$$\int_0^T \frac{FK_0}{KV}(1 - e^{-Kt})\,dt = -\frac{FK_0T}{KV} + \frac{FK_0}{KVK}(1 - e^{-KT}) \quad \text{(Eq. 9)}$$

Placing Eq. 9 into Eq. 8 gives:

$$AUC_2 = \frac{FK_0}{KVK}(1 - e^{-KT}) \quad \text{(Eq. 10)}$$

Equation 10 is equal to the plasma concentration at time T, divided by K:

$$AUC_2 = \frac{C_T}{K} \quad \text{(Eq. 11)}$$

This expression may be recognized as being equal to AUC_3 which is conventionally used to calculate the postinfusion area and, therefore, $AUC_2 = AUC_3$.

The relationship indicated in Eq. 4, that area is the product of C_{ss} and T, appears not to have been previously reported. Furthermore, assuming linear kinetics, this relationship is model independent. The only difference in calculation for multicompartment models is that AUC_0^∞ is the product of T and the sum of all the appropriate coefficients (*e.g.*, $A + B$, in a two-compartment model).

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Michael Mayersohn
Department of Pharmaceutical Sciences
College of Pharmacy
The University of Arizona
Tucson, AZ 85721

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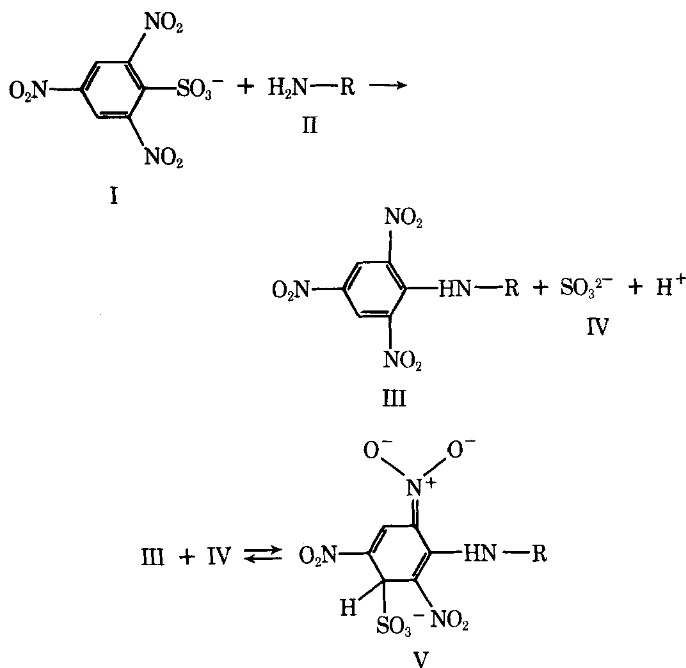
Kinetic Study on Rapid Reaction of Trinitrobenzenesulfonate with Human Serum Albumin

Keyphrases □ Trinitrobenzenesulfonate—kinetics of reaction with human serum albumin □ Kinetics—of reaction of trinitrobenezenesulfonate with human serum albumin □ Albumin, human serum—kinetics of reaction with trinitrobenzenesulfonate

To the Editor:

Trinitrobenzenesulfonate (I) has been used as a reagent for the chemical modification of amino groups in amino acids, peptides, and proteins (1–4). The basic mechanism for the reaction of I with amines (II) was proposed previously (5, 6) (Scheme I). The trinitrophenylated II (III) reacts further with sulfite ion (IV) to form the sulfite monoadduct (V). It was reported that III and V have UV absorption maxima at 340 and 420 nm, respectively, giving an isosbestic point at 367 nm during the reaction of III and IV (5, 6).

The distinction between, and identification of, the drug binding sites on human serum albumin were made previously by us on the basis of the inhibition of the reaction of *p*-nitrophenyl acetate with the albumin caused by several drugs (7). When the albumin was modified with I to characterize the drug binding sites, rapid reaction of I with the albumin was found. In this communication, we describe the localization of the reactive site on the albumin for I and the kinetics and mechanism for the reaction.



Scheme I

All reactions in this study were carried out in pH 7.4, 0.067 *M* phosphate buffer ($\mu = 0.2$, adjusted with sodium chloride) at 25°. To localize the reactive site on the albumin for I, the fluorescence spectra of the albumin excited at 300 nm were measured[1]. The intensity of the emission

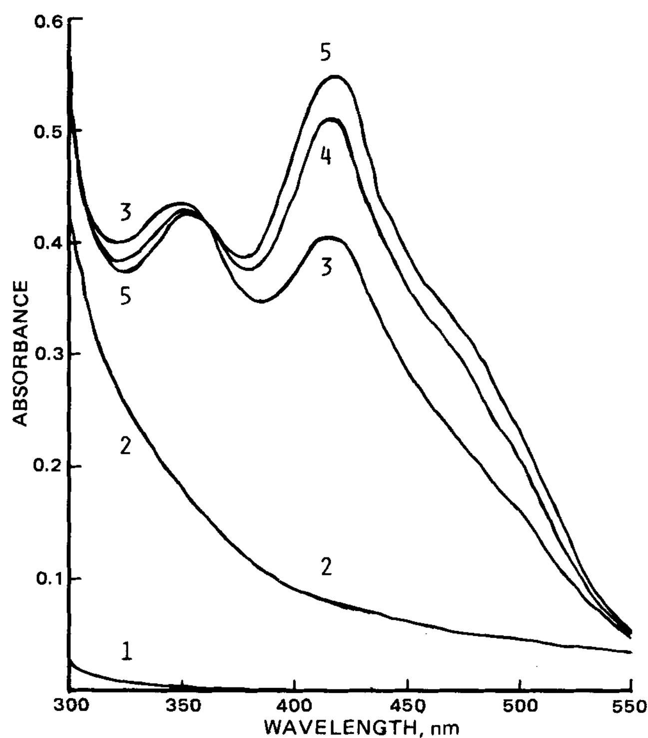


Figure 1—*UV absorption spectra. 1, 2.5 × 10*$^{-5}$ M *I; 2, 1.0 × 10*$^{-4}$ M *albumin; 3, 2.5 × 10*$^{-5}$ M *I and 1.0 × 10*$^{-4}$ M *albumin; 4, 2.5 × 10*$^{-5}$ M *I, 1.0 × 10*$^{-4}$ M *albumin, and 5.0 × 10*$^{-5}$ M *NaHSO*$_3$*; and 5, 2.5 × 10*$^{-5}$ M *I, 1.0 × 10*$^{-4}$ M *albumin, and 1.0 × 10*$^{-4}$ M *NaHSO*$_3$.

[1] RF-510 spectrofluorophotometer, Shimadzu, Kyoto, Japan.

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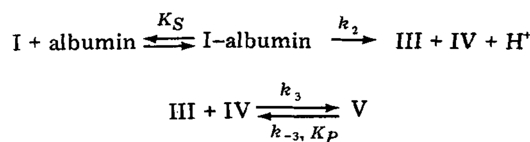
spectrum for the reaction mixture of I and the albumin was smaller than that for the albumin solution. This reduced emission spectrum indicates that the reactive site for I is located close to tryptophan residue 214, which is the lone tryptophan residue of the albumin (7–9). This important drug binding site on albumin was named Site I by Sudlow *et al.* (10) and U site by us (7).

When the reaction of I (2.5×10^{-5} *M*) with albumin (1.0×10^{-4} *M*) was followed spectrophotometrically at 340 nm, the reaction was completed rapidly. In contrast, when the reaction was monitored at 420 nm, the absorbance gradually increased (half-life ~30 sec) after the initial rapid increase in the absorbance up to ~0.25. Thus, the reaction proceeded *via* two steps. Figure 1 shows the UV absorption spectra of each reactant solution and the reaction mixtures after the spectral changes were complete. The spectra of 3, 4, and 5 show the isosbestic point at ~360 nm. These kinetic and spectroscopic results suggest that the reactions similar to Scheme I (trinitrophenylation and sulfite monoaddition) occur in the reaction of I with the albumin.

The rate of the trinitrophenylation of the albumin was followed at 360 nm (isosbestic point of III and V) with a stopped-flow spectrophotometer[2]. The pseudo-first-order analyses could be applied under the excess molar concentration of the albumin over that of I (2:8 concentration ratio). The pseudo-first-order rate constants (~2.5×10^{-1} sec^{-1}) were almost independent of the albumin concentrations. This independence suggests that the trinitrophenylation occurs *via* Michaelis–Menten-type complex formation and that the dissociation constant of the complex is very small. Therefore, the overall reactions of I with the albumin may be represented as in Scheme II, where k_2 is the first-order rate constant of the I–albumin complex for the trinitrophenylation. The rate constants for the formation of V and the elimination of IV from V are expressed by k_3 and k_{-3}, respectively, and K_S and K_P represent the dissociation constants of the complex and V, respectively. Detailed studies on the kinetics and mechanism for the reactions shown in Scheme II are in progress.



Scheme II

[2] Model RA-401, Union Giken, Osaka, Japan.

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Yukihisa Kurono
Kenichi Ichioka
Sawako Mori
Ken Ikeda ˣ

Faculty of Pharmaceutical Sciences
Nagoya City University
Tanabe-dori, Mizuho-ku
Nagoya, 467, Japan

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