **1** and the three nucleophiles in DMSO are 1 for NaSCN, 4.4 for NaI, and 18 for NaN_3.[7] The relative lack of influence of the nature of the nucleophile on k_{cat} suggests significant carbocation character in the transition state of the catalyzed reaction. Next, secondary isotope effects were studied to provide additional evidence for the existence of a carbocation intermediate.[8] The antibody-catalyzed reaction containing 200 μM of the α-dideuterated substrate **5b** and 150 mM NaI is 23% slower than that of substrate **1**. These rate differences lead to a secondary isotope effect of 1.30 ± 0.04. Assuming that both deuteriums contribute equally to the observed isotope effect, the contribution of each deuterium is 1.14 ± 0.02, which is a value typically seen for reactions involving a change from an sp^3 center to an sp^2 center.[9] In comparison, the uncatalyzed reaction of these same substrates (*vide supra*) yielded an isotope effect of 1.03 ± 0.01, which is a typical value for an S_N2 reaction.[10] The β-isotope effect was also investigated using compound **5c** as a substrate. A β-isotope effect of 1.08 ± 0.01 was observed for the antibody-catalyzed reaction, while a slightly higher isotope effect (1.10 ± 0.02) was observed for the uncatalyzed reaction. Taken together, the α and β secondary isotope effects suggest the formation of a carbon atom that is sp^2 but has diminished capacity for hyperconjugation with the β-carbon−hydrogen/deuterium bond. The lack of a significant β-isotope effect is not surprising since the antibody is induced to a charged, chair-shaped hapten where hyperconjugation is disfavored by a combining site which reduces the electrophilicity of the sp^2 carbon with a counter-charge and/or favors a staggered conformation of the substituents on the α- and β-carbons, thereby precluding the requisite orbital overlap.[11]

A mechanism which requires the formation of a contact ion pair is consistent with our experimental evidence.[12] In this model, we suggest that 16B5 catalyzes the formation of a contact ion pair within substrate **1** as outlined in Scheme 1. In the absence of a properly bound nucleophile, a nonproductive equilibrium exists between the contact ion and **1**. However, when a properly oriented nucleophile is allowed to attack the contact ion from the back side, nucleophilc substitution products are observed. This mechanism is further supported by the fact that compound **6** failed as a substrate for this reaction. In accordance with our hapten design, binding of substrate **6** in a chair conformation in the antibody cleft would block back-side attack with the extra methyl group and thus preclude product formation.

Acknowledgment. We thank Diane M. Schloeder, Ping Fan, and Jon A. Ashley for technical assistance. We would also like to thank Professor Andrew Streitwieser for a preprint of his work before publication and Professors Jack Kirsch and Richard L. Schowen for helpful suggestions. This work was supported in part by NIH Grant GM-43858 (K.D.J.) and a fellowship (K.D.J.) from the A. P. Sloan Foundation.

Supplementary Material Available: Experimental procedures for the synthesis of substrates, determination of the stereochemistry, and secondary isotope effect measurements (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA943647Q

(7) A strict comparison between the catalyzed and the uncatalyzed rates requires knowledge of IgG16B5's combining site. Without this knowledge, we suggest the nonprotonic solvent, DMSO, to be representative of the microenvironment of an antibody's binding pocket. However, if one wants to compare the relative rates of the uncatalyzed reaction under our biphasic conditions, they are 1 for NaSCN, 0.3 for NaI, and 0.5 for NaN_3. We advise caution in applying these rates in a direct comparison with the relative k_{cat} values since they are affected and thus controlled by the different partition behaviors of these nucleophiles under our biphasic conditions.

(8) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*; Harper & Row: Cambridge, 1987; Chapter 2. See also: Jencks, W. P. *Catalysis in Chemistry and Enzymology*; Dover Publications, Inc.: New York, 1987; Chapter 4.

(9) Dahlquist, F. W.; Rand-Meir, T.; Raftery, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *61*, 1194. Smith, L. E. H.; Mohr, L. H.; Raftery, M. A. *J. Am. Chem. Soc.* **1973**, *95*, 7497.

(10) We would like to thank one referee and Professor Richard L. Schowen for bringing to our attention the ranges that can be observed in secondary isotope effects and the dependence on the nature of the leaving group. However, our value approaches the classical number for the conversion from an sp^3 to an sp^2 center when the leaving group is a sulfonate (Gray, C. H.; Coward, J. K.; Schowen, K. B.; Schowen, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 4351). It should be noted that in this isotope experiment there are no differences in assay conditions between the catalyzed and the uncatalyzed reactions. Thus the differences observed between the secondary isotope effects of the catalyzed and the uncatalyzed reactions point to a difference in mechanism.

(11) DeFrees, D. J.; Hehre, W. J.; Sunko, D. E. *J. Am. Chem. Soc.* **1979**, *101*, 2323.

(12) Harder, S.; Streitwieser, A.; Petty, J. T.; Schleyer, P. v. R. Ion Pair SN2 Reactions; Theoretical Study of Inversion and Retention Mechanisms, to be submitted.