Sodium Cholate-induced Changes in the Conformation and Activity of Rat Pancreatic Cholesterol Esterase*

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Patrick W. Jacobson, Paddy W. Wiesenfeld, and Linda L. Gallo*
From the Department of Biochemistry, The George Washington University Medical Center, Washington, D. C. 20037

Ramon L. Tate
From the Computer Systems Laboratory, National Institutes of Health, Bethesda, Maryland 20892

James C. Osborne, Jr.
From Beckman Instruments, Inc., Palo Alto, California 94304

Pancreatic cholesterol esterase (CEase) regulates dietary cholesterol absorption and is activated in the presence of trihydroxy bile salts while remaining inactive in their absence or in the presence of di- or monohydroxy bile salts. CEase from rat pancreas has been purified by ammonium sulfate precipitation, hydroxylapatite chromatography, and gel filtration on Sephacryl S-200/S-300 columns connected in series, and its homogeneity and Mr (55,418 ± 288) have been determined by sedimentation equilibrium centrifugation. The effects of tri-, di-, and monohydroxy bile salts on the conformation of the purified enzyme in buffer solution and in an in vitro assay system were studied by circular dichroism spectropolarimetry. The CD spectrum of the enzyme in solution shows a curve shape suggestive of an α-helicity, but low mean residue ellipticity (MRE) values may indicate an important β-turn contribution. Sodium cholate, a trihydroxy bile salt, induces a decrease in the negative MRE values of the enzyme in solution at bile salt concentrations of 70–100 mM, with no further spectral changes at concentrations as high as 1 mM. Sodium cholate concentrations higher than 1 mM also induce an increase in the enzyme’s negative MRE values under activity assay conditions, which reverts toward its original value once the reaction reaches equilibrium. These latter changes are interpreted as induced by substrate binding to the enzyme followed by partial substrate depletion after the reaction reaches equilibrium. Sodium deoxycholate, a dihydroxy bile salt, induces unstable transient increases and decreases in the MRE values of CEase in buffer solution and under activity assay conditions. These changes are bile salt concentration-dependent and may reflect self-association of the protein. Sodium taurocholate, a monohydroxy bile salt, does not affect the CD spectrum of CEase, and neither the di- nor the monohydroxy bile salt activates the enzyme.

The role of pancreatic CEase in cholesterol absorption and the relation between bile salts and the activation of CEase from a variety of origins have been studied for a number of years (1–7), as well as the relationship of this enzyme to other cholesterol-esterifying enzymes (8–10). Calame et al. (11) studied the binding of bile salts to purified CEase from rat pancreas and suggested activation mechanisms involving bile salt-induced conformational changes in the enzyme. It was subsequently suggested that this putative conformational change may constitute the rate-limiting step in the enzyme’s catalytic sequence.

We have investigated whether specific bile salts cause conformational changes in CEase and whether observed conformational changes correlate to enzyme activity. The effects of tri-, di-, and monohydroxy bile salts on the conformation of purified rat pancreatic CEase in solution and under in vitro assay conditions are compared, along with the effects of these bile salts on the enzyme’s activation. The information obtained clarifies the mechanism of cholic acid activation of pancreatic CEase.

MATERIALS AND METHODS

CEase Assay—CEase activity was assayed by the method of Vahouny et al. (12) modified as follows. The emulsions used in the activation studies and their corresponding CD determinations contained, in 1 ml of 0.15 M sodium phosphate buffer (pH 6.2), 20 nmol of [14C]cholesterol, 0.15 µmol of cholesterol, 0.53 µmol of oleic acid, 0.66 µmol of ammonium chloride, 0.04 mg of fatty acid-free bovine serum albumin, and 0.2% Tween 20 in place of sodium taurocholate.

The substrate mixture was incubated with an appropriate amount of enzyme preparation at 37 °C in a Dubnoff metabolic shaker. At timed intervals, aliquots 80–70 µl were removed from the reaction mixture and added to 20 volumes of chloroform:methanol (2:1). One volume of 0.5% CaCl2 was then added to this mixture and mixed vigorously. After phase separation, the top layer was discarded, and the bottom layer was dried under nitrogen. The sediment was then resuspended in 75–100 µl of hexanes and processed as described. Betafluor (National Diagnostics, Inc.) was used as scintillation mixture, and the radioactivity in counts/min was measured in a Beckman LS 250 scintillation counter.

Purification—CEase was purified from rat pancreas (Pel-Freez Biologicals) by ammonium sulfate precipitation, adsorption chromatography on hydroxylapatite (Bio-Rad), and gel filtration on Sephacryl S-200/S-300.

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1 Portions of this paper (including part of “Materials and Methods” and Equations 1–4) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

1 The abbreviations used are: CEase, cholesterol esterase; MRE, mean residue ellipticity; SDS, sodium dodecyl sulfate.
crol S-200/S-300 columns (Pharmacia LKB Biotechnology Inc.) con-
ected in series. Unless otherwise indicated, all purification steps
were conducted at 4 °C and pH 6.2, and all buffers contained 0.1 M
NaCl, 0.02% sodium azide, 0.001 M EDTA, 1.5% glycerol, and 1 mg/
liter leupeptin (see Miniprint). Homogeneity of the purified enzyme
protein was determined by SDS-polyacrylamide gel electrophoresis
according to the method of Irwin et al. (13) and by sedimentation
centrifugation (see Miniprint).

Sedimentation Equilibrium Measurements—Sedimentation equi-
librium measurements were performed in a Beckman Model E ultra-
centrifuge equipped with an ultraviolet photoelectric scanner and
temperature control system following the method of Osborne et al.
(14-16), Formisano et al. (17), and Lee et al. (18) (see Miniprint).

Circular Dichroic Measurements—Circular dichroic spectra of sam-
pleus of purified CEase in the presence and absence of tri-, di-, and
monohydroxy bile salts were obtained using a Cary 61 or a Jasco 500
A spectropolarimeter equipped with a thermostated cell holder ac-
cording to the method of Osborne et al. (14-16) (see Miniprint).
The temperature was maintained at 22.5 or 37 °C, and sample as well as
buffer blank spectra were obtained within the wavelength range
of 270-200 nm. Cell path lengths were 0.497 and 0.502 nm for the Cary
61 system and 1.000 nm for the Jasco 500 A system, and protein
concentration ranged from 90 to 600 μg/ml.

Activation of CEase—The effect of increasing concentrations of
sodium cholate on the activation of CEase was studied as described
(see Miniprint). Blank assays contained all the components of the ex-
perimental assays except for the absence of bile salts and were used
to determine residual base-line nonenzymatic esterification. Samples
of reaction mixture were also withdrawn from some of the reaction
sets, and their CD spectra were determined as described below.
Supplementary control assays were carried out in which the enzyme
 aliquots were incubated with sodium deoxycholate or sodium tauro-
lithocholate at final concentrations in the reaction mixture of 1 mM
and 1 μM, respectively.

RESULTS
Purity of CEase—The method described in this manuscript
yields a recovery of over 25% of highly purified enzyme protein
with a specific activity range of 344-694 units/mg of protein. This
represents a significant improvement over the procedure
described previously (11), which produced a yield of 3-6% of
the initial activity with a specific activity range of 210-470
units/mg of protein.
The purified protein migrated as a single band on SDS-
polyacrylamide gels, as seen in Fig. 1. Homogeneity and
apparent molecular weight were determined by sedimentation
equilibrium centrifugation as described above. The use of
Equations 1-3 permits a direct and sensitive estimation of
the homogeneity and apparent molecular weight of the protein
sample. The original concentration versus radius data are
fitted to Equation 3, which assumes the presence of only one
homogeneous species. The unknowns in this equation are the
concentration of the protein at the meniscus and the molec-
ular weight. The least squares values of these two parameters
can then be used to generate a theoretical concentration
(milligrams/milliliter) versus radius (centimeter) profile for
the sample. The deviations between experimental concentra-
tion values and those obtained by the use of Equation 3 are a
random function of the radius and a clear indication of sample
homogeneity. If the sample contained detectable species
which differed in molecular weight, the deviations between
experimental and theoretical data would be a nonrandom
function of the radius. Fig. 2 is a plot of the ln c versus the
square of the radius of purified CEase. The least squares slope
of this plot is used to obtain the apparent weight average
molecular weight by using Equation 2. Fig 3 shows the appar-
ent weight average molecular weight (M̅w) of purified CEase
as a function of protein concentration as measured by sedi-
mementation equilibrium, in terms of molecular weight (M̅w ×
10^-7) versus milligrams/milliliter. The molecular weight
of CEase in guanidinium chloride buffer obtained by this method
was 55,418 ± 288.

![FIG. 1. Demonstration of purity of cholester esterase by
SDS-polyacrylamide gel electrophoresis. Migration positions of
standard molecular weight markers and CEase are indicated. Further
details are under “Materials and Methods.”](http://www.jbc.org/)

![FIG. 2. Sedimentation equilibrium of purified CEase: plot
of natural logarithm of concentration (ln c) versus square of
radius (r²). Details are under “Materials and Methods.”](http://www.jbc.org/)

![FIG. 3. Sedimentation equilibrium of purified CEase: plot
of apparent weight average molecular weight versus protein
concentration. Details are under “Materials and Methods.”](http://www.jbc.org/)
Circular Dichroism — The CD spectra of CEase in the far-ultraviolet is included in Fig. 4 and shows negative transitions at 221 and 210 nm as well as a positive maximum at about 200 nm. These features are indicative of a globular protein. However, the relatively low MRE values observed suggest a strong contribution of β-turn conformation to the spectrum, presumably given by the disulfide bonds and the carbohydrate in the enzyme. This can contribute strongly positive ellipticity bands with maxima at 224 and 202 nm and thus partially cancel the negative α-helical maxima at 220–222 and 208–210 nm (26).

Effect of Sodium Cholate — At concentrations of 100 nM to 1 mM, this trihydroxy bile salt induced a reduction in the negative MRE values of CEase, particularly noticeable at the two transitions, as shown in Fig. 4. The general shape of the spectral curve was not significantly altered. The effect is observed when the enzyme is in a simple buffer solution or under activity assay conditions. In the latter situation, the enzyme also undergoes an increase in the negative MRE values at bile salt concentrations of 1 µM to 1 mM, as seen in Fig. 5. The MRE values of the pertinent spectral curves tend to approach their initial values after 2 h of reaction time, as shown in Fig. 6.

Fig. 4. Effect of sodium cholate on CD spectrum of purified CEase. The CD spectra of purified CEase in the presence of sodium cholate at concentrations of 0–1 mM were obtained with a Cary 61 spectropolarimeter. Further details are under “Materials and Methods.”

Fig. 5. Effect of sodium cholate on CD spectrum of purified CEase under activity assay conditions. The CD spectra of CEase in the presence of sodium cholate at concentrations of 0–1 mM were obtained with a Jasco 500 A spectropolarimeter. Further details are under “Materials and Methods.”

Fig. 6. Effect of sodium cholate on CD spectrum of purified CEase under activity assay conditions after 2 h of reaction time. The CD spectra of CEase in the presence of sodium cholate at concentrations of 0–1 mM were obtained with a Jasco 500 A spectropolarimeter. Further details are under “Materials and Methods.”

Fig. 7. Effect of sodium deoxycholate on CD spectrum of purified CEase. The CD spectra of CEase in the presence of sodium deoxycholate at concentrations of 0–1 mM were obtained with a Cary 61 spectropolarimeter. Further details are under “Materials and Methods.”

Fig. 8. Effect of sodium deoxycholate on CD spectrum of purified CEase under activity assay conditions. The CD spectra of CEase in the presence of sodium deoxycholate at concentrations of 0–1 mM were obtained with a Jasco 500 A spectropolarimeter. Further details are under “Materials and Methods.”
proaches equilibrium at about 30 min of reaction time and that, under the assay conditions, no significant increase in esterification was determined from control mixtures in the absence of terol. Each reaction mixture (0.5 ml) contained 62 pg of CEase and included 0.2% Tween 20 as substrate-solubilizing agent. Substrate described under "Materials and Methods." Nonenzymatic cholesterol emulsions were prepared, and assays were performed and analyzed as described above (30).

**Effect of Sodium Deoxycholate**—This dihydroxy bile salt induced large increases in the enzyme’s negative MRE values at concentrations of 1 μM to 100 μM. These changes were particularly noticeable at the 208-210 nm transition and suggest an increase in α-helicity. Further increases in bile salt concentration caused a reversion of the changes described toward the MRE values of the bile salt-free enzyme. These effects are shown in Fig. 7. The same type of spectral changes was observed with the enzyme under activity assay conditions, as seen in Fig. 8, where the transitions at 220 and 210 nm became very prominent, suggesting once again an increase in the α-helical character of the enzyme’s spectrum.

**Effect of Sodium Taurocholate**—This monohydroxy bile salt showed no detectable effect on the CD spectrum of CEase within the bile salt concentration range used, as shown in Fig. 9.

**Activation of CEase**—Table I summarizes the results of typical CEase titration with sodium cholate. It is apparent that enzyme activity becomes clearly detectable when the concentration of sodium cholate reaches 100 μM in the reaction mixture. It is also apparent that the reaction rate approaches equilibrium at about 30 min of reaction time and that, under the assay conditions, no significant increase in CEase activity is detected with sodium cholate concentrations higher than 500 μM in the reaction system. No esterifying activity was detected in CEase systems in which the enzyme was preincubated with sodium deoxycholate or sodium taurocholate.

**DISCUSSION**

The molecular weight of CEase in the presence of denaturants, as determined by sedimentation equilibrium, was 55,418 ± 288. This value is lower than that of 69,000–70,000 reported previously (11) as obtained by SDS-polyacrylamide gel electrophoresis or gel filtration chromatography. This difference can be attributed to the presence of carbohydrate associated with the enzyme protein. The carbohydrate content of purified CEase (4.5%) has been recently determined and is presumably covalently attached to the protein. Glycoproteins have a lower charge-to-volume ratio in the presence of SDS and do not migrate as rapidly as standard proteins on SDS gels. Molar mass determinations by sedimentation equilibrium centrifugation are based on first principles and do not depend upon the use of known standards. In addition, a recent report by Hui et al. (28) describes the cloning of rat pancreatic CEase with a reading frame coding for a 67,000 Mr protein. The difference between the Mr values of the purified enzyme and the cDNA-coded protein may also be accounted for by the existence of a Mr 17,000 signal peptide which puts CEase into a secretory route and by a signal peptide which signals the attachment of glycosylphosphatidylinositol but is itself cleaved. In other proteins with glycosylphosphatidylinositol attachments, this peptide contains 17–33 residues. Glycosylphosphatidylinositol attachment to CEase has been recently suggested by Gallo and Wiesenfeld (29), and the topic of glycosylphosphatidylinositol anchoring has been recently reviewed as well (30).

The contribution of the β-turn component to the conformation of CEase needs to be further clarified. The studies described above show that the ligand-associated changes in the CD spectrum of this enzyme affect the MRE values more significantly than the overall shape of the spectral curve. This could be interpreted as related to a change in the contribution of the β-turn component of the spectrum, rather than a shift toward a more β-sheet or random coil conformation, which would most probably involve a change in the shape of the spectral curve as well. In addition, the relatively low MRE values at its λmax of 221 and 210 nm indirectly point to an effect of the β-turn on the overall conformation of the enzyme since its positive ellipticity bands between 235 and 212 nm (λmax = 224 nm) and 210 and 190 nm (λmax = 202 nm) could partially cancel the negative ellipticity bands precisely at their λmax regions. An essentially α-helical CD spectral curve would be expected to show more negative MRE values if no significant β-turn contribution would make them less negative. The CD spectrum of CEase in buffer solution presents interesting features. Although the general shape of the spectral band suggests an important α-helical contribution, the low MRE values between 230 and 210 nm point to the possibility of an important β-turn component, with strong positive ellipticity maxima at 224 and 193 nm and negative ellipticity maxima at 202–205 nm. The concentration of all β- and monohydroxy bile salts on the CD spectrum of the enzyme are clearly distinguishable. Sodium cholate at concentrations of 70–100 μM induces a single conformational change indicative of a decrease in the MRE values of the enzyme. Ellipticity changes plateau at higher concentrations, probably due to the binding of one or a few molecules of this bile salt to specific

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**TABLE I**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Sodium cholate</th>
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<tr>
<td></td>
<td>100 μM</td>
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<tr>
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<td>0.5</td>
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<tr>
<td>0.8</td>
<td>0.8</td>
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<tr>
<td>6</td>
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<tr>
<td>10</td>
<td>1.7</td>
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<tr>
<td>15</td>
<td>2.1</td>
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<tr>
<td>20</td>
<td>2.9</td>
</tr>
<tr>
<td>30</td>
<td>3.9</td>
</tr>
<tr>
<td>60</td>
<td>4.4</td>
</tr>
<tr>
<td>90</td>
<td>≈0</td>
</tr>
</tbody>
</table>

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3 P. W. Wiesenfeld, unpublished results.
site in the enzyme. In contrast, the effect of sodium deoxycholate, a dihydroxy bile salt, on the CD spectrum of CEase in buffer solution is complex and indicative of transient unstable conformational changes. The greater negative MRE values of the CD spectrum of the enzyme without bile salt as well as the induced spectral changes may also be due in part to a protein concentration-dependent reversible self-association of the individual enzyme molecules. Concentration-dependent changes in α-helicity has been reported for several apoproteins (14, 16, 21, 20). Monohydroxy bile salts such as sodium taurothiocolcholate do not show any detectable effect on the conformation of CEase in solution.

Evaluation of the enzyme’s conformation under activity assay conditions at varying concentrations of bile salts revealed some interesting effects. In the first place, sodium cholate at concentrations of 70–100 mM in the reaction mixture induced conformational changes similar to those observed in buffered solution. Higher concentrations of this bile salt caused a second conformational change which was not detected in buffer solution. This change suggests an increase in the α-helical contribution to the enzyme’s conformation and is reversed when the enzyme reaction reaches equilibrium. This tendency to revert toward a more stable conformation is indicative of the transient character of this second conformational change, which could be interpreted as induced by substrate binding to the enzyme. After a maximum rate or esterification is reached and the reaction is allowed to continue in time, a point can be reached at which less substrate becomes available for binding to free enzyme. The free enzyme molecules may have a different conformation, and the MRE values observed in the reaction system at any given time after reaching equilibrium would depend on the percentage of free enzyme molecules to those bound with substrate.

The effects of sodium deoxycholate on the conformation of CEase under activity assay conditions, on the other hand, show no significant differences from those shown by the enzyme in solution. This may reflect substrate binding to a conformationally inactive enzyme and would agree with the observation that CEase is not enzymatically activated in the presence of the dihydroxy bile salt.

The effects of bile salts on the conformation of rat pancreatic CEase observed in these studies are in sharp contrast with those recently reported by Tsujita et al. (19) for pig pancreatic carboxylic-ester lipase, viz. cholesterol esterase. Their monomeric enzyme (M, 74,000) showed no conformational changes upon addition of sodium cholate and sodium deoxycholate at single concentrations of 200 and 100 μM, respectively. However, these findings are difficult to evaluate for lack of important methodological information. On the other hand, we wish to point out two areas of general agreement with the findings of Tsujita et al. The first is the 1:1 stoichiometry between CEase and cholate-type bile salts which was suggested by our laboratory in 1975 (11). The second is the fact that interaction between CEase and bile salts can be detected and quantitated at bile salt concentrations below 2.5 mM, reaching its maximum at about 8 mM. Although the activities measured were synthesis of cholesterol esters for rat CEase and their hydrolysis for the human enzyme, the difference in bile salt concentration required for activation may still be significant for the question of dimerization concomitant with activation, as reported for the human enzyme. In addition, the specific importance of the 7α-hydroxyl group for activation of rat CEase, as shown for the human enzyme, would be worth investigating. Lombardo and Guy (7) have also suggested that deoxycholate-type bile salts have no effect on the enzyme, but act on the substrate’s (cholesterol ester) conformation. In contrast, we observed that sodium deoxycholate did modify the conformation of rat pancreatic CEase both in the presence and absence of the substrate for the esterification reaction.

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REFERENCES

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D. Gel Filtration Chromatography

Two Pharmacia F 250 100 columns, containing a total volume of approximately 500 ml of Pharmacia Blue agarose 100-50. Mucopolysaccharides and mucoproteins are eluted in two major fractions, which were collected in series. The mobile phase was 20 mm Tris-HCl buffer at pH 8.0 and containing (per liter) 0.6 mm EDTA, 0.6 mm NaCl, 0.2 mm CaCl₂, 0.02 mm ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 0.6 mm p-nitrophenyl phosphate at pH 8.0. The samples were loaded onto the column in 3.5 ml of the mobile phase, and the elution was performed in 1.5 ml of the mobile phase. The fractions were collected in 500-ul fractions and assayed for activity.

Sedimentation equilibrium

Samples of purified ChEs containing 100 to 400 mg/ml were dialyzed against 0.15 M sodium phosphate buffer, pH 7.4, and centrifuged (42,000 g, 1 h, 0°C). The resulting glycerol was removed, and the sample was centrifuged at 30,000 g, 1 h, 0°C. The supernatant was phase-buffered in 0.15 M sodium phosphate buffer, pH 7.4, containing 2% glycerol, and 0.6 mm p-nitrophenyl phosphate at pH 8.0. The samples were assayed for activity after being centrifuged at 30,000 g, 1 h, 0°C. The supernatant was phase-buffered in 0.15 M sodium phosphate buffer, pH 7.4, containing 2% glycerol, and 0.6 mm p-nitrophenyl phosphate at pH 8.0. The samples were assayed for activity after being centrifuged at 30,000 g, 1 h, 0°C.
CIRCULAR DICHROIC MEASUREMENTS

A. C1ase in Buffer Solution

C1ase was titrated with NaCl, a tributyrin bile salt or with NaOOCl, a tributyrin bile salt, whose purity had been previously checked by gas chromatography or a United Technologies Beckman Model 110A system, as follows. After an initial CD spectrum of the C1ase sample (usually 0.1 mg/ml) was obtained using a Cary 61 spectropolarimeter under the conditions described in the Main Text section, a small volume of the stock solution of the bile salt, made in 0.1 M succinate phosphate buffer, was added to the sample in a cuvette and mixed for approximately 1 minute. The CD spectrum of the solution was then immediately obtained as an average of 2 to 5 scans at 0.1 nm intervals. The bile salt concentrations in the cuvette were usually 20, 80, 150, or 300 mM in 0.1 M succinate phosphate buffer. The titration of C1ase with NaCl, NaOOCl, a monobutyrin bile salt, was performed as described above. However, the two buffers were used for these titrations; the NaCl concentration was 0.1 M, and the NaOOCl concentration was 0.5 M. As experimental controls, chymotrypsinogen (250 mg/ml) and bovine serum albumin (250 mg/ml) were titrated with NaCl as described above. The raw data ellipticity was processed according to the reference of C1ase using a microcomputer based data acquisition system RT/1L, Malvern developed at the Computer Systems Laboratory, National Institutes of Health, Bethesda, Maryland. Each CD spectra were then averaged and converted to MCD values at each wavelength according to Equation (6) below, after correction for appropriate buffer blanks, baseline shifts, and protein concentration changes.

\[ \delta = \frac{\theta - \theta_0}{c} \]  

where \( \delta \) is the Mean Residue Ellipticity in deg cm²/mole, \( \theta \) is the observed ellipticity in mdeg/deg/nm, \( \theta_0 \) is the baseline shift, and \( c \) is the protein concentration in mg/ml. The ellipticities were then transformed by means of a G value elliptic function.

B. C1ase in Substrate/Reaction Mixture

Samples of purified C1ase (0.1 to 0.3 ml) were incubated with small aliquots of NaCl or NaOOCl stock solutions for 1-2 minutes and then mixed with volume-controlled aliquots of substrate assay mixture as described in the Main Text section above, and containing 10-20 (50%, v/v) in place of bile salt as substrate solubilizing agent. The CD spectrum of the reaction mixture, when titrated as above, was obtained as an average of 2 to 5 scans at 0.1 nm intervals. The bile salt concentrations were usually 15, 30, 50, 75, and 100 mM. The NaCl concentration in the reaction mixture was maintained at 10-20 mM, and the NaOOCl concentration was maintained at 10-20 mM. The conditions were 50, 60, 70, 80, and 90 minutes, respectively. The data was usual to 50, 100, 120, 140, and 160 minutes. The solution was then titrated as described above, and the final product was the CD spectra of the C1ase activity determination. The raw data ellipticity was processed according to the reference of C1ase using a microcomputer based data acquisition system RT/1L, Malvern developed at the Computer Systems Laboratory, National Institutes of Health, Bethesda, Maryland. Each CD spectra were then averaged and converted to MCD values at each wavelength according to Equation (6) below, after correction for appropriate buffer blanks, baseline shifts, and protein concentration changes.

ACTIVATION OF C1ase

A aliquots of purified C1ase were incubated with small amounts of NaCl stock solutions for 5 minutes and used with aliquots of substrate mixture as described in the Main Text section. The C1ase concentration in the reaction mixture was maintained at 10-20 mM. The CD spectrum of the reaction mixture, when titrated as above, was obtained as an average of 2 to 5 scans at 0.1 nm intervals. The bile salt concentrations were usually 15, 30, 50, 75, and 100 mM. The NaCl concentration in the reaction mixture was maintained at 10-20 mM. The conditions were 50, 60, 70, 80, and 90 minutes, respectively. The data was usual to 50, 100, 120, 140, and 160 minutes. The solution was then titrated as described above, and the final product was the CD spectra of C1ase activity determination. The raw data ellipticity was processed according to the reference of C1ase using a microcomputer based data acquisition system RT/1L, Malvern developed at the Computer Systems Laboratory, National Institutes of Health, Bethesda, Maryland. Each CD spectra were then averaged and converted to MCD values at each wavelength according to Equation (6) below, after correction for appropriate buffer blanks, baseline shifts, and protein concentration changes.

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