molecular weight 33 000 after reduction. We suggest from our model that this discrepancy in results between chicken and mammalian species arises because in mammals, during pepsin digestion, cleavage occurs at all three chains of HMW at the location of the kink, resulting in the isolation of only the long arm of HMW.

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Zinc-Sulfur Bonds of Aspartate Transcarbamylase Studied by X-ray Absorption Spectroscopy†

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ABSTRACT: X-ray absorption spectra have been recorded for aspartate transcarbamylase [unligated and ligated with the transition-state analogue *N*-(phosphonoacetyl)-L-aspartate] and for the model compound zinc dimethyldithiocarbamate. The spectra confirm that, in the enzyme, the zinc atom is ligated to four sulfur atoms, with a mean distance of 2.34 ± 0.03 Å. A spread in bond lengths of 0.1 ± 0.03 Å is possible, due to thermal and/or static disorder. No significant difference was found between the spectra of the ligated and unligated enzymes.

Aspartate transcarbamylase (ATCase)[1] from *Escherichia coli* [EC 2.1.3.2; reviewed by Jacobson & Stark (1973)] is a widely studied allosteric enzyme composed of six catalytic polypeptide chains (33 000 daltons each) and six regulatory chains (17 000 daltons each). The catalytic chains form two polar trimers which have only a restricted contact with each other (Monaco et al., 1978; Monaco, 1978). Instead, the principal connections between the trimers occur via the regulatory dimers, so the catalytic trimers and regulatory dimers are released when the regulatory-catalytic bonding domains are disrupted by treatment with mercurials (Gerhart & Schachman, 1965, 1968). This reaction breaks, in each regulatory chain, the bonds between a zinc atom and four cysteinyl sulfur atoms—bonds which maintain the structure of the region that binds to a catalytic trimer. Through their central position at this site, the zinc atoms must lie on or near the path of information transmission used when activation at a catalytic site on one trimer stimulates activation on the other trimer (Gibbons et al., 1976; Yang & Schachman, 1980).

Substitution of the zinc by other metals has produced changes in the optical spectrum over certain regions [250-280 nm in the circular dichroism after substitution by cadmium (Griffin et al., 1973); around 360 and 440 nm in the visible absorption spectrum after substitution by nickel (Johnson & Schachman, 1980)]. By use of the modified spectral region as a probe specific for the metal site, changes were found after activation of ATCase by carbamyl phosphate and aspartate (Griffin et al., 1973) or by PALA (Johnson & Schachman, 1980).

No crystal structure is yet available for the ligated form of ATCase, so there is no information from this source about any related structural changes, which in any case might easily be too small to be observed by this technique. However, information about metal-ligand distances in noncrystalline samples of enzymes can be obtained from extended X-ray absorption fine structure (EXAFS) measurements, permitting the detection of changes not only in the coordination number but also in bond lengths to below 0.1 Å (Shulman et al., 1978a; Stern, 1978; Cramer & Hodgson, 1979; Doniach et al., 1980). Moreover, this technique can be applied without needing to

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[1] Abbreviations: ATCase, aspartate transcarbamylase; EXAFS, extended X-ray absorption fine structure; PALA, *N*-(phosphonacetyl)-L-aspartate.

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substitute the zinc by another metal.

Materials and Methods

Materials. PALA was a gift from Dr. G. R. Stark, and boron nitride was from Merck, Darmstadt. Zinc dimethyldithiocarbamate (from Fluka, Buchs) was recrystallized as described by Klug (1966), and an X-ray powder pattern was recorded to confirm that the crystal form was identical with that in Klug's study. ATCase was prepared by a modification (A. M. Foote, Leberman, and M. F. Moody, unpublished experiments) which avoids the heat step of the standard procedure (Gerhart & Holoubek, 1967).

Preparation of Specimens for EXAFS. Zinc dimethyldithiocarbamate powder was finely ground and diluted by mixture with finely ground boron nitride, before being placed in an aluminum cell 1 mm thick with Mylar faces.

Purified ATCase, 15 mg/mL in 0.09 M Tris–0.08 M borate–HCl buffer, pH 8.35, was spun down overnight at 45000 rpm in a Spinco SW 50.1 rotor (240000*g* at the pellet). Six tubes (5 mL each) were filled, in three of which the ATCase was ligated with PALA (four molecules per active site of ATCase). The pellets, having the consistency of hard honey, were dug out with a small spatula and transferred, by scraping, into a cell. This was made of a sheet of Lucite, 2 mm thick, and with a slot 45 mm long and 20 mm high, to the under face of which was glued a sheet of thin Mylar. After the slot was filled with enzyme pellet, another Mylar sheet was glued on top. After EXAFS data collection, the unligated pellet was checked for enzyme activity [assayed by the method of Prescott & Jones (1969), as modified by Savage et al. (1973)], and it was confirmed that the X-ray scattering patterns of the unligated and PALA-ligated enzymes were as reported earlier (Moody et al., 1979).

EXAFS Data Collection. Data for zinc dimethyldithiocarbamate were collected at room temperature while DORIS was running at 2.2 GeV and 60 mA. The absorption EXAFS apparatus [similar to that described in Bordas et al. (1979)] used a channel-cut Si (111) monochromator and gave six usable scans in 20 min.

The ATCase data were collected at room temperature while DORIS was running at 3.3 GeV and 40 mA. We used a Si (220) monochromator with a harmonic rejection servomechanism (Hart & Rodrigues, 1978; P. Gill, J. Phillips, and J. Bordas, unpublished experiments). The EXAFS spectra were recorded in the fluorescence mode with an array of 10 scintillation detectors (Phillips, 1981), and absorption EXAFS data were recorded simultaneously. For unligated ATCase, 16 scans were taken in the EXAFS region, 4 of which covered the edge region in fine steps; for ligated ATCase, 13 scans were taken in the EXAFS region, 4 of which covered the edge region in fine steps. For each specimen, a scan took about 0.5 h. On average, the detectors were operating at 51 kHz above the edge, 12 kHz of which was due to fluorescence. Counting time was approximately 2 s/data point but varied with beam current to accumulate constant counts per scan. Thus 2.5×10^6 and 1.7×10^6 fluorescent counts were recorded for the native and ligated spectra, respectively. Spectra were calibrated against a Zn foil, the inflection point at the edge being assigned the value 9660.7 eV (Bearden, 1967).

Data Averaging. All scans were checked (and, if necessary, corrected) for shifts of the monochromator calibration before averaging. The fluorescence spectra from individual detectors were examined for signs of exceptional noise and saturation (Phillips, 1981). Signals from eight detectors were averaged by using the weighting factor (change of count rate across edge)/(count rate above edge). [Similar weighting schemes have been used by Shulman et al. (1978b) and K. O. Hodgson (private communication).]

Background Subtraction. The absorption due to zinc was isolated from the total absorption by the standard procedure of fitting a polynomial in the preedge region and extrapolating the polynomial through the whole spectrum. (For data taken in the absorption mode, the polynomial was fourth order; for fluorescence data, it was linear.) The EXAFS oscillations were then extracted from the total zinc absorption with a computer program which smoothed the oscillations by making each point of the spectrum equal to the mean of its nearest neighbors, with iteration to convergence. A cubic spline fitted to the smoothed data was subtracted from the original spectrum. Oscillations were scaled to the jump at the absorption edge and then renormalized to account for the falloff of atomic absorption with increasing energy by using a Victoreen function with the coefficients in Table 3.2.2C of *International Tables for X-ray Crystallography* (Macgillavry & Rieck, 1968).

Spectrum Fitting. The observed spectra were fitted with theoretical spectra calculated by using model zinc environments and the theory of Lee & Pendry (1975).

Phase shifts were calculated by using programs developed by Pendry (1974), Lee & Pendry (1975), and S. Gurman and J. B. Pendry (unpublished work). The excited zinc atom was represented by neutral gallium without the $4p^1$ electron. The zinc environment was taken to be four neutral sulfur atoms arranged as in the model compound zinc dimethyldithiocarbamate (Klug, 1966). Wave functions tabulated by Clementi & Roetti (1974) were used. [This approach has been successfully applied to xanthine oxidase (Bordas et al., 1979, 1980).]

The calculated phase shifts were used as input to a least-squares fitting program for EXAFS analysis, EXAFSFIT (R. Pettifer and A. D. Cox, unpublished results). Fitting of a model to the observed spectrum was accomplished by varying the radii, occupation number, and Debye–Waller factors of shells of ligands and also the residual background and the energy zero. Cycles of parameter adjustment were alternated with recalculation of the amplitudes and phases of the backscattering, based on the adjusted distances. The program has recently been used in an EXAFS study of lithium germanate glasses (Cox & McMillan, 1981).

Data averaging and background subtraction was done with a PDP11/45 at the EMBL Outstation; an IBM 370/168 system at the DESY Rechenzentrum was used for spectrum fitting.

Results

Figure 1 shows the near-edge region of the absorption spectra of the two forms of the enzyme and of the model compound. All three are extremely similar. There are no bound-state transitions, as is to be expected from the electronic configuration of Zn^{2+}. Of particular note is the low-energy peak 6.5 eV above the white line present in all three spectra. This indicates a similarity between the protein and model compound zinc environments, though it is difficult to fit this region of the spectrum theoretically.

The background-subtracted EXAFS oscillations are shown in Figure 2. It is apparent that the spectra for unligated and ligated ATCase are virtually identical except for the signal:noise level and the effects of monochromator "glitches".[2]

[2] These are artifacts which occur where a secondary reflection contaminates the main beam.

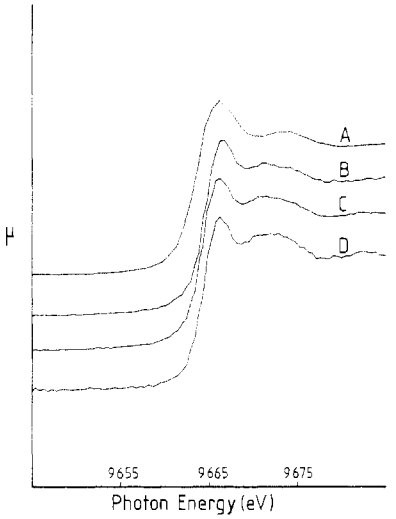

FIGURE 1: EXAFS spectra of ATCase and a model compound (zinc dimethyldithiocarbamate) showing the region around the absorption edge. The absorption coefficient μ is plotted against the incident X-ray photon energy (eV). (A) Model compound (zinc dimethyldithiocarbamate) measured by absorption. (B) Ligated ATCase spectrum measured by fluorescence. (C) Unligated ATCase spectrum measured by fluorescence. (D) Unligated ATCase spectrum measured by absorption.

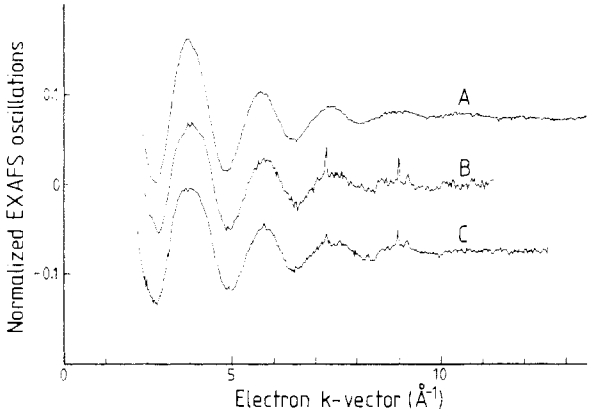

FIGURE 2: EXAFS spectra of ATCase and a model compound (zinc dimethyldithiocarbamate) plotted against the electron **k** vector, showing the oscillations. (A) Model compound (zinc dimethyldithiocarbamate) (absorption). (B) ATCase ligated with PALA (fluorescence). (C) Unligated ATCase (fluorescence).

The model compound spectrum is also similar, except that the amplitudes of the oscillations are approximately 25% greater, and the phase is very slightly shifted to lower k. All three spectra appear dominated by a single decaying wave.

By use of the EXAFSFIT program, the model spectrum was simulated by assuming an environment of four sulfur atoms at the distances found by Klug (1966), with a fixed occupation parameter of one atom, and varying only the Debye–Waller factors, the energy zero, and the residual background. (Background variation was permitted only for data taken in the absorption mode on the model compound; the fluorescence data for the protein was considered to have a sufficiently good background subtraction.) The experimental spectrum was fitted in the range 90–460 eV, the data points being given a weight varying exponentially with energy. This fit is shown in Figure 3A. So that the stability of the fit to variation in the bond distances could be tested, all parameters except the occupation number and the bond length of one ligand were fixed. When this bond length was perturbed by ±0.1 Å, it refined back to the original crystallographic value to within ±0.0015 Å. This gives confidence that the computational

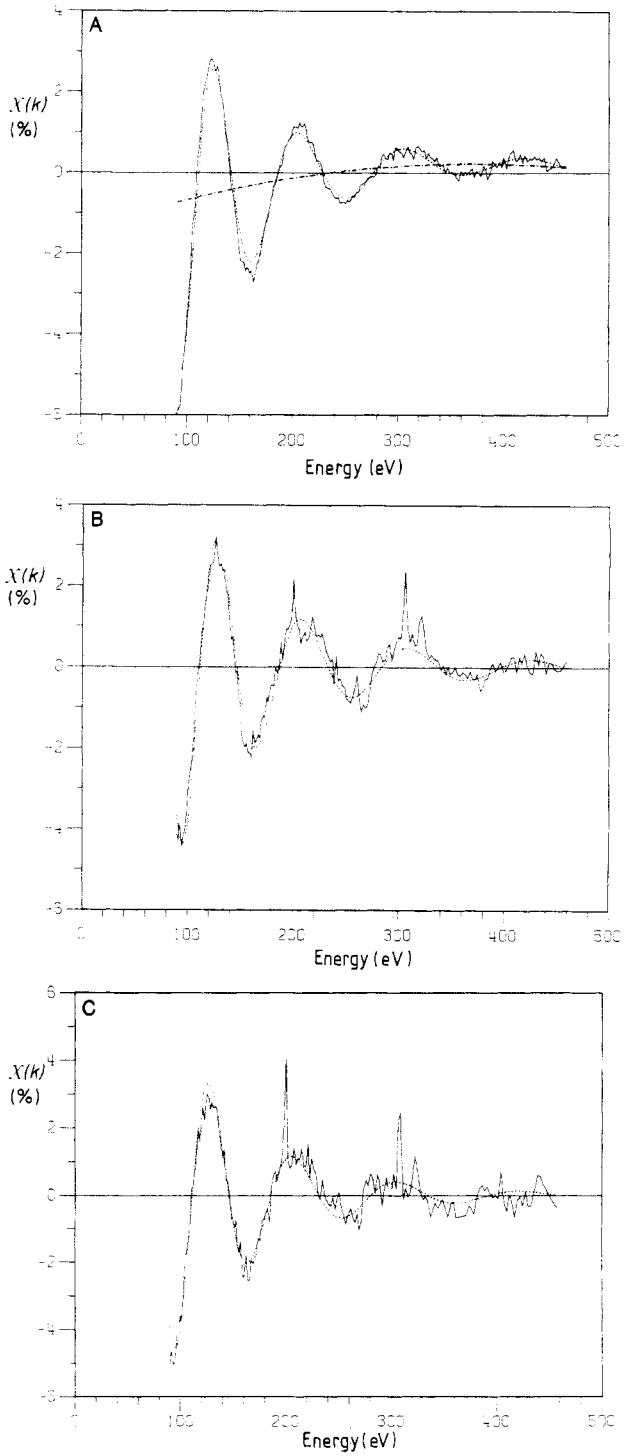

FIGURE 3: Experimental EXAFS spectra (solid line) and theoretical fits (broken line). (A) Model compound (zinc dimethyldithiocarbamate). [The residual background is shown by (···).] (B) Unligated ATCase. (C) ATCase ligated with PALA.

fitting procedure works well. The model compound fit accounts for all features of the spectrum; there appears to be little contribution to the EXAFS from any other neighboring atom.

The protein spectra were fitted over the same energy range and with the same weighting as the model spectrum. The following fitting procedure was adopted. First, the Debye–Waller factors of the four sulfur atoms were refined, taking the following parameters from the model compound refinement: bond lengths, the energy zero, and the Debye–Waller starting values. This produced reasonable agreement with the observed spectra; during refinement the Debye–Waller factors

Table I: Final Refined Values for Zn-S Bond Lengths in Aspartate Transcarbamylase

	unligated		ligated	
bond	length (Å ± 0.03)	Debye-Waller factor[a] (×10³ Å² ± 4.0)	length (Å ± 0.03)	Debye-Waller factor (×10³ Å² ± 4.0)
Zn-S(1)	2.39	20.5	2.35	11.0
Zn-S(2)	2.33	12.0	2.34	12.3
Zn-S(3)	2.32	11.0	2.36	9.9
Zn-S(4)	2.31	4.0	2.29	7.3

[a] The Debye-Waller factor is defined to be σ^2 entering the EXAFS expression as $e^{-2\sigma^2\mathbf{k}^2}$, where k is the photoelectron wave vector.

rose above those for the model compound, as was only to be expected, since the spectra are more strongly damped for the protein. Then each sulfur atom bond length and Debye–Waller factor was allowed to vary in turn. The shortest bond length and its Debye–Waller factor were allowed to vary first, the final values being used and fixed in the next refinement when the longest bond length and its associated Debye–Waller factor were allowed to vary. The remaining two bond lengths were then varied in turn, and the process was repeated once more, varying the bond lengths in the same order. After the second cycle, the stability of the fit was tested by perturbing one bond length by ±0.1 Å, with the result that the original value was again obtained to within ±0.011 Å (unligated) or 0.009 Å (ligated). Finally, all four bond lengths and Debye–Waller factors were allowed to vary simultaneously, resulting in minor adjustments of the parameters. The spectra and the final fit obtained are shown in parts B and C of Figure 3, and the results of the refinement are displayed in Table I.

Error Estimates. Bunker & Stern (1977) and Shulman et al. (1978b) have discussed the factors affecting the accuracy of assignment of mean bond distances and the spread of bond distances within a shell. Both groups used the plane-wave approximation for data analysis, and their error estimates do not carry over exactly into our study, which used curved-wave theory. Nevertheless, their broad conclusion, that a spread in bond distances within a shell is difficult to distinguish from thermal motion, is valid for our study.

We take the spread in bond distances produced by the refinement program to be the error in estimating the mean shell radius (2.34 ± 0.03 Å) and the mean Debye–Waller factor to indicate the sum of the static and thermal spread of individual bonds about this mean (0.1 ± 0.03 Å).

Bunker & Stern (1977) suggested that a temperature-dependence EXAFS study could distinguish between static and thermal disorder, and they carried out such studies on a model compound.

Discussion

EXAFS spectra give only an averaged picture of the environment of the zinc atoms present in the sample, and such data might be difficult to interpret for a protein with six zinc atoms. However, because of the D_3 point group symmetry of ATCase in both the unligated and ligated forms (Monaco et al., 1978; Monaco, 1978), all the zinc atoms are equivalent and must therefore have identical ligand environments.

The similarity between the spectra of ATCase and of the model compound confirms the already well-established fact (Rosenbusch & Weber, 1971; Nelbach et al., 1972; Monaco et al., 1978) that the zinc atom is ligated with four sulfur atoms. A comparison of the EXAFS spectra of the unligated and ligated forms (parts B and C of Figure 2) indicates that any difference between the Zn–S bond lengths in the two forms would be hidden by noise. We therefore interpret those differences found by the fitting program not as definitive structural results but rather as a measure of the uncertainty permitted by the noise level.

The mean bond length found for unligated and ligated ATCase (2.34 ± 0.03 Å) is quite reasonable for tetrahedral zinc–sulfur bonds, for which the mean value usually lies between 2.32 and 2.38 Å. The rather large static/dynamic spread in bond lengths (0.1 ± 0.03 Å) is also reasonable. In small molecules, the static spread often reaches 0.04 Å [zinc dimethyldithiocarbamate (Klug, 1966); zinc diethyldithiocarbamate (Bonamico et al., 1965)] or even 0.067 Å [tetraethylammonium tris(dimethyldithiocarbamato)zincate (Ashworth et al., 1976)]. A static spread of bond lengths is hardly surprising since there is no symmetry in the environment of the zinc atom of ATCase (Weber, 1968; Monaco et al., 1978).

Because small changes of bond length may occur following the allosteric transition, and because any changes in bond angle would have been unobservable in our experiments, our results are consistent with the interpretation of the optical spectroscopic changes following the allosteric transition (Griffin et al., 1973; Johnson & Schachman, 1980). However, the EXAFS data make it clear that these optical changes are not the result of any drastic alteration in the zinc environment, such as a change in the number of ligands, despite the large quaternary structure change (Gerhart & Schachman, 1968; Dubin & Cannell, 1975; Moody et al., 1979) and the greatly increased reactivity of the cysteine residues (Gerhart & Schachman, 1968).

It therefore appears that the zinc–sulfur center of ATCase functions like a rivet, holding together the otherwise rather disordered bonding region of the regulatory chain, but holding it in a flexible fashion to permit conformational change. Another tetrahedral zinc–sulfur center occurs in liver alcohol dehydrogenase (Eklund et al., 1976), and similar centers (with an additional redox function) are found in iron–sulfur proteins (Carter, 1977). In both classes of protein the metal center often contains the sequence Cys-X-X-Cys [one of which occurs in the regulatory subunit of ATCase (Weber, 1968)], and in the latter class the possible changes in bond length following redox reactions are also undetectable by EXAFS (Shulman et al., 1978b).

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Electric Dichroism of Deoxyribonucleic Acid in Aqueous Solutions: Electric Field Dependence†

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ABSTRACT: The ionic strength and molecular weight dependence of the electric field dichroism of DNA, using native and sonicated calf thymus DNA, has been examined in the range of 0.09-1.0 mM NaCl solutions for molecular weights ranging from 1.24×10^5 to 44×10^5. The application of a classical theoretical analysis implies that the orientation results from an induced moment which appears to saturate at moderate fields of several killivolts per centimeter and that the induced moment is at least partly characteristic of that expected of a polyion whose charge is partially counterion compensated. The orientation leads to dichroism values which are in accord with other observations and are lower than expected for a B-form rod of DNA. These values, however, are not uniquely interpretable as resulting from base tilting.

Structural and conformational studies of DNA have long been pursued by various physical methods under vastly different conditions because of the vital importance of DNA in biological activity. Nevertheless, there remain a number of serious questions to be resolved with respect to the polyelectrolyte properties and the structure of DNA in low salt containing aqueous solutions. We report here a series of measurements made on sonicated fragments and "native" (i.e., unsonicated) calf thymus DNA to help resolve some of these questions. The experimental part of the present work was completed before restriction- and endonuclease-digested monodispersed fragments of DNA became available. The sonicated DNA samples used were very carefully treated and characterized. The polydispersity presents some problems of data interpretation but is by no means wholly restrictive. Firm semiquantitative results and conclusions can be drawn.

In an early exploration of the electric dichroism and birefringence of DNA (Yamaoka & Charney, 1973), we demonstrated that native calf thymus DNA did not appear to obey the classical theoretical prescription (Langevin, 1910; Yamaoka & Charney, 1972) that the orientation be quadratic in field strength. This anomaly was attributed to the flexibility of DNA partly on the basis that an earlier though preliminary report (Charney & Yamaoka, 1971) proved that the orien-

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