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SERUM ALBUMIN AND THE METABOLISM OF DISULFIRAM

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Abstract—The effectiveness of tetraethylthiuram disulfide (DSF) as a drug used in the treatment of alcohol abuse has been limited by the fact that it is degraded rapidly in the tissues and in the serum. Hence, a useful dose–response curve for this drug cannot be determined easily. The degradation in the tissues has been well characterized; however, its fate in the serum is less well understood. Here we kinetically describe the first steps in the degradation of DSF in the serum which results from a covalent interaction of this drug with the free sulfhydryl of serum albumin. DSF and its cleavage product diethyldithiocarbamate (DDC) both absorb significantly in the ultraviolet region. The reduction of DSF with mercaptoethanol to two molecules of DDC resulted in a large change in absorption in this region. The reaction of serum albumin with DSF produced a similar but much *slower* change in the ultraviolet absorption. As a result of the existence of this slow spectral change, we have been able to directly and continuously monitor the interaction of serum albumin and DSF and have determined that it is an overall first-order process. A model is proposed wherein DSF and serum albumin rapidly form a noncovalent adduct and, subsequently, in a slow unimolecular process, DSF is reduced to one mole of free DDC and one mole of the serum albumin–DDC mixed disulfide. At pH 9 the half-time for this process was 30 to 40 sec, and at pH 7.4 the half-time for this process was 1 to 1.5 min. These results suggest that degradation of DSF by serum albumin is physiologically and clinically important since the drug is maximally active only many *hours* after administration.

A major function of serum albumin in the plasma is the binding and transport of small molecules [1]. In most cases, the interactions with drugs [2], fatty acids [3], and other ligands [4] are non-covalent. However, in some cases the interactions of serum albumin with small molecules are covalent and may even result in enzymatic activity. Albumin has been shown to act as a thioesterase [5] and as an esterase [6]. In particular, several prostaglandins have been shown to undergo specific transformations (hydrolysis, dehydration, isomerization) catalyzed by albumin [7, 8]. In other cases, only the stability of the molecule is affected. Serum albumin increases the half-life of thromboxane A_2 [9] and stabilizes prostaglandin I_2 [10]. Here we report the specific *destabilization* of a drug, tetraethylthiuram disulfide (DSF¶, Antabuse or disulfiram), known to bind to serum albumin. This destabilization probably results from the covalent interaction of DSF with the free sulfhydryl (cys 34) of the protein.

Disulfiram has been widely used in the treatment of alcohol abuse since its introduction over 35 years ago [11]. Consumption of alcohol while taking a therapeutic dose of disulfiram results in a subjectively unpleasant experience characterized by facial flushing, nausea, tachycardia, rapid respiration and hypotension (the disulfiram–ethanol reaction). This reaction has been ascribed mainly to the inhibition of the enzyme aldehyde dehydrogenase (EC 1.2.13) in the liver, with the result that the consumption of alcohol is promptly followed by the accumulation of toxic amounts of acetaldehyde in the blood [12].

The inhibition of aldehyde dehydrogenase by DSF has been proposed to be due to a covalent modification of thiol groups of this enzyme [13]. However, despite the dramatic response elicited by the administration of disulfiram, its usefulness as a clinical tool has been complicated by the fact that it has been difficult to determine a specific dose–response relation for this compound. The reason for this is that DSF is rapidly degraded both in the serum and in the tissues.

The metabolism of DSF in the tissues has been elucidated by Stromme [14] and Gessner and Jakubowski [15]. The first step in the degradation of DSF is the reduction of the disulfide yielding two molecules of diethyldithiocarbamate (DDC). DDC is subsequently degraded to carbon disulfide and a number of other products including glucuronic acid conjugates. In the tissues of mice this degradative metabolism occurs to the greatest extent in the liver and kidneys [15, 16]. The metabolism in the tissues is very rapid, with DDC being nearly totally degraded within 40 min [16]. In addition to degradation in the tissues, disulfiram is also rapidly broken down in the serum. A number of groups [16–18], in addition to

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¶ Abbreviations: DSF, tetraethylthiuram disulfide, disulfiram; DDC, diethyldithiocarbamate; and SA, serum albumin.

our own [19], have demonstrated that DSF is rapidly degraded when added to human plasma, the initial breakdown product being DDC. Specifically, Agarwal *et al.* [19] have shown that DSF is degraded in human plasma in an apparent two-step process. The first process has a half-time of 1–2 min and the second process has a half-time of 1–2 hr. The breakdown product, DDC, has been shown to accumulate free in the plasma as well as to be covalently associated with a protein component. This protein component is probably serum albumin [17–19].

However, in contrast to the catabolism of disulfiram in the tissues, the degradation of disulfiram in the serum is less well understood. In an effort to characterize in detail the processes involved in the degradation of DSF in the serum which have been outlined by Stromme [17], Pedersen [18] and by Agarwal *et al.* [19], the current studies were begun. Here we specifically examine the kinetics of the initial rapid interaction of DSF with serum albumin directly visualizing the process by the technique of ultraviolet difference spectroscopy.

MATERIALS AND METHODS

Reagents. Disulfiram was supplied by Ayest Laboratories (New York, NY). Sodium diethyldithiocarbamate (DDC), iodacetic acid, Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)] and bovine serum albumin were purchased from Sigma (St. Louis, MO). Activated charcoal (Darco) was obtained from Atlas Chemical Industries, Inc. (Wilmington, DE). All other reagents were reagent grade and were used without further purification. Double-deionized water, obtained from the Millipore water purification system (Millipore Corp., Bedford, MA), was used throughout. Dialysis tubing (Spectropor, Spectrum Medical Industries, Inc., Los Angeles, CA) was soaked in 10 mM EDTA (pH 8) for 72 hr and washed extensively before use. Aqueous solutions of organic reagents were bubbled with purified nitrogen to remove dissolved oxygen before use. Albumin solutions were prepared in oxygen-free aqueous buffers.

Solubilization of DSF. Solutions of DSF were freshly prepared in aqueous buffers by sonication of a suspension of the drug for 10 min on a model W-220F sonicator obtained from Heats System-Ultrasonic, Inc. (Plainview, NY). The suspension was allowed to stand at room temperature for 30 min, and undissolved drug was removed by filtration. DSF concentrations higher than 15 μM were prepared by the addition of 50 μl of an ethanol solution of the drug to 10 ml buffer. These solutions were stable at concentrations of less than 50 μM of DSF as reported by Stromme [17].

Electrophoresis. Polyacrylamide gel electrophoresis was used to ascertain that albumin preparations used in the study were free of other macromolecules. Electrophoresis was performed in sodium phosphate (0.1 M, pH 7.2) containing 10% sodium dodecyl sulfate (SDS) by the method descibed by Weber and Osborn [20].

Absorbance measurements. Ultraviolet absorption spectra were recorded on a Beckman DU-8 spectrophotometer. Double sector mixing cells (quartz, 1 cm light path), obtained from Helma Cells, Inc. (New York), were used to study spectral changes. These cells consist of two compartments. Reagents (1 ml) were pipetted separately into the two half-cells, and the openings at the top of the cuvette used for introducing solutions were sealed with Teflon stoppers. The spectrophotometer was blanked with the above cell containing the two solutions in separate compartments. The solutions were then mixed by inverting the cell, and the absorption spectrum of the reaction mixture was recorded. The spectrophotometer was programmed so that u.v. scans (240–320 nm) were repeated with fixed intervals. To study kinetics of reactions, absorbance of reaction mixtures was measured as a function of the time of incubation.

Computation of rate constants. The analysis of the time-dependent change in the ultraviolet difference spectrum of the serum albumin–disulfiram mixture was done using a Gauss–Newton non-linear least squares regression [21, 22]. The kinetic data were fit to the following equation:

$$A_t = A_0 + (A_\infty - A_0)(1 - e^{-k_2 t}) \qquad (1)$$

where A_t, A_0, and A_∞ are the absorbances at a time, t, at time zero, and at infinite time respectively; k_2 is the apparent first-order rate constant for the reduction of DSF in the presence of albumin.

Modification of sulfhydryl groups of albumin. Sulfhydryl groups of bovine serum albumin were blocked by treatment with iodoacetic acid [23]. BSA (40 mg) dissolved in 10 ml Tris–HCl (0.05 M, pH 7.4) was mixed with 10 mg iodoacetic acid dissolved in 10 ml of the same buffer, thus providing a 100-fold molar excess of the reagent over albumin. The reaction mixture was allowed to stand in the dark at room temperature for 1 hr. The solution was then dialyzed for 72 hr at 4° against a 25-fold excess of Tris–HCl (0.05 M, pH 9) containing 1 mM EDTA (Tris–EDTA) with five changes of the buffer. The concentration of albumin was determined by the method of Lowry *et al.* [24].

Removal of fatty acids from albumin. Fatty acids were removed from bovine serum albumin by treatment with activated charcoal as described by Chen [25]. Briefly, albumin (0.5 g in 10 ml water) was mixed with charcoal (0.5 g), adjusted to pH 3.0 by the addition of 0.2 N HCl, and stirred magnetically on ice for 1 hr. Charcoal was removed by centrifugation at 20,000 *g* for 30 min at 4°. The clarified protein solution was raised to pH 7 by the addition of 0.2 N NaOH and dialyzed against Tris–EDTA as described above.

Analysis of DSF. The unreacted DSF and DDC (free and protein-bound) formed in reaction mixtures were estimated by high performance liquid chromatogaphy performed on a Waters Associates (Milford, MA) liquid chromatograph by the method described previously [19]. In this way we were able to quantitate the concentration of DSF and DDC absolutely.

Estimation of free SH content in albumin. The number of free sulfhydryl groups per mole of bovine serum albumin was estimated with Ellman's reagent [26].

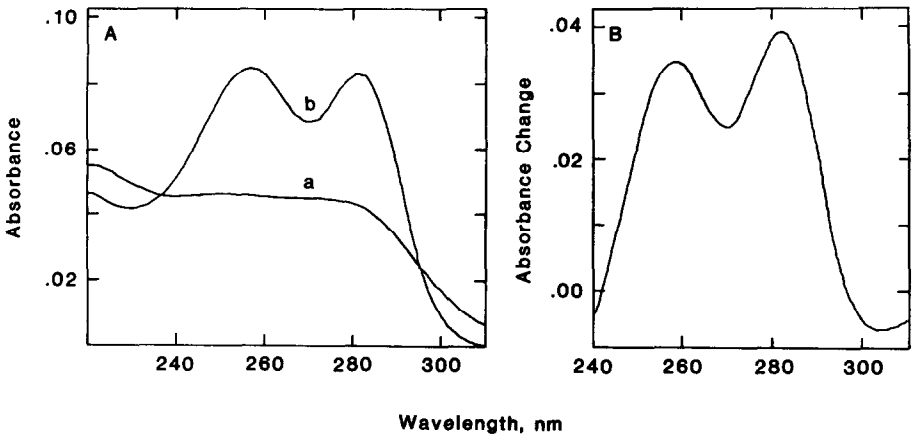
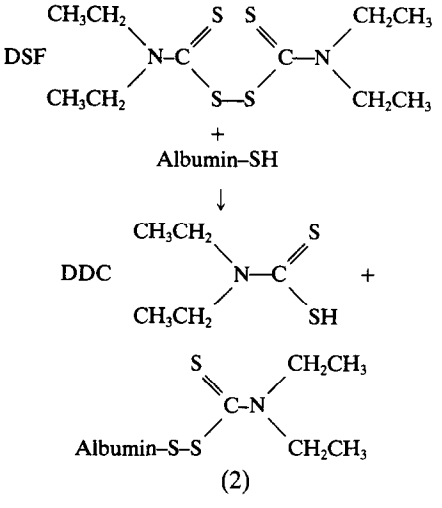


Fig. 1. (A) Ultraviolet spectra of 30 μM DSF (a) and 60 μM DDC (b) in 50 mM Tris–EDTA, pH 9.0. (B) Ultraviolet difference spectrum which resulted from the reduction of 30 μM DSF with 1 mM mercaptoethanol under the same buffer conditions.

RESULTS

Ultraviolet difference spectra. The results of Agarwal *et al.* [19] suggest that disulfiram rapidly interacts with serum albumin forming a covalent serum albumin–DDC adduct and releasing free DDC into solution. This chemistry is outlined in equation 2. It is known that serum albumin, DSF and DDC absorb in the ultraviolet region [27]. It therefore might be anticipated that the proposed reaction of DSF with serum albumin would produce an ultraviolet difference spectrum. Figure 1A shows the absolute spectra of DSF (a) and DDC (b) in 50 mM Tris–EDTA, pH 9.0. Figure 1B shows that reduction of DSF to DDC does produce a change in the u.v. spectrum. This difference spectrum resulted when 30 μM DSF was mixed with 1 mM mercaptoethanol producing 60 μM DDC. The difference spectrum had maxima at 258 and 282.5 nm.



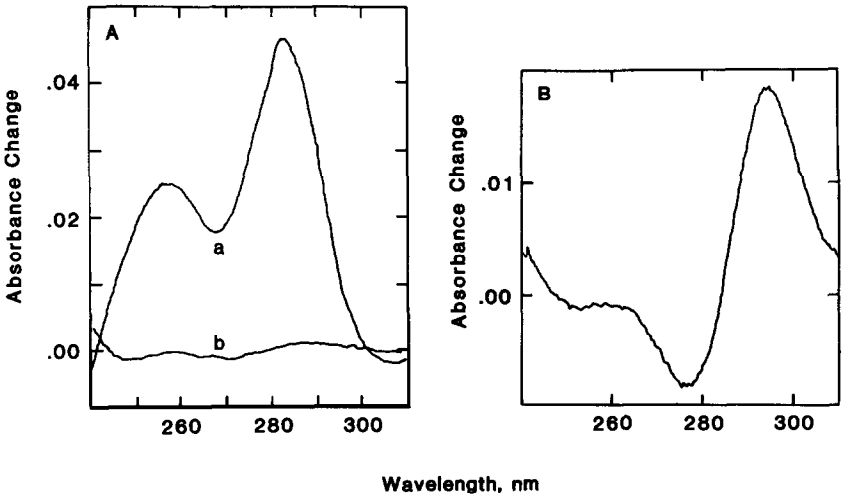


Fig. 2. (A) Ultraviolet spectrum which resulted from the interaction of 30 μM DSF with 30 μM serum albumin in 50 mM Tris–EDTA, pH 9 (spectrum a). Spectrum (b) resulted from the interaction of DSF and serum albumin under the same conditions as in spectrum (a) except that serum albumin was covalently modified with iodoacetic acid (see Materials and Methods). (B) Ultraviolet difference spectrum which resulted from the interaction of 30 μM serum albumin and 30 μM DDC in 50 mM Tris–EDTA, pH 9.0

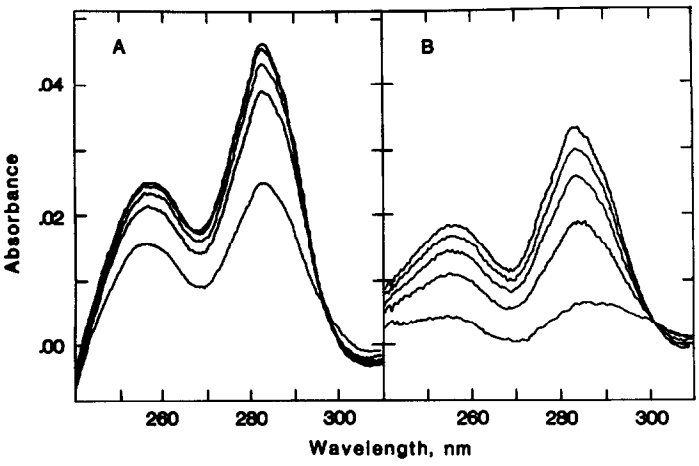


Fig. 3. (A) Time dependence of the spectral change which resulted from the interaction of 30 μM serum albumin and 30 μM DSF in 50 mM Tris–EDTA, pH 9.0. The spectra were recorded every 30 sec. (B) A similar experiment to that in Panel A except that both serum albumin and DSF were dissolved in 50 mM Tris–EDTA, pH 7.4

Reaction of DSF with serum albumin. Figure 2A [spectrum (a)] shows that the ultraviolet difference spectrum obtained upon the mixing of disulfiram with serum albumin was similar to that produced by the interaction of DSF with mercaptoethanol (Fig. 1B). This spectrum also had maxima at 258 and 282.5 nm. Since serum albumin has a single free sulfhydryl [28], these results suggest that this sulfhydryl (cys 34) is responsible for the reduction of DSF to DDC. This suggestion is supported by the fact that

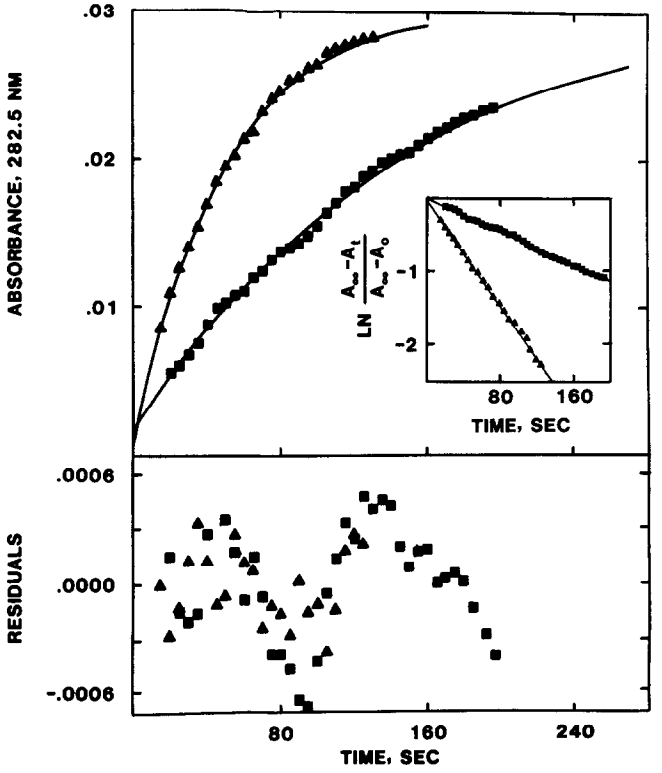


Fig. 4. Time dependence of the absorption change at 282.5 nm. This change resulted from the interaction of 20 μM serum albumin and 30 μM DSF in 50 mM Tris–EDTA, pH 9.0 (▲). The slower kinetic data (■) resulted from the interaction of 20 μM serum albumin and 30 μM DSF in 50 mM Tris–EDTA, pH 7.4. Theoretical curves drawn through the data points result from the best fits of the data to equation 1. For the triangles, A_0 is equal to 0.0012, A_{00} is equal to 0.0309, and k_2 is equal to 0.0189 sec^{-1}. The variance of the fit was 4.59×10^{-8}. For the squares, A_0 is equal to 0.0019, A_{00} is equal to 0.0344, and k_2 is equal to 0.0052 sec^{-1}. The variance of the fit was 1.13×10^{-7}. The residuals from these fits are plotted in the lower panel. The inset shows the logarithmic transform of the primary data.

no spectral change was observed by the interaction of DSF with serum albumin which was previously modified with iodoacetic acid [spectrum (b), Fig. 2A]. A similar spectrum to that in spectrum (b) results from the interaction of DSF with serum albumin reacted with *N*-ethylmaleimide or *p*-chloromercuribenzoate (data not shown). These latter data also provide support for the suggestion that the difference spectrum in (a) results from the covalent interaction of the free sulfhydryl of serum albumin with DSF. It also suggests that DSF does not react with any internal disulfide bond in the protein. Figure 2B shows the ultraviolet difference spectrum which resulted from mixing of DDC with serum albumin. This spectrum has a maximum at 294 nm and a large minimum at 276 nm. These spectra are qualitatively distinct.

Kinetics of the spectral change. Figure 3 shows that the spectral change that resulted from the interaction of DSF with serum albumin was time dependent. Panel A shows the time dependence of the spectral change at pH 9.0 and panel B shows the time dependence of the spectral change at pH 7.4. The change in absorbance at 282.5 nm was fit to equation 1 (see Materials and Methods). As can be seen from Fig. 4, this process was first order for at least three half-times for the reaction at pH 9.0 and for nearly two half-times at pH 7.4. Shown also is the distribution of residuals, which is random. The inset to Fig. 4 shows that the natural logarithm of the fractional increase in absorbance at 282.5 nm was a linear function of time, again demonstrating an overall first-order process.

Concentration dependence of k_2. In the above experiment, concentrations of DSF and serum albumin were roughly equimolar. These are second-order conditions, but the rate process observed by the ultraviolet difference spectroscopy was first order. This suggests that a rapid bimolecular binding process may precede the slower unimolecular reduction. This process is described in equation 3. If the binding

$$\begin{array}{ccccc} \text{DSF} & & \text{DSF} & & \text{DDC} \\ + & \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} & & \overset{k_2}{\rightarrow} & + \\ \text{Albumin–SH} & & \text{Albumin–SH} & & \text{Albumin–S–S–DDC} \end{array} \quad (3)$$

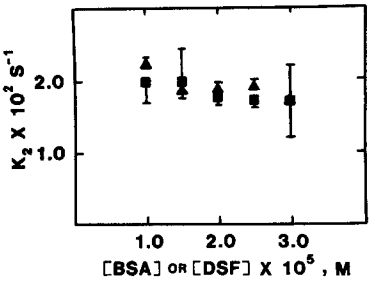


Fig. 5. Dependence of k_2 on serum albumin and DSF concentration at pH 9.0. Key: (▲) these data represent k_2 measured at a fixed serum albumin concentration (30 μM) with DSF concentrations varying from 10 to 30 μM; and (■) these data represent k_2 measured at a DSF concentration of 30 μM with the BSA concentration varying from 10 to 30 μM. The reaction conditions were the same as those in Fig. 3.

equilibrium strongly favors the formation of the noncovalent adduct, the observed rate constant for the reduction will be independent of the concentration of the reactants. If the adduct is significantly dissociated, this rate constant will be concentration dependent. Figure 5 shows the dependence of the rate constant, k_2, on the concentrations of DSF and serum albumin. In this figure, k_2 was determined first at 30 μM serum albumin with DSF concentrations varying from 10 to 30 μM (triangles). Subsequently (squares), the DSF concentration was fixed at 30 μM, and the serum albumin concentration was varied from 10 to 30 μM. These are the limits of the experimentally accessible concentration ranges. As can be seen in Fig. 5, with an experimental error, k_2 was independent of the concentration of DSF or serum albumin.

DISCUSSION

In this study we take advantage of the fact that the reduction of DSF to two molecules of DDC produces an ultraviolet difference spectrum. We use this spectral change to monitor the reduction of DSF in the presence of serum albumin. We have observed that the difference spectrum was time dependent with a maximum extinction change at 282.5 nm. The kinetics of the reduction of DSF by the single sulfhydryl group in serum albumin are first order with a half-time of 30 to 40 sec at pH 9.0 and a half-time of 1 to 1.5 min at pH 7.4. Though the reaction is performed under conditions where serum albumin and DSF are roughly equimolar, under the conditions examined here the rate of reduction of DSF was first order. Figure 5 shows that the apparent first-order rate constant, k_2, was independent of either the DSF concentration at fixed serum albumin concentration or the serum albumin concentration at fixed DSF concentration. These results suggest the model proposed in equation 3 in which DSF and serum albumin form a noncovalent adduct in a rapid and reversible step followed by the unimolecular reduction of DSF to two molecules of DDC. This results in one free molecule of DDC and the mixed disulfide of DDC and serum albumin.

DSF and serum albumin have been known for some time to interact. Stromme [17] showed that ^{35}S-labeled disulfiram, when added to serum, eluted on a Sephadex G-25 gel filtration column in a position identical with that of serum albumin. More recently Agarwal *et al.* [19] have examined the kinetics of the interaction of DSF with serum proteins. This latter study demonstrated that the reduction of DSF occurred in a two-phase process, the first phase being substantially complete in 1–2 min and the second phase being complete in 1–2 hr. Their results suggest that, in human plasma, albumin is the protein which contributes most significantly to the reduction of DSF. The results presented here, therefore, confirm the above observations and provide a quantitative description of the first rapid phase of the reduction of DSF in plasma.

The difference spectrum (Fig. 1B) obtained by the reduction of one molecule of DSF with excess mercaptoethanol to two molecules of DDC had maxima at 258 and 282.5 nm. The extinction at these two

wavelengths was roughly equivalent. Spectrum (a) in Fig. 2A is the difference spectrum between one mole of DSF with one mole of serum albumin versus one mole of DDC with one mole of the serum albumin–DDC mixed disulfide. In this spectrum, the extinction at 258 nm was decreased. This may either result from changes in extinction of the serum albumin as a result of the covalent modification of cysteine 34 or from changes in the extinction of DDC covalently bound to serum albumin as the mixed disulfide. The bound mixed disulfide may also have different absorption properties when compared to a free molecule of mixed disulfide since DDC covalently attached to serum albumin may be in a significantly different microenvironment.

Figure 2B shows the difference spectrum which resulted from the presumed non-covalent [19] adduct of DDC and serum albumin. This spectrum was significantly different from that of Fig. 2A or Fig. 1B. The difference spectrum in Fig. 2B had a maximum at 294 nm and a minimum at 276 nm. Since this difference spectrum is qualitatively distinct from that of Fig. 2A or Fig. 1B, it may be inferred that the change in absorbance which is a consequence of the reduction of DSF by serum albumin results primarily from the reduction of DSF to the mixed disulfide and a free molecule of DDC. If the interaction of DSF with serum albumin is specific, one might speculate that the binding site for DSF (and presumably DDC) might be blocked by formation of the DDC–serum albumin mixed disulfide. Hence, the molecule of free DDC which results from the reduction of DSF apparently remains free in solution.

Figure 3 shows the time dependence of the spectral change in the ultraviolet region which resulted from the interaction of DSF with serum albumin at pH 9.0 (Panel A) and pH 7.4 (Panel B). The reaction at pH 9.0 was approximately four times faster than that at pH 7.4. Because the extinction change at 282.5 nm was of low amplitude and, hence, much noisier at pH 7.4, much of the data discussed here was obtained at pH 9.0. Figure 4 shows that, with the exception of the absolute magnitude of the rate constant, k_2, there was no qualitative difference between the reduction of DSF at pH 7.4 and pH 9.0. As was shown in Fig. 5, k_2 was independent of either DSF or serum albumin concentration, albeit over a small range. Similar results (data not shown) were obtained for k_2 as a function of DSF or serum albumin concentration at pH 7.4 The fact that k_2 is independent of the concentration of DSF or serum albumin supports the model presented in equation 3. This can also be seen from an examination of equation 4.

$$k_{obs} = k_2 K_1[([DSF] + [SA])/(1 + K_1([DSF] + [SA])) \quad (4)$$

Here k_{obs} is the observed rate constant for the reduction of DSF to DDC, k_2 is the intrinsic rate constant for the reduction of DSF, K_1 is the association constant for the binding of DSF to serum albumin (k_1/k_{-1}) and [SA] is the serum albumin concentration. This equation comes from an analysis of the model in equation 3 in terms of relaxation kinetics [29]. As a consequence of linearization assumptions, equation 4 formally applies to the last 10% of a progress curve such as is shown in Fig. 4. However, as is indicated by the inset in Fig. 4, the apparent rate constant which describes the last 10% of the reaction is the same as that which describes the whole reaction. Therefore, equation 4 is an appropriate definition of the rate constant measured here. In the limit that the DSF and the serum albumin concentration are below the dissociation constant ($K_1([DSF] + [SA])$ much less than 1), equation 4 applies directly, and k_{obs} will be a function of the concentration of DSF and albumin. However, in the limit that the concentrations of DSF and albumin are above the dissociation constant ($K_1([DSF] + [SA])$ much greater than 1), k_{obs} will increase to k_2 and will be independent of concentration. As discussed above, the data in Fig. 5 demonstrate that k_2 is independent of concentration. Hence we can infer that the association constant (K_1) is greater than $10^5\ M^{-1}$. The above data and discussion do not prove the model in equation 3; however, they are consistent with it and with a number of similar (but non-quantitated) observations from other laboratories [17–19].

The dependence of the reaction rate on pH is not surprising since it is presumably the thiolate form of cysteine which is reacting with DSF. The pK of the cysteine thiol varies from 8.5 to 10.1 depending on the state of ionization of its alpha-amino and alpha-carboxyl groups. However, a better model of cysteine in a protein would be glycylcysteinylglycine. For this species, the cysteine side chain has a pK of 8.0 to 8.7 [30]. If the pK of the thiol in cysteine 34 in serum albumin was 8.0, a 4-fold reduction in the rate constant for reduction of DSF ($1.89 \times 10^{-2}\ sec^{-1}$ at pH 9.0 to $5.23 \times 10^{-3}\ sec^{-1}$ at pH 7.4) might not be unexpected.

It is worth considering the clinical implications of these observations. The results presented here, as well as the results of Agarwal *et al.* [19], demonstrate that, in addition to the liver and kidneys, serum proteins may make a significant contribution to the preliminary steps in the degradation of disulfiram. Serum albumin is the main protein component of blood plasma, and each serum albumin molecule contains 0.2–0.6 free sulfhydryls per polypeptide chain [31]. The albumin used here had 0.5 sulfhydryls per chain (data not presented). Hence, an average adult blood volume contains enough serum albumin reducing equivalents to degrade approximately 250 mg of DSF within a few minutes. The normal half-life of circulating albumin, measured by radioiodine labeling, is 19 days [32]. Hence if the DDC–serum albumin complex was stable, the contribution of serum albumin to the degradation of DSF would be negligible after the first dose of this compound. However, the results of Stromme and Eldjarn [16] using mice demonstrate that [^{35}S]DDC is cleared from the plasma almost completely in 40–60 min. In rats the time for the removal of the mixed disulfide of serum albumin and DDC was increased to 30–40 hr [18]. These results suggest that some other system exists in the serum for the re-reduction of serum albumin to the free sulfhydryl form. Therefore, serum albumin may well continue to make a significant contribution to the degradation of disulfiram.

Furthermore, alcoholic patients with impaired hepatic function may have significantly reduced concentrations of albumin in the serum [33]. It is possible that this might result in slower degradation of circulating disulfiram with a concomitant prolongation of the clinical effect of the drug. It is of interest to note that when the dose of disulfiram is discontinued, the disulfiram ethanol reaction persists for less than 48 hr in alcoholics with normal serum albumin levels; however, in alcoholics with reduced serum albumin, the clinical effects of disulfiram may persist for 96 hr or longer [34].

In summary, we have presented data consistent with the suggestion that serum albumin specifically reduces DSF to one mole of DDC and one mole of the mixed disulfide of DDC and serum albumin. At physiological pH the half-time for this process was 1 to 1.5 min. These results indicate that, in the absence of other effects, the drug would be rapidly and *totally* degraded soon after entering the serum. Deitrich and Hellerman [35] have shown that DDC is not a potent *in vitro* inhibitor of aldehyde dehydrogenase from rat liver; however, Deitrich and Erwin [36] have shown that DSF will inhibit 80% of the mitochondrial aldehyde dehydrogenase activity 40 hr after administration. Further, they have shown that *in vivo* DDC will inhibit aldehyde dehydrogenase by 80% 20 hr after administration. These results suggest that new emphasis should be placed on an attempt to understand in detail the physiological mechanism by which DDC–serum albumin mixed disulfide is reduced in the serum and the mechanism by which DDC is reoxidized to the active drug, DSF, in the serum and in the tissues. It is these processes which are evidently responsible for the efficacy of and possibly the idiotypic response to, the drug. Such mechanisms have been suggested [37–39].

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