evident in the complex suggest that chelate complexes of heavier metals may exhibit selectivity.

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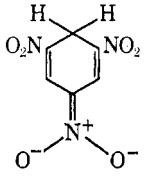
Bovine Serum Albumin as a Catalyst. Accelerated Decomposition of a Meisenheimer Complex[1]

Sir:

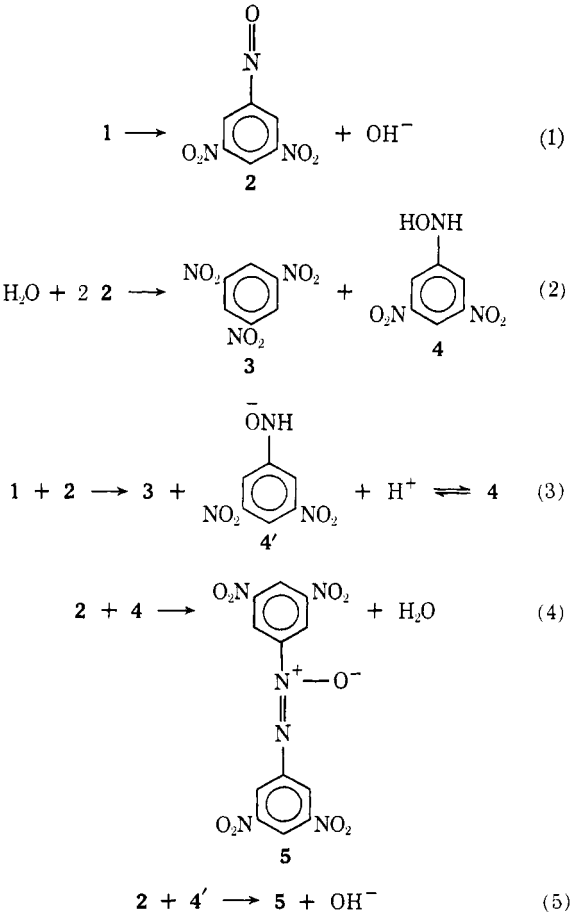
Recently we have begun investigating various Meisenheimer complexes (MC) as possible biophysical probes[2] for studying the physical chemistry of immunoglobulins specific for polynitrophenyl haptens.[3] In the course of this work we have discovered that bovine serum albumin (BSA) significantly increases the rate of the base-catalyzed decomposition of MC **1** and we now report the results of our preliminary studies.



1

The tetramethylammonium salt of **1** was prepared by previously published methods.[4] It is relatively stable in water, and its decomposition (observed by following the decrease in absorbance at 478 mμ of aqueous solutions of **1**) is base catalyzed (Table I). In the presence of BSA, this decomposition is accelerated by factors of *ca.* 10^4, in the neutral to slightly basic region (Table I). At higher pH values (~11–12) the base-catalyzed decomposition of **1** is rapid ($t_{1/2}$ ~105 sec at pH 11.5) and in this region, where BSA is known to undergo a conformational transition,[5] rate accelerations due to this protein are eliminated.

Decomposition of **1** in the presence of BSA liberates 1 mol of hydroxide anion/mol of **1**. The final product isolated from this decomposition reaction was 3,5,-3′,5′-tetranitroazoxybenzene (**5**).[6] This product was identified by its nmr spectrum ((Me$_2$SO-d_6) τ 9.37 (d, rel intensity 2, J = 2.1 Hz), 9.25 (d, rel intensity 2, J = 2.1 Hz), 9.10 (t, rel intensity 1, J = 2.1 Hz), 8.87 ppm (t, rel intensity 1, J = 2.1 Hz)) and by high resolution mass spectroscopy (calcd mol wt, 378.0195; obsd mol wt, 378.0190).[7] In addition, trinitrobenzene (**3**) was isolated from the reaction mixture by ether extraction. The precipitated product obtained from the high pH (~11–12) base-catalyzed decomposition of **1** was also **5**. The results suggest the following possible scheme (eq 1–5) for the decomposition of **1**, in the



(1) Publication no. 86 from the Laboratory for Biophysical Chemistry.

(2) R. P. Taylor and S. B. Farnham, *J. Chem. Phys.*, to be submitted for publication.

(3) J. R. Little and H. N. Eisen, *Biochemistry*, 5 3385 (1966).

(4) R. P. Taylor, *Chem. Commun.*, 1463 (1970).

(5) D. Wetlaufer and R. Lovrien, *J. Biol. Chem.*, **239** 596 (1964).

(6) BSA (526 mg) was used to decompose 781 mg of **1** (previously dissolved in ~50 ml of acetonitrile) in ~3 l. of water. The pH was maintained at ~7–9 by periodic addition of HCl. The decomposition (decolorization) was over in about 20 min. The cloudy yellow solution was stirred for 48 hr at room temperature and the precipitate was isolated through filtration on a fine sintered glass filter. No precipitate is obtained if filtration is attempted immediately after the decomposition. Precipitated product (272 mg) (containing about 10% water by weight after drying at room temperature for a few hours) was obtained. This represents (after subtracting out the water contribution) a yield of about 72%, based on eq 1–3.

Table I. Rates of Decomposition of **1**[a,b]

pH	7.0	7.5	8.0	8.5
k_1, sec^{-1} [c]	1.9×10^{-5}	3.7×10^{-5}	4.2×10^{-5}	7.2×10^{-5}
k_{BSA}, sec^{-1} [d]	0.11	0.26	0.46	0.52

[a] 25°, 0.05 M phosphate, 1% or less acetonitrile. [b] The molar extinction coefficient of aqueous solution of **1** is 2.0×10^4 at 478 mμ. Under the conditions of the experiment (initial concentration of **1** was 8×10^{-5} M) the product had essentially no OD at 478 mμ after decomposition was complete. [c] First-order rate constants were determined based on the time needed for 50% decomposition. Kinetics were approximately first order over this time range; these results are to be taken as estimates to be compared to the BSA-catalyzed rates. [d] These are "turnover numbers" which were determined under conditions of saturation of "enzyme" (BSA) by "substrate" (**1**). A $\Delta\epsilon$ of 2.0×10^4 was used to calculate rate constants by standard "initial rate" methods (B. Zerner, R. P. M. Bond, and M. L. Bender, *J. Amer. Chem. Soc.*, **86**, 3674 (1964)). Conditions: [BSA] $\simeq 10^{-6}$ M; [**1**] $\simeq 8 \times 10^{-5}$ M. At lower substrate concentrations the rates decreased; the rate was reduced to 50% at a concentration of **1** of 17 μM; the rate was 25% of maximum at substrate concentrations of 6 μM. Uncertainties in rate constants are 10% or less.

(7) Mass spectra were taken on an MS 30 double beam mass spectrometer at 10,000 resolution.

presence or absence of BSA. The initial product of the reaction is presumably 3,5-dinitronitrosobenzene (**2**) (eq 1). This is consistent with the fact that the first two-electron step in the reduction of nitrobenzene is nitrosobenzene,[8] and in this case **1** can be considered as a combination of hydride anion (a potential two-electron reducing agent) and **3**. Disproportionation of **2** (eq 2) or reduction of **2** by **1** (eq 3), followed by condensation reactions (eq 4 or 5), should lead to the final isolated products; other pathways are possible. The work of Hutchins, *et al.*, on the reduction of aromatic nitro compounds by sodium borohydride leads to a similar analysis.[9]

All our evidence to date suggests that BSA is acting as a macromolecular catalyst in this reaction (presumably catalyzing the first step, eq 1); our results indicate that the protein must be in its "native" conformation and that the reaction depends upon the binding of **1** to BSA. (1) Other molecules which bind to BSA[10] (sodium dodecyl sulfate, picrate anion, phenol red) inhibit the catalysis.[11] The nature of this inhibition must be determined. (2) BSA is able to "turnover" significant quantities of substrate. In ~20 minutes, 8×10^{-6} mol of BSA decomposed a total of 2.7×10^{-3} mol of **1** in 3 l. of solution. (3) The reaction is characterized by saturation kinetics typical of enzyme–substrate reactions (Table I). (4) A variety of samples of BSA from different companies (Schwarz/Mann, Pentex, Sigma) and of different degrees of purity (fraction V, crystallized, fatty acid free, defatted by Chen's method[12]) exhibited similar catalytic properties. (5) No catalysis is observed in 8 *M* urea, where BSA is presumably unfolded. (6) Other animal serum albumins, *e.g.*, sheep and horse, exhibit similar, though lower activities. Human serum albumin (Sigma fraction V, Schwarz/Mann) was apparently inert. This catalysis does not only depend upon binding of **1** to a protein with a hydrophobic binding site. Other proteins which bind hydrophobic molecules (β-lactoglobulin,[13] α-chymotrypsin[14]) show no catalysis at all. (7) Isolated fragments A and B[15] of BSA are ineffective alone (residual activities alone are ~0.5% or less) in catalyzing the reaction. When they are combined in aqueous solution at a concentration of about 2×10^{-6} *M* each, ~30% of full activity is restored.

Further studies on this most interesting reaction and its possible physiological implications are in progress.

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(8) N. V. Sidgwick, I. T. Millar, and H. D. Springall, "The Organic Chemistry of Nitrogen," Clarendon Press, Oxford, 1966, p 387.
(9) R. O. Hutchins, D. W. Lamson, L. Rua, C. Milewski, and B. Maryanoff, *J. Org. Chem.*, **36**, 803 (1971).
(10) J. Steinhardt and J. A. Reynolds, "Multiple Equilibria in Proteins," Academic Press, New York, N. Y., 1969.
(11) At a concentration of **1** of $\sim 7.5 \times 10^{-5}$ *M*, the following concentrations of inhibitors are needed for ~50% inhibition: sodium dodecyl sulfate, 1.5 μM; picrate anion, 13 μM; phenol red, 140 μM.
(12) (a) R. F. Chen, *J. Biol. Chem.*, **242**, 173 (1967); (b) M. Sogami and J. F. Foster, *Biochemistry*, 7 2172 (1968).
(13) A. Wishnia and T. W. Pinder, Jr., *Biochemistry*, **5**, 1534 (1966).
(14) R. Lumry in "Enzymology in the Practice of Laboratory Medicine," P. Blume and E. Frier, Ed., Academic Press, New York, N. Y., 1973.
(15) T. P. King, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **32**, Abstr. 542 (1973).

Polymer-Based Sensitizers for Photooxidations

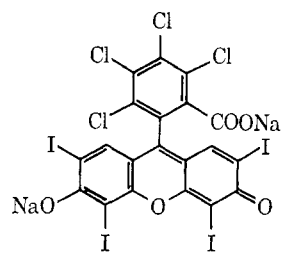
Sir:

Insoluble polymer supports were introduced several years ago by Merrifield[1] and by Letsinger[2] to facilitate polypeptide synthesis. The technique involves the use of an insoluble styrene–divinylbenzene copolymer bead to provide a foundation upon which successive chemical transformations can be carried out.

For some time we have been interested in the use of insoluble polymer supports in photochemical reactions. In this report, we describe the preparation and use of the first example of a synthetically applicable, polymer-based photosensitizer. The reagent, polymer-based Rose Bengal (Ⓟ-Rose Bengal), is utilized to sensitize the generation of singlet molecular oxygen. Rose Bengal[3] is attached to a chloromethylated polystyrene support *via* the following procedure: Rose Bengal, 2.0 g (2.1 mmol), was stirred at reflux in 60 ml of reagent grade dimethylformamide with 2.0 g of chloromethylated styrene–divinylbenzene copolymer beads (1.38 mequiv of CH_2Cl, 50–100 mesh). After 20 hr, the polymer (now dark red) was filtered and washed successively with 150-ml portions of benzene, ethanol, ethanol–water (1:1), water, methanol–water (1:1), and methanol. After these washings, the final filtrate was colorless. The polymer beads[4] were dried in a vacuum oven to a final weight of 2.17 g.

Singlet molecular oxygen exhibits three modes of reaction with alkenes:[5] 1,4-cycloaddition with con-

(1) R. B. Merrifield, *Science*, **150**, 178 (1965).
(2) R. L. Letsinger, M. J. Kornet, V. Mahedevon, and D. M. Jerina, *J. Amer. Chem. Soc.*, **86**, 5163 (1964).
(3) Rose Bengal



(4) The Rose Bengal is probably attached to the polymer as the carboxylate ester.
(5) (a) C. S. Foote, *Accounts Chem. Res.*, **1**, 104 (1968); (b) D. R. Kearns, *Chem. Rev.*, **71**, 395 (1971); (c) K. Gollnick and G. O. Schenck in "1,4-Cycloaddition Reactions," J. Hammer, Ed., Academic Press, New York, N. Y., 1967, p 255; (d) K. Gollnick, *Advan. Photochem.*, **6**, 1 (1968); (e) W. R. Adams in "Oxidation," Vol. 2, R. L. Augustine and D. J. Trecker, Ed., Marcel Dekker, New York, N. Y., 1971, p 65; (f) J. T. Hastings and T. Wilson, *Photophysiology*, **5**, 49 (1970).