Kinetic Study of the Interaction between Aspartate Aminotransferase and *threo*-β-Chloroglutamate*

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ABSTRACT: When the aldimine form of aspartate aminotransferase is allowed to react with β-chloro-DL-glutamate in a rapid mixing apparatus, fast optical density changes due to the coenzyme chromophore occur in the region from 300 to 450 nm. These fast optical density changes are followed by slower ones associated with the reappearance of the spectrum of the free enzyme as substrate is exhausted. The kinetics of the fast process can be accommodated in a simple scheme, reflecting the formation of an enzyme–substrate complex: $E + S \rightleftharpoons ES$. The following values for the kinetic constants have been obtained: $k_1 = 7 \times 10^2$ M^{-1} sec^{-1} and $k_{-1} = 7.5$ sec^{-1}. The apparent equilibrium constant can be determined from the amplitude of the spectral change related to the fast process as a function of substrate concentration; the value thus obtained, 10^{-2} M for β-chloro-DL-glutamate, agrees well with the ratio of the off and on rate constants. The standard thermodynamic parameters for the formation of the enzyme–substrate intermediate are: $\Delta G° = -3$ kcal/mole at 307°K, $\Delta H° = 2$ kcal/mole, $\Delta S° = +16$ eu. The spectrum of the transient intermediate (ES) has been calculated from the kinetic difference spectrum and that of the initial form of the enzyme; its spectral features combined with chemical considerations suggest that ES may be tentatively identified with a coenzyme–substrate Schiff base. No evidence was found for the formation of any intermediate absorbing around 500 nm. Consideration of the kinetic and spectrophotometric data suggests that the rate-limiting step is the removal of the α proton from the substrate.

It has been recently shown (Manning *et al.*, 1968) that a substrate analog containing a strongly electronegative group in the β position, *threo*-β-chloroglutamate, undergoes, in the presence of catalytic amounts of aspartate aminotransferase,[1] a β-elimination reaction, with release of Cl^-, NH_4^+, and α-ketoglutarate. There is already quite strong evidence that this β-elimination reaction is specifically catalyzed by the aminotransferase itself, and not by some impurity (Manning *et al.*, 1968).

In the present study, the enzyme intermediates (ES) formed when *threo*-β-chloroglutamate reacts with soluble aspartate aminotransferase have been studied by rapid-mixing techniques. Events occurring immediately after mixing the aldimine form of the enzyme with the amino acid substrate were followed by measuring the changes in optical density occurring in the spectral region where the protein-bound coenzyme is known to absorb (300–500 nm).

Quite apart from the specific interest of this study in the mechanism of action of pyridoxal dependent enzymes, the special (kinetic) features of the system allow one to study the kinetics of a reversible part of the enzyme–substrate interaction as distinct from the other steps involved in the overall reaction mechanism. Therefore the system offers the opportunity for a detailed study of such reaction steps, information on which is still limited to a few enzyme systems.

Materials and Methods

Soluble aspartate aminotransferase was isolated in the aldimine form from pig heart as previously described (Martinez-Carrion *et al.*, 1967). *threo*-β-Chloro-DL-glutamate was synthesized according to Khomutov (Khomutov, 1966). All other compounds were reagent grade commercial samples from Merck, A. G., Darmstadt, or from C. Erba, Milano.

Absorption spectra were recorded in a Beckman DK-2 or in a Cary 14 spectrophotometer, provided with a thermostated cell holder (25 ± 1°). pH measurements were carried out in a Radiometer Model 4 potentiometer. Kinetic experiments were carried out in a Gibson–Durrum stopped-flow spectrophotometer, equipped with a 2-cm observation tube. The dead time of the instrument was approximately 3 msec.

Solutions of the aldimine form of soluble aspartate aminotransferase at concentrations from 5×10^{-5} to 10^{-4} M (expressed as coenzyme) were rapidly mixed with solutions of *threo*-β-chloro-DL-glutamate at concentrations between 10^{-3} and 4×10^{-2} M. All solutions were at pH 8.05 ± 0.05 in 0.1 M potassium phosphate buffer. The changes in transmission occurring after mixing were monitored at various wavelengths between 300 and 500 nm. Unless otherwise stated the kinetic experiments were performed at 20°.

Results

Upon mixing the enzyme with the substrate, optical density changes were observed in the range between 315 and 430 nm. In this spectral region the pyridoxal phosphate bound to aspartate aminotransferase shows absorption bands which

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[1] The abbreviation used is: AAT = L-aspartate–2-oxoglutarate aminotransferase, EC 2.6.1.1.

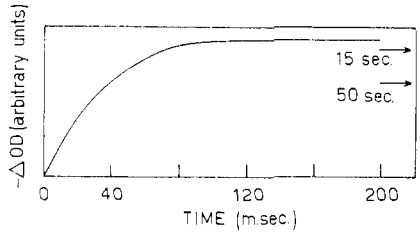

FIGURE 1: Time course of the optical density changes occurring at 370 nm upon mixing the aldimine form of aspartate aminotransferase (5×10^{-5} M) and 5×10^{-3} M *threo*-β-chloro-DL-glutamate. The arrows indicate the values of absorbancy 15 and 50 sec after mixing. Subsequently the absorbancy at this wavelength continues to increase slowly with time and tends to approach the original value at infinite time.

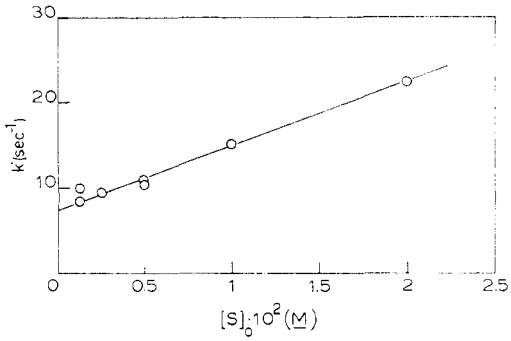

FIGURE 3: Substrate concentration dependence of the first-order rate constant for the fast step of the reaction between aspartate aminotransferase and *threo*-β-chloro-DL-glutamate.

change in position and extinction coefficients upon interaction of the enzyme with substrates and analogs (Jenkins *et al.*, 1959; Fasella and Hammes, 1967). Neither the substrate nor the products of the enzymic reaction under investigation absorb appreciably in this region, with the exception of the product α-ketoglutarate, which displays a weak band around 315 nm. Therefore, the contribution of α-ketoglutarate formation to the observed changes is relevant only at wavelengths lower than 350 nm and at product concentrations higher than 10^{-5} M. Under appropriate conditions, the observed changes in absorbancy can then be attributed uniquely to events occurring at the active site of the enzyme.

At all wavelengths and at the different concentrations of enzyme and substrate studied here, the overall time course of the process showed a fast phase, reaching an asymptote in less than a second, followed by slow changes over a time range of about 60 sec. This is illustrated in Figure 1 which shows the progress of the optical density change at 370 nm on mixing the enzyme with substrate.

At higher concentration of substrate, the possibility of distinguishing between the fast phase and the following processes is enhanced. Therefore at most of the substrate concentrations employed in the experiments, the early fast step may be analyzed independently of the subsequent events. The present investigation has been focused on this fast process. Under any set of conditions, the absorbancy changes

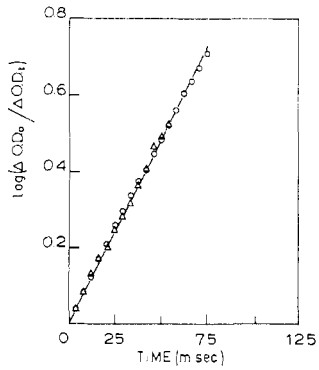

FIGURE 2: Pseudo-first-order plot of the optical density change associated with the fast step in the reaction between aspartate aminotransferase and *threo*-β-chloro-DL-glutamate: (△) data at 370 nm; (○) data at 330 nm.

related to the fast step followed a first-order time course provided that the concentration of substrate was much greater than that of the enzyme. In addition they were characterized by an identical time constant (k') at different wavelengths. Typical results are shown in Figure 2.

Figure 3 reports the dependence of the first-order rate constant for the fast step upon the initial substrate concentration (S_0). These results indicate that the data can be analyzed within the framework of a simple reversible bimolecular reaction. Accordingly, the data in Figure 3 allow the calculation of two rate constants, a binding (k_1) and a dissociation (k_{-1}) velocity constant (see Discussion).

The effect of temperature on these two rate constants calculated from data similar to those shown in Figure 3, but obtained at different temperatures, is reported in Figure 4.

The amplitude of the fast change was found to be dependent on the initial concentration of substrate, at constant enzyme concentration. This is shown in Figure 5, where the reciprocal of the total optical density change (at 370 nm), between time 0 and about 2 sec after mixing, is plotted against the reciprocal of the substrate concentration. Assuming that this amplitude represents the degree of binding attained in the (metastable) equilibrium reached in the fast process, this plot may be used to estimate an apparent equilibrium constant for the reaction involved, as will be shown in the Discussion.

Discussion

The results reported above indicate that *threo*-β-chloroglutamate reacts rapidly with the enzyme to give a spectroscopically distinct compound which slowly disappears; the spectrum of the aldimine enzyme is eventually restored. Thus, the initial rapid phase can be attributed to the formation of an enzyme–substrate complex and the slow phase to the transformation of this complex and, eventually, to the regeneration of free enzyme and formation of products. In a previous paper (Manning *et al.*, 1968), it had been shown that when *threo*-β-chloroglutamate was mixed with aminotransferase the reaction products were chloride, ammonia, and α-ketoglutarate; at the completion of the reaction the catalytic properties of the enzyme were unchanged.

The optical density changes at various wavelengths associated with the rapid initial process permit the identification of the spectroscopic properties of the transient intermediate which is taken to represent an enzyme–substrate complex

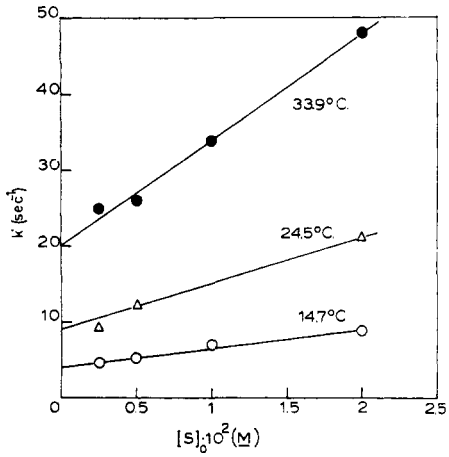

FIGURE 4: Temperature dependence of the first-order rate constants describing the fast step in the reaction between aspartate aminotransferase and *threo*-β-chloro-DL-glutamate.

(ES) (Figure 6). The main features of this spectrum and its relation to the possible structures of the enzyme–substrate complex will be discussed later. Here we want to emphasize that the time course of the rapid process is the same at different wavelengths, indicating that no significant amounts of other spectroscopically distinct intermediates are present. It should be pointed out that the contribution of α-ketoglutarate to the spectral change is negligible during the early phases of the reaction.

A quantitative analysis of the slow optical density changes, which should correspond to the transformation of the complex into other enzyme–substrate intermediates and their breakdown to free enzyme and product, has not been attempted here. Instead, the data relative to the fast step lend themselves to important qualitative and quantitative considerations. All available evidence relative to the fast step can be interpreted in a satisfactory way assuming that it corresponds to the establishment of the following fast equilibrium

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \dashrightarrow E + P \qquad (1)$$

(fast) (slow) (to be neglected)

where E represents the aldimine form of the aminotransferase, S the substrate, ES an enzyme–substrate complex, and P the products. According to the above scheme, the approach to the equilibrium starting from the free enzyme and substrate should follow a first-order time course if the initial substrate concentration is much higher than that of the enzyme. The apparent rate constant (k') is related to the initial substrate concentration by the linear equation

$$k' = k_{-1} + k_1(S_0) \qquad (2)$$

where k_{-1} is the first-order rate constant for the dissociation of the ES complex, k_1 is the second-order rate constant for the formation of ES, and (S_0) is the initial concentration of the substrate. Under the experimental conditions used, (S_0) is in large excess over (E), and remains nearly constant during the approach to equilibrium.

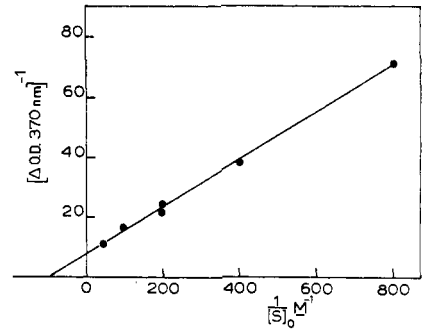

FIGURE 5: Double reciprocal plot of the amplitude of the optical density change associated with the fast step *vs.* the free substrate concentration.

The results reported in Figure 3 show that the experimental data fit eq 2 well; the graph is a straight line where the ordinate intercept is equal to k_{-1} and the slope is equal to k_1; the equilibrium constant is given by the ratio of these two kinetic constants, $K_{eq} = k_{-1}/k_1$. The values calculated from Figure 3 at 20° are: $k_1 = 7.2 \times 10^2$ M^{-1} sec^{-1}; $k_{-1} = 7.5$ sec^{-1}; $K_{eq} = 1.04 \times 10^{-2}$ M.

It must be pointed out that these data have been obtained with a preparation of racemic *threo*-β-chloroglutamate. If the reasonable assumption is made that only the L isomer binds (Jenkins *et al.*, 1959) the values become $k_1 = 1.44 \times 10^3$ M^{-1} sec^{-1}; $k_{-1} = 7.5$ sec^{-1}; $K_{eq} = 5.2 \times 10^{-3}$ M. The activation energies for the two rate constants, as calculated from the data in Figure 4, are

$$\Delta E_1^{\ddagger} = 14.6 \text{ kcal/mole}$$

$$\Delta E_{-1}^{\ddagger} = 16.3 \text{ kcal/mole}$$

The equilibrium dissociation constant can be determined independently from the results reported in Figure 5. Thus, for the system represented in eq 1, the following linear relationship exists between the reciprocal of the free substrate concentration (S) and the reciprocal of the concentration of the enzyme–substrate complex (ES)

$$\frac{1}{(ES)} = \frac{K_{eq}}{(E)_T(S)} + \frac{1}{(E)_T} \qquad (3)$$

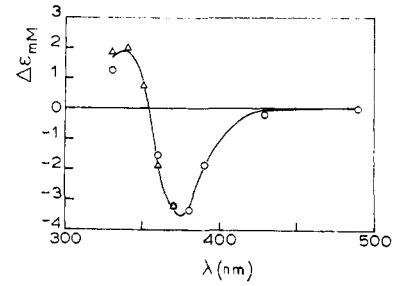

FIGURE 6: Wavelength dependence of the amplitude of the optical density change related to the fast step of the reaction between aspartate aminotransferase and *threo*-β-chloro-DL-glutamate. The values at each wavelength were calculated from the ordinate intercept of plots like that of Figure 5 using data obtained at 21° with 2.35×10^{-5} M (○) and with 1.5×10^{-5} M (△) enzyme.

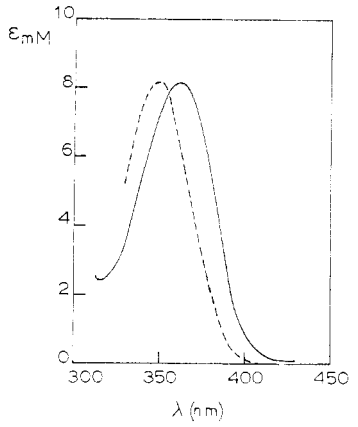

FIGURE 7: Absorption spectrum of the aldimine enzyme (——) and of the enzyme–substrate complex formed in the fast step of the reaction between aspartate aminotransferase and *threo*-β-chloro-DL-glutamate (-----).

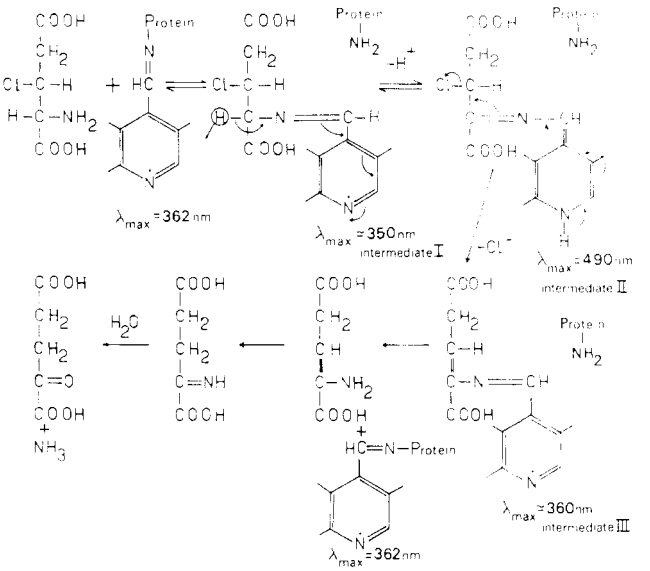

FIGURE 8: Tentative scheme for the reaction between AAT and *threo*-β-chloroglutamate. The absorption maximum attributed to each intermediate is given under the corresponding formula.

where $(E)_T$ is the total enzyme concentration, and (ES) and (S) are the equilibrium concentrations of the enzyme–substrate complex and of the free substrate, respectively. It can be shown that

$$(ES) = \frac{\Delta OD}{\Delta\epsilon} \tag{4}$$

where $\Delta\epsilon = \epsilon_{ES} - \epsilon_E$ is the difference in the molar extinction coefficients of the enzyme–substrate complex and the free enzyme, and ΔOD is the experimentally determined difference between the absorbancy at equilibrium (when the enzyme is present partly as free enzyme and partly as enzyme–substrate complex) and the absorbancy at time zero (when all the enzyme is in the free form ((ES) = 0)). Substitution of eq 4 into eq 3 gives eq 5.

$$\frac{1}{\Delta OD} = \frac{K_{eq}}{\Delta\epsilon \times (E)_T} \times \frac{1}{(S)} + \frac{1}{\Delta\epsilon \times (E)_T} \tag{5}$$

The plot in Figure 5 shows that the experimental data fit eq 5 quite well. The value of K_{eq} calculated from this plot is 1.05×10^{-2} M for the racemate or 5.25×10^{-3} M for the L isomer. This value is in excellent agreement with the value calculated from the kinetic constants (k_{-1}/k_1) and is consistent with the K_M value obtained from steady state measurements, taking into account the differences in the conditions of pH and ionic strength at which the latter was determined (Manning *et al.*, 1968).

The agreement between the values of K_{eq} obtained from the data in Figures 3 and 5 may be taken as a further evidence for the absence of detectable amounts of other spectroscopic intermediates and for the consistency of the experimental data with the simple eq 1. It is possible, however, that other rapidly equilibrating enzyme–substrate intermediates may be formed at concentrations which, under the experimental conditions used, are too low to be detected. Since at the concentrations used in this study (from 5×10^{-5} to 1×10^{-4} M) the enzyme is made up of two subunits, each containing one molecule of coenzyme (Jenkins *et al.*, 1959; Martinez-Carrion *et al.*, 1967), these results provide rather convincing evidence for the functional equivalence and independence of the two sites in the enzyme molecule, at least for what concerns the interaction with *threo*-β-chloroglutamate. It is interesting that substrate activation related to interaction between the active sites has been postulated to occur in the system aspartate aminotransferase plus α-methyl-L-aspartate (Hammes and Haslam, 1968).

It is evident from eq 5 that the ordinate intercept of the plot shown in Figure 5 is equal to $1/\Delta\epsilon \times (E)_T$. Thus, from experiments performed at various wavelengths, it is possible to obtain the kinetic difference spectrum between the enzyme–substrate complex and the free enzyme, and therefore the spectrum of the postulated enzyme–substrate complex. This spectrum is shown in Figure 7.

It is not possible to ascribe unequivocally a definite chemical structure to the observed enzyme–substrate intermediate. Its spectral features, however, are worthy of comment. According to the mechanism proposed for pyridoxal phosphate catalyzed β-elimination reactions (Braunstein, 1960; Morino and Snell, 1967; Snell and Morino, 1968), the present reaction might well proceed as shown in Figure 8.

The wavelengths of the absorption maxima, tentatively attributed to each intermediate on the basis of previous experience with pyridoxal enzymes (Jenkins *et al.*, 1959; Velick and Vavra, 1962; Jenkins and D'Ari, 1966; Jenkins, 1964; Snell and Morino, 1968), are written under the corresponding formula. The enzyme–substrate complex observed by us presents an absorption maximum around 350 nm and might on this basis be identified with either compound I or with compound III of Figure 8. However, chemical considerations suggest that the transformation of compound II into compound III is an essentially irreversible process; consequently, compound III could not be in equilibrium with the free enzyme and substrate. It seems reasonable, therefore, to identify tentatively the intermediate observed in the present study as compound

I. This would indicate that the rate-limiting step in the β elimination catalyzed by aspartate aminotransferase is the labilization of the substrate α-hydrogen; conversely in the β elimination catalyzed by tryptophanase it has been shown that the rate-limiting step is the removal of the β substituent from the enzyme-bound substrate (Morino and Snell, 1967); this leads to the accumulation of an enzyme–substrate intermediate with an absorption maximum around 500 nm, identified with compound II of Figure 8. In the present study with aspartate aminotransferase and *threo*-β-chloroglutamate, no evidence was found for the formation of detectable amounts of material absorbing in this region.

The rate-limiting step for the reactions catalyzed by soluble aspartate aminotransferase varies with the substrate. Thus, in the transamination between the aldimine enzyme and L-glutamate or L-aspartate (Banks *et al.*, 1968), as well as in the β elimination from *threo*-β-chloroglutamate, the rate-limiting step probably coincides with the labilization of the bond between the α carbon atom of the substrate and hydrogen, while in the transamination between the aldimine enzyme and *erythro*-β-hydroxy-L-aspartate the rate-limiting step has been tentatively assigned to the hydrolysis or to a conformational change of the enzyme–substrate ketimine intermediate (Hammes and Haslam, 1969).

Assuming that the intermediate observed is an enzyme–substrate aldimine complex, the values of the thermodynamic and kinetic parameters obtained with *threo*-β-chloroglutamate may be compared with those obtained with α-methyl-L-aspartate which forms a similar complex. Such a comparison is reported in Table I. The equilibrium dissociation constant for the complex with *threo*-β-chloroglutamate (assuming that the D isomer does not interact with the enzyme) is about half the dissociation constant for the complex with L-glutamate (Jenkins and D'Ari, 1966) (Fasella and Hammes, 1967), of the same order as the constant for the complex with α-methyl-L-aspartate (Hammes and Tancredi, 1967), and considerably larger than that for *erythro*-β-hydroxyaspartate (Jenkins, 1964; Hammes and Haslam, 1969). Some caution must be used when comparing these data, since they have been obtained with different buffers, pH, and ionic strength, and it is known (Jenkins and Tsai, 1968) that these factors can affect the parameters under consideration.

The formation of a Schiff base between the enzyme and the amino acid substrate has a positive enthalpy change both with β-chloroglutamate and with α-methylaspartate. A simple interpretation of this finding (Hammes and Tancredi, 1967) is that the internal coenzyme–protein Schiff base is energetically more stable than the Schiff base between the protein-bound coenzyme and the substrates. The latter complex is formed only because of the favorable entropy change which may be due to desolvation of the amino acid and/or to an increased freedom of the enzyme structure due to the breakdown of the internal Schiff base.

The rate constants for the formation and dissociation of the enzyme–substrate complex are much lower for *threo*-β-chloroglutamate than for the real substrate L-glutamate and of the same order as those for the analog α-methyl-L-aspartate. Thus, the apparent bimolecular rate constant for the formation of the complex is $>10^7$ M^{-1} sec^{-1} for L-glutamate and L-aspartate (Fasella and Hammes, 1967), 3×10^6 M^{-1} sec^{-1} for β-hydroxy-L-aspartate (Hammes and Haslam, 1969), 1.2×10^4 M^{-1} sec^{-1} for α-methyl-L-aspartate (Hammes and Haslam, 1968), and 1.44×10^8 M^{-1} sec^{-1} for *threo*-β-chloro-L-glutamate.

TABLE I: Thermodynamic Parameters for the Interaction of AAT with α-Methyl-L-aspartate and with *threo*-β-Chloro-L-glutamate.

	$\Delta G°$ (kcal/mole (°K))	$\Delta H°$ (kcal/mole)	$\Delta S°$ (eu)
α-Methyl-L-aspartate[a]	−3.5 (298)	5.9	+31
threo-β-Chloroglutamate	−3.0 (307)	2.0	+16

[a] Hammes and Tancredi, 1967.

When comparing these values, it is important to remember that the data for *threo*-β-chloroglutamate do not exclude a reaction scheme of the type $E + S \underset{\text{fast}}{\rightleftharpoons} ES_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES_2$ (Hammes and Haslam, 1969), where the equilibrium constants are such that under the experimental conditions used ES_1 is present at negligibly low concentrations. It is evident that whenever the latter scheme applies, the apparent second-order rate constant for the formation of the enzyme–substrate complex contains both the equilibrium constant for the formation of ES, and the kinetic constant for the conversion of ES_1 into ES_2. For these reasons a direct comparison among the above data may not be unequivocal. It seems evident, however, that the presence of substituents in the α or β positions of the analogs markedly affects the "apparent" second-order rate constants. This might be an indication that the efficiency with which the enzyme catalyzes the formation of the coenzyme–substrate Schiff base is strictly dependent upon the substrate structure.

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Molecular Conformation of Egg-White Lysozyme and Bovine α-Lactalbumin in Solution*

W. R. Krigbaum and F. R. Kügler

ABSTRACT: Small angle diffraction measurements are reported for hens egg-white lysozyme and bovine α-lactalbumin. The amino acid sequences of these two enzymes exhibit considerable homology, which has led to the suggestion that they may have similar tertiary structures. Lysozyme has a radius of gyration, R, of 14.3 Å, and its equivalent scattering body is a prolate ellipsoid having dimensions 28 × 28 × 50 Å, while α-lactalbumin has R = 16.7 Å, and its equivalent ellipsoid is oblate with dimensions 22 × 44 × 57 Å. We therefore conclude that lysozyme and α-lactalbumin have quite different molecular conformations in solution. The observed R value for lysozyme is quite close to that calculated from the crystallographic coordinates assuming the molecule to be in a vacuum, R = 13.8 Å. This observation, in conjunction with the good agreement between the scattering curves and the electron pair radial distribution functions observed for the molecule in solution and calculated from the crystallographic coordinates, indicates that lysozyme undergoes very little conformational change on dissolution.

Lysozymes form a class of widely distributed enzymes found in a number of organs, tissues, and secretions of vertebrates, as well as in bacteria, phages, and plants. Members of this class may differ considerably in molecular weight, but all exhibit a common capability to rapidly lyse bacterial cell walls by their action as muramidases. Comparison of the amino acid sequence of T4 phage lysozyme (Tsugita and Inoye, 1968) and hens egg-white lysozyme (Jollès *et al.*, 1963; Canfield, 1963) revealed no common primary structure, although there was some compositional similarity in terms of the relative numbers of basic, acidic, and hydrophobic side chains. In the following we shall refer to hens egg-white lysozyme as lysozyme.

Lactose synthetase catalyzes the reaction:

$$\text{UDP-galactose} + \text{glucose} \longrightarrow \text{lactose} + \text{UDP}$$

The soluble enzyme from milk may be separated into two proteins, A and B, both of which are required for lactose synthesis. Ebner, Brodbeck, and coworkers (1966, 1967) have identified the B protein as α-lactalbumin.

Although α-lactalbumin and the lysozymes perform different functions, some evidence for structural similarity of bovine or guinea pig lactalbumin and hens egg-white lysozyme has been provided by Brew and Campbell (1967) and Yasunobu and Wilcox (1958). This inference was confirmed when Brew *et al.* (1967) determined the amino acid sequence of bovine α-lactalbumin. The latter workers observed that over 40 of the residues in α-lactalbumin were identical with the corresponding residues in egg-white lysozyme, and that an additional 27 residues could be classified as conservative replacements (Smith and Margoliash, 1962). Further, each of the two molecules has four disulfide bonds formed by the same half-cystinyl residues. Thus approximately 40% of the residues of α-lactalbumin and lysozyme are identical (Hill *et al.*, 1968), as compared to 44% for the α and β chains of human hemoglobin (Ingram, 1963). In view of the fact that the α- and β-hemoglobin chains have very similar tertiary structures, and that the four disulfide bonds in α-lactalbumin and lysozyme are formed in the same way, there is a possibility that the latter two proteins may have very similar tertiary structures. Browne *et al.* (1969) have investigated the possibility of building an hypothetical molecular model of α-lactalbumin by appropriate alteration of the known structure of lysozyme (Blake *et al.*, 1965). Since α-lactalbumin contains six fewer residues, it was necessary to delete certain residues from the lysozyme structure. A few additional deletions were necessary to achieve maximum homology of the two structures. It was found that the residues to be deleted occurred in loops or at the end of the helical regions, so that it was possible to build a speculative molecular model of α-lactalbumin which retained essentially all of the features of the secondary and tertiary structures of lysozyme.

Evidence that lysozyme and α-lactalbumin have similar conformations in solution was provided by Aune (1968), who found that these two proteins have indistinguishable optical rotatory dispersion curves between 206 and 233 mμ,

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