Optical Rotatory Dispersion of Catalase*

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It is well recognized that the measurements of optical rotatory dispersion of proteins with strong absorption bands are extremely difficult because of the diminishing intensity of the emergent light. Until very recently, these studies were made possible only by improving the light source and also frequently by a wide opening of the slit width on the monochromator at the expense of spectral purity. Unfortunately, many of the observations of Cotton effects as reported in the literature were found to be merely rotatory artifacts which can easily be produced in regions of high absorbance (1). Now that better instruments are available, more accurate data on optical rotatory dispersion of proteins such as myoglobin and hemoglobin (2-4) are beginning to accumulate. In this paper, we will present the optical rotatory dispersion of both native and denatured catalase. Our results seem to differ significantly from the recent findings of Osbahr and Eichhorn (5), whose measurements perhaps suffered the handicap of instrumental limitation. With the present data, we will also estimate the helical content of catalase by various optical rotation analyses.

EXPERIMENTAL PROCEDURE

Materials—The preparation of bovine liver catalase has been described in the preceding paper (6). The protein was dissolved in 0.1 M phosphate buffer (pH = 7), and its concentration was determined spectrophotometrically, with ε = 13.5 at 405 μm. Denaturation was performed either by adjusting the solution at pH 3.0 with dilute HCl (plus 0.1 M KH₂PO₄) or by adding 8 M urea to the buffered solution at neutral pH. The chemicals used were of reagent grade.

Optical Rotatory Dispersion—All ORD measurements were made at room temperature (22-26°) in a Rudolph model MSP 4 manual spectropolarimeter, over the wave length range of 190 to 650 μm. The light source was an Osram XBO-450-watt xenon lamp. The photomultiplier tube was an EMI/US type 7200, and its sensitivity could be bolstered by an applied voltage varying from 420 to about 900 volts; the upper limit could reach 2000 volts, but in practice it was limited by the noise level of the tube. The cells used were 10, 1, 0.1, and 0.01 cm long. The concentrations of the protein solutions were so adjusted that their absorbance was always below 2 (1). (To take into consideration the large absorption by solvent and various ions below 200 μm, the total absorbance of the solution below 210 μm was measured under nitrogen flush against a reference cell which contained no solvent.) Measurements were usually made at 10-μm intervals, except in the Cotton effect regions where the readings were taken every 2 to 3 μm. The exit slit width was kept at about 0.02 to 0.03 mm in the visible region and gradually raised to about 0.5 mm near 210 μm, thus maintaining the spectral purity to ±1 μm or better. Below 210 μm, however, the slit width had to be further opened, and at 200 μm or lower, it was 1 to 2 mm, which corresponded to about ±1.5 to 3 μm. Several readings were taken at each wave length and averaged; the experiments were at least duplicated for each concentration measured. The data were found to be very reproducible, and the specific rotation showed no significant concentration dependence beyond normal experimental errors.

The measurements in the Cotton effect regions were, however, found to be dependent on the slit width used (see “Discussion”). Accordingly, the reported specific rotations in Figs. 1 and 2 are the extrapolated values (to zero slit width) similar to those to be described in Fig. 4. This precaution, however, still does not eliminate some unavoidable stray light either within the instrument or from external sources, which could cause serious errors in the experimental data. As a matter of fact, the room light (a fluorescent lamp) was found to affect the magnitude and even sign of the readings in the far ultraviolet regions, presumably because the instrument was not airtight and stray light from the lamp was introduced into the cell compartment. This error was corrected simply by turning the room light off or covering the polarimeter with a black cloth. To test the stray light inside the instrument, we made two experiments. In one, the rotation of a sucrose solution (National Bureau of Standards grade) was measured with and without a cell containing a K₂Cr₂O₇ solution in series with the sample cell. The wave lengths used were 373 and 313 μm, which represent the absorption maximum and minimum of the K₂Cr₂O₇ solution. As shown in Table I, the results were unaffected by the presence of an absorbing material; furthermore, they were in very good agreement with those reported in the literature. In a second experiment, the rotation of the same sucrose solution was measured at 233 and 195 μm by placing the sample cell in series with another cell containing formamide-water. The concentration of formamide was so adjusted that it gave an absorbance of about 1.6 at the said wave lengths. All the measurements were extrapolated to zero slit width. The data at 233 μm was again in accord with the literature value. On the other hand, the rotation at 195 μm turned out to be about 20 to 30% higher than the calculated value. This discrepancy could be the result of instrumental limitation, but it is also not certain whether...
TABLE I
Calibration of the spectropolarimeter with 10% (w/v) sucrose solution in 1-cm cell

<table>
<thead>
<tr>
<th>λ (µm)</th>
<th>Absorbing cell in series with sample cell</th>
<th>A</th>
<th>[α]observed</th>
<th>[α]calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>373</td>
<td>(a) None</td>
<td>1.0</td>
<td>185.9</td>
<td>185.1</td>
</tr>
<tr>
<td>313</td>
<td>(b) K₃[CrO₄] solution</td>
<td>0.07</td>
<td>284.3</td>
<td>285.7</td>
</tr>
<tr>
<td>253</td>
<td>(a) None</td>
<td>0.07</td>
<td>606</td>
<td>676</td>
</tr>
<tr>
<td>198</td>
<td>(b) HCONH₃·H₂O</td>
<td>1.6</td>
<td>672</td>
<td>1280</td>
</tr>
</tbody>
</table>

* Except at 198 µm, c = 1%.
* Based on the data at 589 and 546 µm provided by the National Bureau of Standards, which in turn give [α] = 21.615/(λ² - 0.0223).
* Based on the data (between 260 and 670 µm) of Harris, Hirst, and Word (7): [α] = 21.676/(λ² - 0.0213).
* Concentration, 0.004% (w/v) in 0.05 M NaOH in 1-cm cell.
* Concentration, 0.075% (w/v) in 1-mm cell.

sucrose solution still obeys the one-term Drude equation at such a low wave length. The data in Table I seem, however, to rule out the possibility of significant stray light inside the particular instrument used. Indeed, stray light, if present in large amounts, would become most pronounced in the presence of an absorbing material due to the reduction of the light intensity at the chosen wave length and the transmission of unwanted wave lengths. This would be more serious with higher concentrations of the absorbing material. The fact that both K₃[CrO₄] and HCONH₃ solutions did not affect the rotation of sucrose plus the absence of concentration dependence of the rotation led us to believe that the observed Cotton effects at 233 and 198 µm (to be described) were genuine.

RESULTS

In Figs. 1 and 2 are shown the ORD curves of catalase in both native and acid- and urea-denatured states. Two features immediately emerge.

1. Native catalase displays a very strong double peak Cotton effect in the Soret band (near 400 µm), which disappears completely on denaturation. The shape of the curve for the native protein resembles that for ferrimyoglobin and ferrihemoglobin (2-4), but the specific rotations of catalase are negative at all wave lengths from 210 to 650 µm (the experimental points above 600 µm are not shown in Fig. 1). This is in contrast to the results of the other two heme proteins which are dextrorotatory above 410 µm.

The ORD of native catalase above 600 µm was found to be a featureless extension of that in Fig. 1. We are unable to detect any Cotton effect around 620 µm, as reported by Osbahr and Eichhorn (5), whose measurements were made in an old model of the Rudolph spectropolarimeter. Also, the absorption spectrum of our preparation has a millimolar extinction coefficient of 28.7 at 622 µm, which is much higher than that obtained by Osbahr and Eichhorn. This might be attributed to the different preparations used which can possess a wide range of activities and spectral properties (8).
Optical Rotatory Dispersion of Catalase

2. The Cotton effect in the ultraviolet region shows that the acid-denatured catalase at pH 3.0 still retains a large levorotation at the trough (233 mp), which according to the findings of Simmons et al. (9, 10) indicates the presence of α-helix. On the other hand, in 8 M urea, this minimum reduces to about −2000, which is very close to the value for random coils (9, 10). Thus, catalase at low pH still contains certain amount of helicity in spite of its gross conformational change (6). This is in agreement with the ORD analysis based on the Moffitt equation (11) to be further elaborated on in “Discussion.” Supporting evidence also comes from the difference in levorotation in the visible region, for example, [α] 226 was about −77 at pH 3.0 and −105 in 8 M urea, a fact indicative of more ordered structure in the acid medium than in urea.

In Figs. 1 and 2 are also included the experimental points (−−−X) of the reconstituted protein molecule by the rapid dilution method (6). The Cotton effects and the general shape of the dispersion curve are identical with those of the native protein, a fact indicative of the complete recovery of the original conformation of catalase on renaturation. This is in good agreement with other evidence obtained from the studies of enzymic activity, absorption spectrum, hydrodynamic properties, and the determination of sulfhydryl groups (6).

Due to the presence of strong absorption bands, the concentrations of the proteins used in this study were limited to 0.005 to 0.15% and the absolute degrees of rotations at many wave lengths were very small. Thus, the precision of the data becomes a serious problem. For example, the degree of rotation for the peak of the Cotton effect at 400 mp was of the order of −0.001°. Indeed, the splitting of the peak might easily be attributed to normal experimental errors. Likewise, a smooth monotonic curve could be drawn for the native protein between 430 and 470 mp, had one taken into consideration a possible variation of ±0.002° in the measurements. Nevertheless, we are led to believe that these observations might not just be experimental errors, since repeated experiments appeared to give the same shape for the dispersion curve in the said regions. There is still the possibility that the particular instrument used in this study might have some inherent defects in certain ranges of wave lengths. On the other hand, the dispersion curves for the denatured proteins were smooth and featureless in these same regions. Tentatively, these findings in Fig. 1 are therefore accepted as they are, unless future studies indicate otherwise.

DISCUSSIONS

The data in Figs. 1 and 2 (above 240 mp) can be analyzed by both the Drude equation

\[\alpha = k/\lambda^2 - \lambda^2\]  
(1)

and the Moffitt equation (11)

\[m'\lambda = [3M_n/100(n^2 + 2)] [\alpha],\]

\[= \alpha \lambda^2 / (\lambda^2 - \lambda^2) + b \lambda^4 / (\lambda^2 - \lambda^2)^2\]  
(2)

The constants \(k\) and \(\lambda\) in Equation 1 are determined from a plot of \([\alpha]_\lambda\) \(\lambda^2\) versus \([\alpha]_\lambda\). The parameters \(a_0\) and \(b_0\) in Equation 2 are obtained by plotting \([m']\lambda\) \((\lambda'/\lambda - 1)\), against \(1/(\lambda^2/\lambda_0^2 - 1)\), provided a correct \(\lambda_0\) can be chosen. In our calculations, the dispersion of the refractive indices, \(n\), of the solvent was assumed to be the same as that of pure water. The refractive indices of 8 M urea solution were based on the data of Foss et al. (2). The mean residue weight, \(M_n\), was taken as 129 according to the data of Schnebel (12). For native catalase, the graphic determinations were limited to the wave length range of 240 to 340 mp away from the strong Cotton effect in the visible region. In Table II are listed the various parameters of Equations 1 and 2, from which one can then proceed to calculate and compare the helical contents by six methods.

The \(b_0^H\) and \(a_0^H\) Methods

The so-called \(b_0^H\) method based on Equation 2 is by far most frequently used, mainly because a mean \(b_0^H\) value of −630 has been obtained experimentally for a right-handed perfect helix, and, further, it is solvent-independent as predicted by Moffitt (11). The results of catalase are shown in Fig. 3 (with \(\lambda_0\) preset at 212 mp); the dispersion plots are linear over the range of wave lengths between 240 and 600 mp, except in the case of the native protein where the upper limit is 340 mp away from the Cotton effect region. To make certain that the experimental \(b_0^H\) only represents the helical rotation, one can recast Equation 2 into a general expression

\[m'\lambda = a_0^H \lambda^2 / (\lambda^2 - \lambda^2) + f a_0^H \lambda^4 / (\lambda^2 - \lambda^2) + f b_0^H \lambda^4 / (\lambda^2 - \lambda^2)^2\]  
(3)

Here the first Drude term on the right side represents the configurational rotation due to amino acid residues. If \(\lambda_c = \lambda_0\), Equation 3 reduces to the form of the Moffitt equation and the fraction of helicity, \(f\), can be calculated simply by dividing the experimental \(b_0^H\) (=\(fb_0^H\)) by −630. If \(\lambda_c = \lambda_0\), the first Drude term in Equation 3 can be expanded into an inverse power series of \((\lambda^2 - \lambda^2)^2\), thus yielding a nonzero \(b_0^H\) which does not represent the helical rotation (neglecting the terms higher than the second

\[a_0^H = k/\lambda^2 - \lambda^2\]  
(1)

and the Moffitt equation (11)

\[m'\lambda = [3M_n/100(n^2 + 2)] [\alpha],\]

The constants \(k\) and \(\lambda\) in Equation 1 are determined from a plot of \([\alpha]_\lambda\) \(\lambda^2\) versus \([\alpha]_\lambda\). The parameters \(a_0\) and \(b_0\) in Equation 2 are obtained by plotting \([m']_\lambda\) \((\lambda'/\lambda - 1)\), against \(1/(\lambda^2/\lambda_0^2 - 1)\), provided a correct \(\lambda_0\) can be chosen. In our calculations, the dispersion of the refractive indices, \(n\), of the solvent was assumed to be the same as that of pure water. The refractive indices of 8 M urea solution were based on the data of Foss et al. (2). The mean residue weight, \(M_n\), was taken as 129 according to the data of Schnebel (12). For native catalase, the graphic determinations were limited to the wave length range of 240 to 340 mp away from the strong Cotton effect in the visible region. In Table II are listed the various parameters of Equations 1 and 2, from which one can then proceed to calculate and compare the helical contents by six methods.

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(3)

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order) and therefore should be subtracted from the experimental $b_0$. This correction, however, is usually small if $\lambda_0$ is not too much different from $\lambda_e$. In the present case catalase has a $\lambda_e$ of 208 m$\mu$ in 8 M urea solution in which the protein molecule is regarded to be completely denatured, and the nonhelical $b_0$ correction amounted to about +40 (Table II) (assuming a constant $\alpha_h$ before and after denaturation).

The estimated helicity of the native and acid-denatured catalase based on Equation 3 and the data in Table II were found to be about 37 and 25%, respectively (Table III). More recently, Urnes, Imahori, and Doty (2) found it necessary to use $\lambda_0 = 216 m\mu$ instead of 212 m$\mu$ over a wide range of wave length, which in turn gave a new reference $b_0^H$ of about $-540$. We have therefore also replotted our data using $\lambda_0 = 216 m\mu$ (not shown here), which turned out linear, as did those in Fig. 3. The corresponding helicity of the native and acid-denatured catalase became about 40 and 25%, which were not too different from those mentioned above. Nevertheless, it should be pointed out that the original $\lambda_0$ of 212 m$\mu$ was based on the rotation measurements above 350 m$\mu$. It is not certain whether the same value is equally well applicable at lower wave lengths, especially since our calculations were confined only to the region of 240 and 340 m$\mu$ as in the case of myoglobin (2-4). Suffice it to say that the choice of a correct $\lambda_0$ is still not entirely settled, although from all the available evidence in the literature, it does not seem to differ too much from 212 m$\mu$.

The use of Equation 3 becomes uncertain if structural elements other than the $\alpha$-helix are present in the protein molecule. This is why an independent check is highly desirable (13). Again by assuming that the data in 8 M urea represent the configurational rotations in both the native and denatured proteins and by subtracting them from Equation 3, one can then calculate $f$ from the experimental $f_{\alpha H}$, $\alpha_h^H$ being taken as $+650$. The estimated helicity from this method turned out to be 80 and 35% for the native and acid-denatured catalase, which were much higher than those from other analyses (Table III). It is, however, not known whether the catalase molecule has other nonhelical, but periodic structural units such as the $\beta$ aggregates. Alternately, the numerical value of $\alpha_h^H$ might be in error; indeed, the too high estimates could be accounted for if the reference $\alpha_h^H$ were adjusted to greater than $+650$. On the other hand, the fact that the $\alpha_h^H$ method has been fairly successful for many proteins (1) but fails in other cases seems to argue against this possibility. A third explanation may be found in the assumption of a constant mean $\alpha_h$ before and after denaturation. In a compact protein molecule, many side groups are buried inside an environment quite different from the solvent medium, but upon denaturation these same groups will be exposed and in direct contact with the surrounding solvent (13, 14). This solvent effect on the residue rotations has already been observed for polypeptides and proteins (11, 15) and, more recently, for simple amino acid derivatives (14), although it is still not known to what extent it will affect the $\alpha_h^H$ in proteins upon denaturation. The estimates based on the $\alpha_h^H$ method appear to most cases to be somewhat higher than those from the $b_0^H$ method (see Table XII of Urnes and Doty (1)), which could be accounted for if $\alpha_h$ were less negative in the native proteins than in the denatured ones. All these problems are still not solved at present. In the meantime, the $\alpha_h^H$ method serves a useful purpose not so much as the precise determination of helical contents as in providing information concerning the conformational changes of proteins beyond the breaking of $\alpha$-helices, especially when there are enormous discrepancies between the helicities calculated by this method and by the $b_0^H$ method.

The Trough and Peak Methods; Effect of Spectral Purity

Two new methods recently proposed (9, 10) are based on the intensity of the trough and peak of the Cotton effect in the ultraviolet region. Empirically, Simmons et al. (9) found that $[m']_{233}$ at the trough for poly-L-glutamic acid was approximately $-13,000$ for a perfect helix and $-2,000$ for the random coil. In a recent communication (10), using a better instrument, Blout, Schmier, and Simmons raised the magnitude to about $-15,000$ for the helix (as read from their Fig. 1 (10)). Jirgensons (15) further found that the specific rotations at the trough was concentration dependent. In order to have precise values for these reference states, we have therefore repeated the rotation measurement of PGA at both pH 4.3 (helical) and 7.0 (coiled). Furthermore, we have intentionally varied the slit width at 233 m$\mu$ to determine the effect of spectral purity on the rotations. (The concentrations of the polypeptide were determined on the basis of nitrogen contents by the micro-Kjeldahl methods. By checking the dry weight of the sample (after overnight heating at 105°), the sodium salt of polyglutamic acid was found to retain one molecule of water per amino acid residue.)

The results of the helical poly-L-glutamic acid are shown in the Figure 3. The dispersion plot of catalase. $\lambda_0 = 212 m\mu$

<table>
<thead>
<tr>
<th>Type of catalase</th>
<th>pH</th>
<th>Method</th>
<th>$J_0$</th>
<th>$[m']_{233}$</th>
<th>$[m']_{233}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>7.0</td>
<td></td>
<td>57</td>
<td>44</td>
<td>52</td>
</tr>
<tr>
<td>Acid-denatured</td>
<td>3.0</td>
<td></td>
<td>25</td>
<td>18</td>
<td>34</td>
</tr>
</tbody>
</table>

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Fig. 4, where the half-intensity band width was calculated according to the data supplied by the manufacturer, i.e. the half-intensity band width is 5.2 mμ per mm of slit width at 233 mμ (the entrance, intermediate, and exit slit widths being kept identical). The magnitude of rotation decreases with increasing slit width; the extrapolated value for zero slit width and that with wide open slit could differ by as much as 20% or more. This finding is not unexpected when one considers the strong Cotton effect at 233 mμ similar to that shown in Fig. 2. On both sides of the trough, the levorotation drops sharply. Since any widening of the slit increases the unwanted light at wave lengths both higher and lower than 233 mμ, the measured mean rotation would accordingly diminish with spectral purity.

At fixed slit widths, the levorotation was found to decrease with increasing concentration when half-intensity band width was greater than 5 mμ and, further, the concentrations used were fairly high, but the data in Fig. 4 show a reverse trend at low half-intensity band width for reasons still not clear to us. The extrapolated value (to zero slit width) converges toward a common intercept, irrespective of the concentrations used. In this study, [m']233 (helical) = -18,000; its magnitude is about 20% higher than that reported by Blout et al. (10). Actually, this difference is not as great as it appears when one takes our extrapolation procedure into consideration (see Fig. 4).

f = (1/2,000) [m']233

Here, [m']233 refers to the extrapolated value at zero slit width. For native and acid-denatured catalase, the estimates from Equation 4 indeed agree fairly well with those obtained from the hλ method (Table III).

Blout et al. (10) also reported that [m']233 at the peak for PGA was about +80,000 for the helix and -5,000 for the coiled form. Due to instrumental limitation the measurements at 198 mμ were made possible only when the slit width was wide open, and with our instrument the readings were taken for half-intensity band width at 4.8 and 6 mμ. The estimated [m']233 at zero slit width for helical PGA was found to be about +115,000 for a 0.015% solution and +107,000 for a 0.007% solution. On the basis of the findings in Fig. 4, where the dependence of optical rotation on spectral purity is less drastic with higher concentrations, we tentatively accept the higher value as a more reliable one. (For randomly coiled PGA our value of [m']233 was found to be about -4,500.) Obviously, the discrepancy between our results and that reported by Blout et al. (10) points up the great uncertainty in such determinations, and the true peak rotation is still a subject for future refinement. This disagreement, however, cannot be explained by the presence of stray light in our instrument, since like the widening of slit width significant stray light would actually lower the magnitude of rotation at the peak or trough of a strong Cotton effect. In addition, stronger absorption of PGA at wave lengths lower than 198 mμ plus the cut-off of the far ultraviolet light by the quartz prisms and windows would only permit light of higher wave lengths to reach the sample cell, which again would give smaller magnitude of rotation. On the other hand, the fact that the rotation of coiled PGA at 198 mμ was about 20% or so higher than the calculated value (assuming that the one-term Drude equation is still applicable at this wave length) seems to suggest a lower [m']233 value than what we have reported here. This corrected value could be around +100,000 or even lower, but it is still higher than the value obtained by Blout et al. (10). In their brief communication, these authors did not elaborate on the instrument calibration; thus, we are unable to make any quantitative comparison.

Very recently, Holzwarth, Gratzer, and Doty (17) have calculated the rotatory dispersion of PGA from circular dichroism measurements. From their Fig. 2 (17), the mean residue rotations, [m]α, at 233 and 198 mμ were estimated to be about -20,000 and +80,000, which on refractive index correction (by a factor of 3/(n2 + 2)) give [m']233 = -15,000 and [m']198 = +60,000. The former value seems to agree well with the more recent value reported by Blout et al. (10), whereas the latter is much lower than either +80,000 or +115,000. It is noted that circular dichroism which measures the difference in absorbance, usually a small number, of the left- and right-handed circularly polarized light could possibly involve rather large errors just as the direct measurements of rotatory dispersion at wave length below 200 mμ are. Therefore, any comparison between various reported values in quantitative terms seems to be premature at this stage of development.

In the meantime we may write

f = (1/120,000) [m']233 + 5,000

(5)
Surprisingly, the estimated helicity for catalase based on Equation 5 is in fair agreement with that obtained by the $b$ method and trough methods despite the uncertainty in the magnitude of $[m']_{233}$ for a perfect helix. A correction of, say, 20% in all the measurements at 198 mp would of course give the same $f$ as that in Equation 5. It should also be pointed out that the Equations 4 and 5 are based on the measurements of PGA only, and therefore the numerical values might still be subjected to further tests and refinement when more polypeptides are studied. Neither are we sure that these values will be solvent independent just as $b$ is. It is noted, however, that Simmons et al. (9) have found essentially the same $[m']_{233}$ for poly-$\gamma$-benzyl-L-glutamate in dioxane as for the helical PGA, but the magnitude of $[m']_{233}$ for poly-$\gamma$-methionine in methylene dichloride was lower by about 20%.

The $\lambda_c$ and $[\alpha)_c$ Methods

In passing, we may mention the estimated helicities from these two methods, details of which will not be elaborated here (15, 18). For native catalase, the presence of the Soret Cotton effect prevents the use of either method. For acid-denatured catalase, the helicity based on the $\lambda_c$ (Table II) turned out to be only 10% which was much lower than that from other methods, but the method based on the change of $[\alpha]_c$ gave 25% helical content which was in good accord with that listed in Table III. Suffice it to say, both methods have serious limitations, but, cautiously applied, they could provide a quick, rough estimate of the helicity in proteins.

In summary, the quantitative aspects of the ORD treatment have not yet reached a stage where one can estimate the helicity of proteins without ambiguity. It is still too early to conclude that one method is vastly superior to the others; thus, we have here mentioned all six analyses for comparison, even though the $b$ method has been used almost exclusively in this field. In this paper, we have shown that the trough method at 233 mp could provide an independent check, but for the present instrumental limitation, the same might be true for the peak method at 198 mp. However, the interpretation of these three methods could be complicated by the presence of structural elements other than the $\alpha$-helices in the protein molecules. The $\lambda_c$ method has been found successful in certain cases (1), but it fails miserably in others. Likewise, the $[\alpha]_c$ and $\lambda_c$ methods could only provide a rough estimate and their reliability has frequently been questioned. Actually, any disagreement between the latter three methods and, say, the $b$ method does challenge us to look for the reason for the disparity so that eventually we will have a better understanding of the ORD of proteins.

SUMMARY

1. The optical rotatory dispersions of both native and denatured bovine liver catalase were measured between 190 and 650 mp. The native protein displays a strong Cotton effect in the Soret band, which disappears on acid or urea denaturation.

2. Native catalase also shows strong Cotton effects in the ultraviolet region with a trough at 233 mp, a peak at 198 mp, and an inflection point near 225 mp. The intensities of these effects are reduced to about one-half when the pH of the protein solution is adjusted from neutral to 3.0. The rotatory properties of catalase in 8 M urea resemble those characteristic of random coils and the Cotton effects at 225 mp virtually disappear in this completely denatured state.

3. The spectral purity affects the intensity of the experimental Cotton effect; the levorotation of the helical form at the trough (233 mp) decreases with increasing half-intensity band width. For precise work, the data should be extrapolated to zero slit width (on the monochromator). The specific rotations at several concentrations seem to converge toward a common intercept at zero slit width.

4. The helical contents of both native and acid-denatured catalase were estimated by six methods all based on optical rotations. The native protein has about 50% helix and the acid-denatured one 20 to 25%.

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Jen Tsi Yang and Tatsuya Samejima


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