Evaluation of Secondary Structure of OxlT, the Oxalate Transporter of Oxalobacter formigenes, by Circular Dichroism Spectroscopy

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OxlT, the oxalate/formate exchange transporter of Oxalobacter formigenes, was purified as a histidine-tagged variant, OxlTHis, using Ni$^{2+}$-linked affinity chromatography. OxlTHis was readily obtained in high purity (>95%) and reasonable yield (≥60%), and showed kinetic and biochemical features characteristic of its parent, OxlT, including an unusually high maximal velocity (60 μmol/min per mg of protein at 4 °C). Circular dichroism spectroscopy of purified OxlTHis identified the α-helix as its dominant secondary structural unit, encompassing 60–70% of OxlTHis residues and consistent with a model suggesting 60% of OxlT (OxlTHis) residues are involved in the construction of 12 transmembrane α-helices (Abe, K., Ruan, Z.-S., and Maloney, P. C. (1996) J. Biol. Chem. 271, 6789–6793). In either octyl glucoside/lipid or dodecylmaltoside/lipid micelles, solubilized OxlTHis showed a striking substrate-induced stabilization of function, and at saturating levels of substrate (1000 × K_D) activity recoverable by reconstitution disappeared with a half-life of 7 days at 23 °C. Measurement of changes of ellipticity at 222 nm as a function of time and substrate concentration showed that maintenance of function was attributable to a substrate-induced stabilization of the α-helical ensemble with a K_D of 10 μM for the 1:1 binding of oxalate to OxlTHis.

The anion transporter, OxlT, carries out the exchange of divergent oxalate with monovalent formate at the plasma membrane of the anaerobe, Oxalobacter formigenes (1, 2), and in doing so catalyzes the vectorial portion of a proton-motive metabolic cycle (3) that drives ATP synthesis in this organism (1, 3). While OxlT is of interest for this novel position in cell biology, it also attracts attention for certain of its biochemical features. For example, studies of OxlT purified from O. formigenes suggest this carrier operates with the highest velocity known among transporters of organic molecules (4). Moreover, examination of crude extracts of the solubilized protein suggests that substrate binding energy contributes substantially (≥3.5 kcal/mol) to stabilization of the OxlT-substrate complex (5). As well as showing the soluble protein retains information relevant to substrate binding, this last observation suggests that structural transformation(s) accompanying substrate binding and transport might be investigated by suitable in vitro experiments with solubilized protein.

From analysis of the OxlT amino acid sequence (6) one infers a broad architectural similarity between this protein and other transporters, in both prokaryotes and eukaryotes, that display the reactions of antiport, uniport, or symport (3, 7, 8). This large collection of membrane proteins, now numbering in the several hundreds (3, 4), is presently exemplified by a few well studied cases, such as the erythrocyte Cl/HCO₃⁻ exchange carrier, Band 3 (9), the red cell glucose facilitator, GLUT1 (10), and the Escherichia coli H⁺/lactose symporter, LacY (11, 12). In these three examples, biophysical tests indicate the α-helix as the main secondary structural element (9, 13, 14), and this direct experimental result supports inferences made from more widely available (but indirect) biochemical and genetic studies that point to the presence of 10–12 transmembrane segments in most examples in this group (3, 7, 8, 13, 15, 29).

Indeed, the transmembrane α-helix is often assumed as the dominant structural element in transporters of this type (16, 17), but except for the cases noted this supposition has not been tested directly. For this reason, and because of the unusual kinetic features of OxlT, the initial objective of work reported here was to assess secondary structure in this transporter. To do this, we installed a histidine tag at the OxlT C-terminus and purified the tagged protein, OxlTHis, by affinity chromatography using a Ni$^{2+}$-linked adsorbent. The CD spectrum of solubilized, purified OxlTHis shows that the majority of its residues are organized in an α-helical configuration, supporting a generic model in which this and similar transporters contain 10–12 transmembrane α-helices. Our second objective was to use the purified protein to correlate structure and function. Such studies show that the loss of activity observed in the absence of substrate reflects a spontaneous breakdown of the α-helical ensemble.

EXPERIMENTAL PROCEDURES

Expression Plasmids and Bacterial Strains—pBKoxiT was constructed by insertion into pBluescript II SK⁺ (Amp') of a 1.4-kilobase XbaI-HindIII fragment encoding OxlT (6). In this construct, the first of two in-frame OxlT UAA stop codons are flanked by BamHI and NheI sites. To make an expression vector encoding histidine-tagged OxlT, we digested pBKoxiT with both BamHI and NheI and then ligated the products with a synthetic BamHI-NheI bridging oligonucleotide specifying nine consecutive histidine that would extend from the OxlT C-terminal histidine. The sequence of the resulting vector, pBDoxiT, was confirmed by sequencing of double stranded DNA using the dyeoxy chain termination method of Sanger (18). pBKoxiT and pBDoxIThis were expressed in XL3, a strain that also carries pMS421 (spec⁺, Lac') to ensure repression of OxlT and OxlTHis in the absence of induction (6).

Expression of OxlT and OxlTHis—A single colony of XL3 carrying either pBKoxiT or pBDoxITHis was dispersed in 5 ml of LB broth with antibiotics (100 μg/ml ampicillin, 50 μg/ml spectinomycin); after overnight growth with vigorous shaking, cells were added to 0.5 liter of fresh media and grown until A₆₀₀ reached 0.2–0.3, at which point 1 mM isopropyl-1-thio-β-D-galactopyranoside was added to induce expression of OxlT or OxlTHis. Four hours later, cells were harvested by a 10-min centrifugation at 4,000 × g and lysed by incubation at 37 °C for 15 min in 50 ml of lysis solution (300 μg/ml lysozyme, 40 μg/ml DNase, 0.5 mM freshly dissolved phenylmethylsulfonyl fluoride) (19). The resulting
ghosts were washed twice with iced distilled water, and membrane proteins were extracted at pH 7 by incubation for 60 min on ice with 25 ml of solubilization buffer (20 mM potassium phosphate, 6 mM β-mercaptoethanol, 20% glycerol, 0.42% acetonitrile/ether purified E. coli phospholipid, 1.5% oxalate) containing 10 mM potassium oxalate. The membrane pellets were washed with a detergent solution by centrifugation at 145,000 g for 30 min. The column outlet was sealed with Parafilm, 3 ml of crude detergent mixture was cleared of cell debris and unextractable material by a 2-min centrifugation at 750 g. After centrifugation, 0.5 ml of solubilization buffer (20 mM potassium phosphate, 6 mM β-mercaptoethanol, 20% glycerol, 0.42% acetonitrile/ether purified E. coli phospholipid, 1.5% oxalate) containing 10 mM potassium oxalate, 50 mM potassium phosphate, pH 7), the vacuum was reapplied to the column, and non-specifically bound residual material was removed by a 20-ml wash at 1 ml/min, using solubilization buffer supplemented with 200 mM sodium chloride and 50 mM imidazole, along with OxlTHis substrates (10 mM potassium oxalate or 100 mM potassium formate) as necessary. OxlTHis was eluted by centrifugation after a 20–40 min incubation with 0.2 ml of solubilization solution containing either 500 mM imidazole or 200 mM EDTA, each neutralized by potassium hydroxide, with or without OxlT substrates.

Purification of Histidine-tagged OxlT—To purify OxlTHis, a 5-ml Quik-Sep column (Isobed) was packed with 0.2 ml of Ni²⁺-NTA resin (Qiagen), and the resin was washed with 4°C with 20 ml of distilled water. The column outlet was sealed with Parafilm, 3 ml of crude detergent extract was added, and after sealing the column inlet, the resin and extract were mixed by gentle rotation for 4 h at 4°C. The extract, depleted of OxlTHis, was allowed to drain from the column, and non-specifically bound residual material was removed by a 20-ml wash at 1 ml/min, using solubilization buffer supplemented with 200 mM sodium chloride and 50 mM imidazole, along with OxlTHis substrates (10 mM potassium oxalate or 100 mM potassium formate) as necessary. OxlTHis was eluted by centrifugation after a 20–40 min incubation with 0.2 ml of solubilization solution containing either 500 mM imidazole or 200 mM EDTA, each neutralized by potassium hydroxide, with or without OxlT substrates.

Reconstitution of OxlTHis and OxlT—To compare the transport activity of OxlTHis and OxlT, we first set the two proteins at approximately equal concentrations by mixing 1 volume of purified OxlTHis or a crude cell extract containing OxlT with 149 or 9 volumes of solubilization buffer, respectively. Reconstitution from these mixtures then followed earlier procedures (1, 4, 20). Briefly, the proteins were dispersed in lipid/detergent micelles by a sequential mixing of the following components: 14.6 µl of 100 mM potassium phosphate (pH 7.0); 4.5 µl of 15% octyl glucoside; 35 µl of 45 mg/ml bath-sonicated E. coli phospholipid; and 200 µl of the OxlTHis or OxlT mixtures. The suspensions were kept on ice for 20 min, after which oxalate-loaded proteoliposomes were formed by adding 5 ml of 23°C loading buffer (100 mM potassium oxalate, 50 mM potassium phosphate, pH 7) to bring the detergent level below its critical micellar concentration. After an additional 20 min, proteoliposomes were used to determine OxlTHis or OxlT transport activity in either of the two assays outlined below.

Assays of Oxalate Transport—In routine work, oxalate transport was determined at 23°C by a simplified assay (4) in which replicate 0.1-ml portions of the proteoliposome suspension were applied to the center of a pre-soaked Millipore GS filter (0.22 µm pore size). After removing external loading buffer by a wash with 5 ml of assay buffer (100 mM potassium phosphate, 50 mM potassium phosphate, pH 7), the vacuum was interrupted, and the filter with entrapped proteoliposomes was overlaid with 0.3 ml of assay buffer containing 0.1 ml of [14C]oxalate. The oxalate exchange reaction was complete by 4 min, at which time the assay was terminated by washing the filter twice with 5 ml of assay buffer. For this fixed-time assay, activity is reported as micromoles of [14C]oxalate incorporated per mg of protein during the 4-min incubation.

To measure the kinetic parameters of oxalate transport, proteoliposomes were isolated by centrifugation (20), washed once with assay buffer, and after resuspension in assay buffer, the time course of oxalate transport was measured at 4°C by incubation with [14C]oxalate of the indicated concentrations. Reactions were terminated by filtration and washing (1, 18).

Electrophoresis and Western Blotting—Samples of fractions obtained during purification of OxlTHis were subjected to SDS-PAGE1 using 12% acrylamide, as described (21), and protein content was evaluated by a modification of the method of Schaffner and Weissman (27).

Results

Evaluation of Secondary Structure of OxlT

CD Spectroscopy—CD spectra were collected for OxlTHis which had been passed over a Centri-Spin 20 desalting column (Princeton Separation) to remove the EDTA used for elution. Unless noted otherwise, the column was prehydrated with 0.5 ml of solubilization buffer (with or without added substrate, according to the experimental design), and resin was removed by a 5-ml wash fractionation. After 24 h, EDTA-eluted OxlTHis (80 µl) was placed on the column, and the desalted product was collected by a second centrifugation; as a blank, a solution containing all components except OxlTHis was prepared in parallel.

CD spectra were collected using an AVIV 60DS circular dichroism spectropolarimeter; calibration with (+)-10-camphorsulfonic acid gave a ratio of −2.25 for ellipticities at 192.5 and 290.5 nm. The CD spectrum of OxlTHis (0.2–0.5 mg/ml in protein) was measured in a 0.01-cm path length quartz cell, with temperature set to 25°C unless otherwise noted. Data were obtained at constant slit width (1.5 nm), and each spectrum was acquired as the result of five repeated scans from 260 to 190 nm, using scan intervals of 0.5 nm and an integration time of 1 s at each wavelength. The time for data collection was about 20 min for each set of five scans. Blank and experimental samples showed identical responses to a current of 15% octyl glucoside, suggesting the absence of significant differential scattering of left or right circularly polarized light by detergent/lipid micelles (23); blank values remained at +1 millidegree as the wavelength was reduced to about 215 nm and then rose to +3 millidegrees from 190–195 nm. Experimental samples showed peak negative and positive deflections from the blank at 220–223 nm and 190–195 nm, respectively; depending on protein concentration, at these wavelengths experimental samples showed uncorrected signals of between −4.5 to −12 millidegrees (220–222 nm) and +20 to +50 millidegrees (190–195 nm), indicating adequate signal/noise ratios within the range investigated. Raw spectra were corrected by subtraction of baseline spectra obtained using the blank solution, and after smoothing, the net spectra were used for evaluation of OxlTHis.

CD spectral measurements were expressed as mean residue ellipticity, in units of degree cm² dmol⁻¹, according to: mean residue ellipticity = (100·θ·M)/(c·d·n), where θ is measured ellipticity (deg), M is molecular weight, c is protein concentration (mg/ml), d is path length (cm), and n is the number of residues in OxlTHis (n = 427). Spectral decomposition was done by least-squares fit (Sigma plot) of mean residue ellipticity values at 200–260 nm to the contributions of four components (α-helix, β-strand, β-turn, and unordered) taken from the basis set tabulated by Yang et al. (24) or Park et al. (25), constraining the sum of contributions to equal 1. Because measurements at wavelengths below 200 nm were not considered, only estimates of α-helix content are reported (26). For the basis set of Yang et al. (24), an average helix length of 24 residues was used to account for the dependence of ellipticity on helix length (24).

Protein Estimation—Protein was estimated by a modification of the method of Schaffner and Weissman (27).

Conclusions

Purification and Characterization of OxlTHis—To enable convenient purification of OxlT, we exploited Ni²⁺-linked affinity chromatography. The principle underlying this approach is that a sequence of six or more tandem histidine residues can form a relatively tight binding pocket for the divalent nickel cation, allowing a suitably tagged protein to be retained by a Ni²⁺-containing resin (28). Accordingly, we prepared OxlT variants having 10 consecutive histidine residues at either the N terminus (HisOxlT, not described) or the C terminus (OxlTHis, see “Experimental Procedures”). Because preliminary trials (not given) indicated that the latter showed somewhat tighter binding to the Ni²⁺-linked resin, we focused on purification and characterization of OxlT having the C-terminal histidine tag.

To purify OxlTHis, we first prepared membrane ghosts by lysis of cells overexpressing this protein and then solubilized membranes using octyl glucoside in the presence of excess E. coli phospholipid, with glycerol added as the osmoret stabilant (20). As anticipated (4, 6), the SDS-PAGE profile of the detergent extract showed OxlTHis as a protein with an apparent mass near 35-kDa (Fig. 1, left). OxlTHis was depleted from the extract by nickel ion incubation with the Ni²⁺-linked adsorbent, and reappeared only when wash conditions were altered to release Ni²⁺-linked materials (Fig. 1, left). Western blot analysis verified these findings. Thus, the crude extract and EDTA-eluate contained material reactive with an antibody directed to the OxlT N terminus (Fig. 1, right). In each case, the major element moved as a 35-kDa protein, corresponding to monomeric OxlTHis; dimeric and trimeric OxlTHis (4, 6) were evident in the Western blot (about 75 and 125 kDa, respective-

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1 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; CD, circular dichroism.
The two proteins also had similar Michaelis constants for the time-course of oxalate exchange was the same for OxITHis and OxIT (half-times of about 5 min) and that, as expected for simple antiport, the reaction could be described as an exponential approach to equilibrium (Fig. 2A).

The analysis was done with both octyl glucoside and dodecyl-maltoside as solubilizing agents, since these are the detergents most commonly used for solubilization of bacterial membrane proteins. For each of these preparations, 50–60% of the initial activity was recovered after 7 days incubation (Fig. 3), indicating that purified OxITHis was sufficiently stable for spectral analysis.

CD Spectroscopy of OxITHis—Early work showed that solubilized OxIT retains a capacity to bind its substrates (5), implying that the solubilized material has structural information relevant to the overall process of membrane transport. Consequently, it seemed reasonable to use CD spectroscopy to probe secondary structure of the solubilized protein.

The CD spectra recorded for solubilized OxITHis expected, since OxITHis function had been assessed with purified material, whereas OxIT activity was measured in a crude extract (e.g. Table I). These observations, together with the information noted above, lead us to conclude that the histidine-tagged OxITHis retains the essential aspects of catalytic activity of its parent, OxIT.

Stability of OxITHis—OxITHis had been purified to evaluate its secondary structure by CD spectroscopy, and because this would require extended incubation of solubilized protein at 25 °C (or higher), it was essential to document the stability of OxITHis for these general conditions. For this reason, solubilized (and desalted) OxITHis was dispersed in mixed lipid-detergent micelles in the presence of 10 mM oxalate (to take advantage of substrate-stabilization (5)), and at intervals during incubation at room temperature samples were withdrawn to measure residual activity by reconstitution.

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(Fig. 4, panel 2) showed features expected for a protein with a predominantly helical structure, that is, negative deflections in mean residue ellipticity above about 200 nm, with a minimum near 222 nm, and stronger positive deflections below 200 nm, with a maximum near 195 nm. Accordingly, one may conclude that OxlTHis (and OxlT) contains the α-helix as its main structural element. This overall structure was not influenced by the presence of bulk lipid, since an equivalent spectrum was recorded for protein eluted from Ni\(^{2+}\)-agarose and further processed with lipid-free buffer (Fig. 4, panel 3). It was also evident that the spectra of OxlTHis bound to oxalate or formate were nearly superimposable (Fig. 4, panel 2), while the spectrum taken in the absence of substrate (Fig. 4, panel 4) had both its minimum (near 222 nm) and maximum (near 195 nm) reduced in absolute value. Since there had been gradual decline of these peak values during the repeated scans required for signal averaging (not shown), it appeared that the helical nature of OxlTHis became progressively less prominent with continued incubation in the absence of substrate. This behavior was not studied further in these experiments (but see below), except to note that it correlated with loss of function, as shown by a separate experiment in which activity was monitored subsequent to the recording of CD spectra. In those cases, there was essentially complete recovery of \(^{14}\)Coxalate transport activity for samples incubated with oxalate or formate (95 or 80% recovery, respectively), but only a 30% recovery for the sample processed without substrate.

In the second part of the experiment shown by Fig. 4, each sample was exposed to elevated temperature (55°C) before recording its CD spectrum in a single 3-min scan. Elevated temperature had little apparent effect on samples in which substrate was present, even after a 50–55 min incubation (Fig. 4, panels 6 and 7), but in the absence of substrate, the signal became so severely degraded that heating was discontinued after only 15 min (Fig. 4, panel 8). This maintenance of an organized helical structure at high temperature reinforces the idea that the OxlTHis-substrate complex has an unusual stability (Ref. 5, and see below).

CD spectra collected in this (Fig. 4) and two other experiments (not shown) were used for estimation of secondary structure using basis spectra describing the responses of the α-helix, β-sheet, β-turn, and random coil configurations, constraining the sum of the fractional contributions to equal 1. To reconstruct experimental spectra, a least-squares analysis was performed using the basis set of Yang et al. (24) (Fig. 4, panel 1) or Park et al. (25) (panel 2).
TABLE II

Evaluation of Secondary Structure of OxlT

| Method | Helix content (%) | + Oxalate | + Formate | No addition |
|--------|-------------------|-----------|-----------|-------------|
| I      | 67 ± 6            | 62 ± 7    | 52 ± 11   |             |
| II     | 74 ± 6            | 72 ± 7    | 65 ± 11   |             |

**DISCUSSION**

**Purification and Characterization of OxlT**—The OxlT amino acid sequence (6) indicates it has a global structure resembling any one of a large number of transport systems that mediate the reactions of symport, uniport, or antiport (3). The transmembrane α-helix is usually taken as the main structural element for these and many other transporters, a presumption that is rarely supported by experiment. For this reason, and because early work with crude extracts had shown OxlT to have unusual stability as a solubilized protein (5), we reasoned that direct and useful information could be gained by CD spectroscopy of OxlT if a suitably purified preparation were available.

OxlT had been purified earlier using traditional techniques (4), but as is often the case with membrane proteins, the tendency of OxlT to associate in non-stoichiometric fashion with detergents and phospholipids leads to a heterogeneity in both amino acid sequence (6) and structure. Analysis of OxlT by means of spectroscopy and affinity methods (30–32), and we elected to use Ni2+-linked chromatography of a histidine-tagged variant (OxlTHis), a tactic that enabled the convenient production of highly purified material on a routine basis. Purified OxlTHis has the specific activity expected of the full functional protein (Table I and text), as one might expect from the finding that (with substrate present) OxlTHis lifetime is long compared to the time required for its purification. OxlTHis also retains the kinetic features of its parent, including its unusually high maximal velocity (Fig. 2); this corresponds to a turnover number of about 100 s at 4 °C, since half the protein used for reconstitution is incorporated by proteoliposomes (4). Clearly, analysis of OxlTHis is relevant to understanding its parent, OxlT.

**CD Spectroscopy of OxlTHis**—In extracting structural infor-
mation from CD spectroscopy, one makes four general assumptions (24–26, 33): (i) that the CD spectrum arises as the linear combination of its component spectra (i.e., those of the α-helix, β-sheet, β-turn, unordered regions, etc.); (ii) that these component, or basis, spectra may be deduced from the behavior of reference proteins whose structures are understood; (iii) that an appropriate set of reference proteins is available; and (iv) that the experimental spectrum extends over a sufficiently wide range to provide statistically significant information about the component spectra. Of these assumptions, the last two are at issue in the work described here. It is acknowledged, for example, that basis spectra deduced for the β-pleated sheet, β-turn, and random coil configurations are sensitive to the choice of the reference set (33) (e.g., Fig. 4, panels 1 and 5), and an inability to obtain accurate spectral information below 195–200 nm (as in these experiments) further limits one’s confidence in attempts to evaluate such components (26). These are the likely explanations for failure to derive consistent estimates for these structures in OxlTHis, as well as for the relatively poor fit by theoretical reconstructions when helix content was significantly diminished (e.g., Fig. 4, panel 8). By contrast, it is generally held that the α-helix yields a signal of relative uniformity in different environments (but see below), and since the helix chiral signal dominates in the more readily detected wavelengths, estimates of this parameter are the more likely to be accurate in the range considered here (>200 nm), especially if the target has a substantial helical content (26, 33). In fact, the CD spectrum of solubilized OxlTHis has the profile expected of a largely helical protein (Fig. 4), and calculations using two different basis sets gave similar estimates of OxlTHis helical content (Table II). One might also note that as structural information on membrane proteins is limited, analysis of the OxlTHis CD spectrum relies on component spectra derived from globular proteins, and this may carry potential risk. As an example, it has been proposed that the transmembrane α-helix differs from the α-helix exposed to water in having a somewhat stronger signal than expected (33), and this idea, still under discussion, raises the possibility that standard calculations (e.g., Table II) may overestimate the helix content of membrane proteins. Nevertheless, for the photosynthetic reaction centers of Rhodobacter viridis and Rhodopsseudomonas sphaeroides, whose structures are known from crystallography, such error appeared to be small, and Method I (Table II) overestimated helix content by only about 10% (39% observed versus 42.5% calculated and 51% observed versus 56% calculated, respectively) (33). Similarly, Method I provided a helical content for the nicotinic acetylcholine receptor (23%) (34) consistent with electron diffraction data (35).

It appears probable that the structure of solubilized OxlTHis resembles that adopted by this protein in the lipid bilayer. Although this assumption was not tested here in any specific way, it has been verified for the acetylcholine receptor (34) and the water channel, CHIP28 (36). And for the LacY symporter, presumed to resemble OxlT in general structure, overall helix content was not different for the solubilized and reconstituted protein (cited in Ref. 13). In addition, since tests with crude preparations of OxlT indicate the solubilized protein binds its substrates (5), we take the information reported here as relevant to understanding structure-function relations in both solubilized and membrane-bound OxlT (OxlTHis). Accordingly, given a helical content of 60–70% (Table II), one expects that 256–295 of the 427 OxlTHis residues are organized in this way, suggesting that OxlTHis could have 10–13 α-helices of sufficient length (25–25 residues) to span the lipid bilayer. The OxlTHis CD spectrum is therefore consistent with models derived from hydrophylogy analysis, which predict 11 or 12 transmembrane α-helices (6). Inasmuch as hydrophylogy analysis and other indirect methods generate similar models for a large number of transporters (3, 7, 11), our findings, together with those of the LacY symporter (13), the band 3 anion exchanger (9), and the glucose carrier (14) reinforce this as a generic feature of such transporting systems.

More important than an overall consistency between such models and experiment, this work now establishes a quantifiable relationship between structure and function for OxlTHis. This was first noted in a qualitative way by the parallel reduction of both activity and helical content during spectral analysis (Fig. 4). Subsequently, we explored this explicitly by measuring the kinetics of spontaneous decay of helix content and by showing that a Kp for oxalte could be derived from the substrate dependence of this process (Fig. 5). We had earlier used reconstitution of activity as the method of analysis, and while that gave a similar estimate of Kp (20 μM [5] versus the 10 μM recorded here), such observations remained a phenomenological description without clue as to underlying mechanism. The present work confirms the initial findings (5) and suggests as well that loss of function in the absence of substrate arises from a breakdown in helical structure. Such observations offer strong evidence that the solubilized protein binds substrate, likely in a 1:1 stoichiometry (Fig. 5, legend), and show how CD or other spectroscopic tools might assess secondary structure for the solubilized transporter.

We note in conclusion that purified OxlTHis can be maintained for an extended period in detergent/lipid micelles without loss of function, provided that substrate is present (Fig. 3). Since it has been difficult to study membrane transporters after their extraction from the lipid bilayer, our work establishes OxlT as a robust model with which to investigate correlations between structure and function using the solubilized material.

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