Characterization of a Homogeneous Arginyl- and Lysyl-tRNA Synthetase Complex Isolated from Rat Liver. Arginyl- and Lysyl-tRNA Synthetases Contain Carbohydrates†

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ABSTRACT: An arginyl- and lysyl-tRNA synthetase complex from rat liver has been purified to homogeneity [Dang, C. V., Glinski, R. L., Gainey, P. C., & Hilderman, R. H. (1982) *Biochemistry 21*, 1959–1966]. Both of the synthetases were able to bind concanavalin A, suggesting that the synthetases contain carbohydrates. Gas chromatographic analysis demonstrates that both arginyl- and lysyl-tRNA synthetases contain mannose and *N*-acetylglucosamine. This is the first conclusive evidence that mammalian aminoacyl-tRNA synthetases contain carbohydrates, a major difference between prokaryotic and mammalian aminoacyl-tRNA synthetases.

Recently investigators have presented evidence that suggests that mammalian aminoacyl-tRNA synthetases may be glycoproteins (Glinski et al., 1979; Dignam et al., 1980). Gas chromatographic analysis performed on partially purified rat liver arginyl- and lysyl-tRNA synthetases demonstrated that carbohydrates were associated with these enzymes (Glinski et al., 1979). Other investigators working with threonyl-tRNA synthetase from rat liver showed the synthetase to be positive for the anthrone test for carbohydrate and for the periodic acid–Schiff reagent (Dignam et al., 1980). Both of these reports suggest (but do not demonstrate) that the synthetases contain carbohydrates.

In order to conclusively demonstrate that mammalian synthetases contain carbohydrates, it is necessary to isolate and characterize the carbohydrates associated with homogeneous synthetases. Accordingly, we have purified to homogeneity an aminoacyl-tRNA synthetase complex from rat liver containing activities only for arginine and lysine (Dang et al., 1982). Evidence is presented in this paper which demonstrates that both arginyl- and lysyl-tRNA synthetases have carbohydrates associated with them.

Experimental Procedures

Materials. Female Long Evans rats (120–150 g) were purchased from Charles River Breeding Laboratories, Inc. All radioisotopes were purchased from Schwarz/Mann. Fluorescein isothiocyanate–concanavalin A (FITC–Con A) was purchased from Sigma. All other chemicals and supplies were of analytical grade and were obtained from standard chemical sources.

Methods. The assay conditions for both arginyl- and lysyl-tRNA synthetases and the purification procedure have been described (Dang et al., 1982). Only arginyl- and lysyl-tRNA synthetases demonstrated to be homogeneous by sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis were used in performing the experiments discussed in this paper.

Concanavalin A Chromatography. The sample was divided into two aliquots. One aliquot (0.30 mL) was dialyzed against two changes of 500 mL of buffer A (0.1 M Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$, 10 mM β-mercaptoethanol, 0.5 M NaCl, 0.1% Nonidet P-40, and 10% glycerol) containing 10% mannose. The sample was applied to a Con A column (0.9 by 3 cm) and washed with buffer A plus 10% mannose. Then the column was washed with buffer A containing 10% mannose and 400 mM K_2HPO_4. Fractions of 0.25 mL were collected, and 20-μL aliquots were assayed. Greater than 90% of both enzyme activities were recovered. The second aliquot (0.30 mL) was dialyzed against two changes of 500 mL of buffer A containing 10% galactose. The sample was then applied to a Con A column (0.9 by 3 cm) and washed with buffer A plus 10% galactose. The bound synthetases were eluted from the column with buffer A plus 10% galactose and 400 mM K_2HPO_4. Fractions of 0.25 mL were collected, and 20-μL aliquots were assayed. Greater than 90% of both enzyme activities were recovered.

Staining $NaDodSO_4$ Gels with FITC–Con A for Glycoproteins. Staining for carbohydrates with FITC–Con A was performed as described (Furlan et al., 1979). $NaDodSO_4$ gel electrophoresis was performed as described (Dang et al., 1982). The gels were fixed in methanol–acetic acid–H_2O (1.5:1:17.5) overnight and then washed for 2 days with six changes of buffer B [0.05 M Tris-HCl (pH 7.0), 0.1 M NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$]. The gels were next stained with FITC–Con A (1 mg/mL) in buffer B for 12 h. Destaining was carried out for 2–3 h with buffer B. The gels were illuminated with a long-wave ultraviolet lamp with a peak output at 366 nm.

Preparative $NaDodSO_4$ Gel Electrophoresis for Carbohydrate Analysis. As a control to demonstrate the accuracy of the carbohydrate and protein quantitation, ovalbumin was electrophoresed and analyzed for carbohydrates by the same procedures used for arginyl- and lysyl-tRNA synthetases. $NaDodSO_4$ gel electrophoresis was performed as described (Dang et al., 1982) with the following modification: Slab gels (9 by 14 by 0.15 cm) were used so that preparative electrophoresis could be run. A 1.0-mg sample of pure arginyl- and lysyl-tRNA synthetase was denatured and loaded onto the stacking gel (1 by 14 by 0.15 cm). A constant voltage of 90 V was applied until the sample entered the resolving gel, and then the voltage was increased to 180 V and maintained for about 2 h. The gels were lightly stained by adding a few drops of 0.05% Coomassie Brilliant Blue G-250 to the fixing solution and incubated overnight. The staining procedure revealed two bands.

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The protein bands corresponding to arginyl- and lysyl-tRNA synthetase were cut from the gel. The gels containing the individual polypeptides were put into individual dialysis bags containing electrophoresis running buffer. Each dialysis bag was placed between the anode and cathode of a small rectangular tank containing electrophoresis buffer. The protein was electrophoresed from the gel at a constant voltage of 40 V for 4 h. The gel was then placed in a new dialysis bag and reelectrophoresed. The dialysis bag contents from both electrophoretic runs were dialyzed overnight against distilled water with three changes, pooled, lyophilized, and subsequently used for carbohydrate analysis by gas chromatography. Greater than 90% of both polypeptides were recovered. Gas chromatography, described below, was performed on the intact complex and the individual synthetase polypeptides. Greater than 90% of the *N*-acetylglucosamine and mannose were recovered with both synthetases after $NaDodSO_4$ gel electrophoresis.

Protein Determination. In order to be certain that the protein concentration of synthetase polypeptides analyzed for carbohydrate composition was accurate, we used two different protein quantitation methods, the fluorescamine protein assay (Bohlen et al., 1973) and the method of Bradford (1976). Lyophilized protein was redissolved in physiological saline solution and dialyzed overnight against methanol–acetic acid–H_2O (15:2:83 v/v) to remove contaminating Coomassie Blue. The sample was next dialyzed for 8 h against physiological saline. Protein concentrations were determined by the two methods following the second dialysis. Standard curves were constructed for both methods by using two different standards (ribonuclease A and β-lactoglobulin). In all cases the standard curves had a correlation coefficient of at least 0.99, and the slopes of the standard curves were essentially identical. The values of the synthetase concentrations obtained by both methods deviated less than 10% from each other.

Carbohydrate Analysis. The analysis and quantitation of the neutral and amino sugars were performed according to Mawhinney et al. (1980). Neutral sugars were removed from the synthetases by acid hydrolysis with 0.6 N HCl at 100 °C for 4 h and eluted in sequence with distilled water through 0.8 by 8 cm columns of Dowex 1-4X (CO_3^{2-} form, 50–100 mesh) and Dowex 50-8X (H^+ form, 200–400 mesh). Neutral sugars were then derivatized to their corresponding aldononitrile acetates by the method of Varma et al. (1973). Amino sugars were prepared from the synthetases by acid hydrolysis with 3 N HCl at 125 °C for 45 min. After hydrolysis, amino sugars were eluted from a cationic resin (Dowex 50-8X, H^+ form, 200–400 mesh) by the procedure of Boas (1953). Amino sugars were then derivatized by the method of Mawhinney et al. (1980). Both the neutral and amino sugars were separated on a GLC column of 2% diethylene glycerol adipate (stabilized grade) on a Chromosorb W-HP (100–120 mesh) 6 ft by $^1/_8$ in. column. The instrument used was a Perkin-Elmer Sigma III gas chromatograph equipped with dual-flame ionization detectors with a nitrogen flow rate of 24 mL/min. Injections were made at 180 °C which was maintained for 1 min and then programmed to 220 °C at a rate of 6 °C/min. Peak areas were measured by using a planimeter. Internal standards, 10 μg of α-glucoheptitol, and 10 μg of methyl β-D-glucoside along with 50 μg of protein were used for all analyses.

To demonstrate that contaminating Coomassie Blue was not interfering with the gas chromatographic analysis, we redissolved the lyophilized protein in physiological saline solution and divided the sample into two aliquots. One aliquot was dialyzed overnight against methanol–acetic acid–H_2O (15:2:83 v/v). This sample was then dialyzed for 8 h against physiological saline. The second aliquot was dialyzed only against physiological saline. Protein concentrations were determined, and gas chromatography was performed on both aliquots following dialysis. Gas chromatographic analysis demonstrated that identical amounts of *N*-acetylglucosamine and mannose were obtained from both aliquots.

Results

Earlier we reported that arginyl- and/or lysyl-tRNA synthetase had carbohydrates associated with the synthetases (Glinski et al., 1979). However, these experiments were performed on partially purified enzymes. Therefore, it is necessary to determine if the pure synthetases have carbohydrates associated with them. Arginyl- and lysyl-tRNA synthetases were demonstrated to be homogeneous by $NaDodSO_4$ gel electrophoresis in 9%, 12%, and 15% polyacrylamide. Densitometer tracing of the gels showed that the synthetases were at least 98% pure when 50 μg of protein was applied (Dang et al., 1982). Also, Dr. David Yang furnished us with a sample of purified lysyl-tRNA synthetase isolated from rat liver (Johnson et al., 1980) and a sample of a purified 18S aminoacyl-tRNA synthetase complex containing lysyl-, arginyl-, leucyl-, isoleucyl-, and methionyl-tRNA synthetases (Johnson & Yang, 1981). The lysyl-tRNA synthetases supplied by Dr. Yang from both the purified sample and the complex comigrated with the 73 000-dalton band we have isolated (Dang et al., 1982). Our 65 000-dalton band (Dang et al., 1982) comigrated with a minor species between bands VII and VIII of the 18S complex isolated by Dr. Yang. We can tentatively identify the 73 000-dalton polypeptide as lysyl-tRNA synthetase and the 65 000-dalton polypeptide as arginyl-tRNA synthetase. Assay of the purified arginyl- and lysyl-tRNA synthetase preparation for other aminoacyl-tRNA synthetases using both the assay procedure described by Bandyopadhyay & Deutscher (1971) and the procedure developed in our laboratory demonstrated it to be free (0.3% relative to arginine and lysine) of activity for Ala, Val, Leu, Ile, Ser, Thr, Phe, Tyr, Lys, Met, His, Asp, and Glu (Dang et al., 1982).

The arginyl- and lysyl-tRNA synthetase complex was divided into two aliquots to determine if carbohydrates were associated with the synthetases. One aliquot was extensively dialyzed against buffer A plus 10% mannose and passed through a Con A affinity column equilibrated with the same buffer. Since Con A has tight binding affinity for mannose (Kornfeld & Ferris, 1975), all these sites were blocked. The enzymes did not bind to this column and passed directly through the column (Figure 1A). The second aliquot was extensively dialyzed against buffer A plus 10% galactose and passed through a Con A column equilibrated with the same buffer (Figure 1B). Since Con A has no binding affinity for galactose (Kornfeld & Ferris, 1975), the synthetases did bind to this column and were eluted from the column only after 400 mM K_2HPO_4 was added to the buffer (Figure 1B). These results demonstrate that the synthetases bind to the Con A column through carbohydrate sites and not through nonspecific hydrophobic interactions.

This suggests that the carbohydrates are very tightly associated with the synthetases but does not demonstrate if the carbohydrtes are associated with one or both of the synthetases. For determination of this, $NaDodSO_4$ gel electrophoresis was performed and the gel stained with FITC–Con A. The results of this experiment are shown in Figure 2. Both the arginyl- and lysyl-tRNA synthetase polypeptides were Con A positive

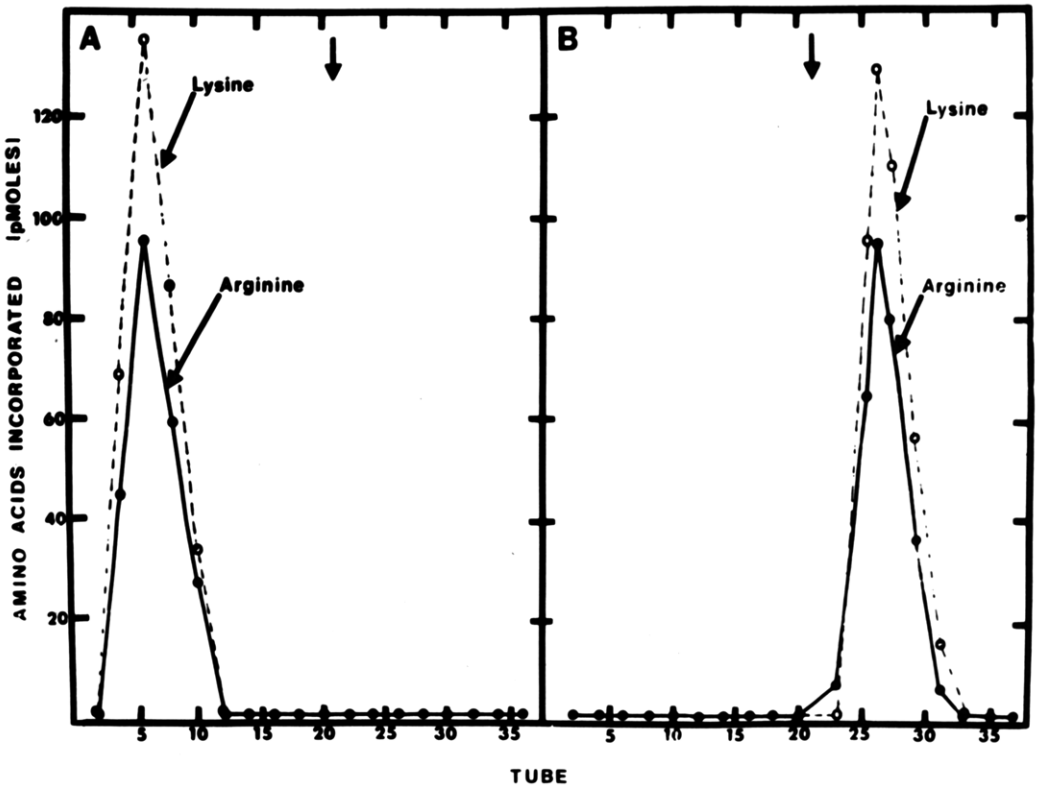

FIGURE 1: Concanavalin A–Sepharose chromatography. The experiments were performed as described under Experimental Procedures. (A) Column was equilibrated and sample applied in buffer A plus 10% mannose. The arrow indicates where buffer A which includes 10% mannose and 400 mM K_2HPO_4 was applied to the column. (●) Arginyl-tRNA synthetase activity and (○) lysyl-tRNA synthetase activity. (B) Column was equilibrated and sample applied in buffer A plus 10% galactose. The arrow indicates where buffer A plus 10% galactose and 400 mM K_2HPO_4 was applied to the column. (●) Arginyl-tRNA synthetase activity and (○) lysyl-tRNA synthetase activity. In both experiments greater than 90% of the activity applied to the column was recovered.

(Figure 2B) as was the positive control protein (thyroglobulin) while the negative control (pyruvate kinase) was Con A negative. Very surprisingly the tracking dye area of this gel was also Con A positive (Figure 2B). This positive Con A reaction was not due to the tracking dye itself because the tracking dye area for the buffer control was Con A negative (Figure 2B, lane 2) as well as the tracking dye areas for the negative and positive controls (lanes 3 and 4). Figure 2A shows the same gel as Figure 2B except the gel was stained with Coomassie Blue. As can be seen, the Con A positive bands coincide with the Coomassie stained polypeptides (lanes 1 and 4). The results shown in Figure 2 indicate that both arginyl- and lysyl-tRNA synthetases most likely contain carbohydrates. However, their corresponding polypeptides must by analyzed by gas chromatography to conclusively demonstrate that both synthetases contain carbohydrates.

Gas chromatography (Table I) showed that both synthetases contain only mannose and *N*-acetylglucosamine. The 73 000-dalton polypeptide, tentatively identified as lysyl-tRNA synthetase, was estimated to contain 2 mol of mannose and 1 mol of *N*-acetylglucosamine per mol of lysyl-tRNA synthetase subunit while the 65 000-dalton polypeptide, tentatively identified as arginyl-tRNA synthetase, was estimated to contain 1 mol of mannose and 1 mol of *N*-acetylglucosamine per mol of arginyl-tRNA synthetase subunit. After the two polypeptides were isolated from $NaDodSO_4$ gels, the polypeptides were individually reelectrophoresed, and gas chromatographic analysis was performed as described. The results (data not shown) were essentially the same as shown in Table I. This demonstrates that the carbohydrates are not contaminants associated with the synthetases and that the two enzymes most likely exist as glycoproteins.

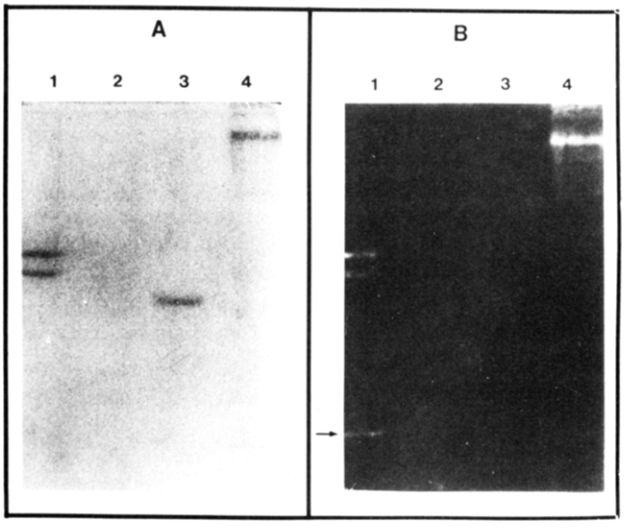

FIGURE 2: Fluorescein isothiocyanate–concanavalin A stained $NaDodSO_4$ gels. $NaDodSO_4$ gel electrophoresis was performed as described (Dang et al., 1982). (A) The gel was stained with Coomassie Brilliant Blue G-250 as described under Experimental Procedures. Lane 1 is arginyl- and lysyl-tRNA synthetases, 40 μg. Lane 2 is a buffer control which contains bromophenol blue tracking dye. Lane 3 is pyruvate kinase, 40 μg, a negative control. Lane 4 is thyroglobulin, 40 μg, a positive control. (B) Same as (A) except the gel was stained with FITC–Con A as described under Experimental Procedures. The arrow indicates the tracking dye area.

Also shown in Table I are the results obtained for ovalbumin prepared and analyzed by the same procedures used for the synthetases. The only carbohydrates detected were *N*-acetylglucosamine and mannose. These are the only carbohydrates found associated with ovalbumin (Fletcher et al.,

Table I: Carbohydrate Composition of Arginyl- and Lysyl-tRNA Synthetases[a]

carbohydrate	73 000-dalton band (lysyl-tRNA synthetase) μg of CHO[b]	mol of CHO/ mol of protein	65 000-dalton band (arginyl-tRNA synthetase) μg of CHO[b]	mol of CHO/ mol of protein	ovalbumin control μg of CHO[b]	mol of CHO/ mol of protein
N-acetylglucosamine	0.136 ± 0.003	0.99[c]	0.157 ± 0.006	1.02[d]	0.66 ± 0.02	2.97,[e] 3[f]
mannose	0.224 ± 0.006	1.82[c]	0.126 ± 0.005	0.91[d]	1.09 ± 0.03	5.45,[e] 5-6[f]
carbohydrate percentage	0.72		0.57		3.5	

[a] The above results were the average acquired from five different enzyme preparations. Carbohydrate content of arginyl- and lysyl-tRNA synthetases was analyzed as described under Experimental Procedures. [b] μg of CHO per 50 μg of protein. [c] Moles of carbohydrate per mole of protein based on molecular weight of 73 000 for lysyl-tRNA synthetases, 200 for *N*-acetylglucosamine, and 180 for mannose. [d] Moles of carbohydrate per mole of protein based on molecular weight of 65 000 for arginyl-tRNA synthetase, 200 for *N*-acetylglucosamine, and 180 for mannose. [e] Moles of carbohydrate per mole of protein based on molecular weight of 45 000 for ovalbumin, 200 for *N*-acetylglucosamine, and 180 for mannose. [f] These are reported values for ovalbumin (Fletcher et al., 1963; Eylar, 1965; Sober, 1970).

1963; Sober, 1970). The total percent carbohydrate was 3.5%, which agrees well with the 3.2% reported for ovalbumin (Eylar, 1965; Sober, 1970). Table I also shows that ovalbumin analyzed by our procedure contains 3 mol of *N*-acetylglucosamine and 5–6 mol of mannose per mol of ovalbumin, in good agreement with the reported values of 3 mol of *N*-acetylglucosamine and 5–6 mol of mannose per mol of ovalbumin (Fletcher et al., 1963; Sober, 1970).

The data obtained for ovalbumin confirm the analytical procedures used in determining the molar ratios of the carbohydrates associated with the synthetases. However, the exact stoichiometry of the carbohydrates must await the determination of the sequence of the carbohydrates.

Discussion

This paper conclusively demonstrates that carbohydrates are associated with some mammalian synthetases. Evidence is presented that shows that homogeneous arginyl- and lysyl-tRNA synthetase complex isolated from rat liver has carbohydrates bound in a constant molar ratio (Table I). Lysyl-tRNA synthetase appears to contain 2 mol of mannose and 1 mol of *N*-acetylglucosamine per mol of lysyl-tRNA synthetase while arginyl-tRNA synthetase appears to contain 1 mol of mannose and 1 mol of *N*-acetylglucosamine per mol of arginyl-tRNA synthetase subunit (Table I). This could indicate an unusual carbohydrate sequence since most glycoproteins contain at least 2 mol of *N*-acetylglucosamine per mol of protein (Montreuil, 1980). However, there are other possible explanations for this unusual carbohydrate stoichiometry. For example, it is possible that the synthetases may be heterogeneous in their carbohydrate composition as demonstrated for porcine RNase (Reinhold et al., 1968). Before an unusual carbohydrate structure can be confirmed, the sequence of the carbohydrates must be demonstrated. The type of carbohydrates associated with both polypeptides suggests that the linkage is most likely an N-glycosidic link between *N*-acetylglucosamine and asparagine. Studies are in progress to determine the linkage and sequence of the carbohydrates associated with both arginyl- and lysyl-tRNA synthetases.

Preliminary gas chromatographic analysis of the Con A positive material isolated from the tracking dye area of NaDodSO$_4$ gels demonstrated the presence of *N*-acetylgalactosamine, *N*-acetylneuraminic acid, glucose, and galactose. The presence of ceramide was also demonstrated by thin-layer chromatography (unpublished observations). Saxholm & Pitot (1974) have suggested that glycolipids are associated with synthetase complexes. The type of carbohydrates present and the fact that ceramide is also present suggest that glycolipids in the form of both cerebrosides and gangliosides are associated with the synthetase complex isolated in our laboratory. At the present time it is not known if indeed these are glycolipids, if they are part of the synthetase complex, or if they are contaminants that copurify with the enzymes. Studies are in progress in our laboratory to answer these questions.

At the present time the function(s) of these carbohydrates is (are) not known. The fact that at least some of the mammalian synthetases have carbohydrates associated with them is a major difference between prokaryotic and mammalian synthetases. Another major difference is that the mammalian synthetases can be isolated as complexes or aggregates (Dang et al., 1982; Bandyopadhyay & Deutscher, 1971; Vennegoor & Bloemendal, 1972; Denny, 1977; Dang & Yang, 1979) while the prokaryotic counterparts are only isolated as free enzymes. It could be possible that the carbohydrates may be involved in the structural organization of these complexes.

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Crystal Structure Determinations of Coenzyme Analogue and Substrate Complexes of Liver Alcohol Dehydrogenase: Binding of 1,4,5,6-Tetrahydronicotinamide Adenine Dinucleotide and *trans*-4-(*N*,*N*-Dimethylamino)cinnamaldehyde to the Enzyme†

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ABSTRACT: 1,4,5,6-Tetrahydronicotinamide adenine dinucleotide (H_2NADH) has been used as a reduced coenzyme analogue to study a simulated transient intermediate of liver alcohol dehydrogenase (LADH) with *trans*-4-(*N*,*N*-dimethylamino)cinnamaldehyde (DACA) as a substrate. X-ray diffraction data of two crystal modifications have been analyzed to 2.9-Å resolution: an orthorhombic complex where the coenzyme analogue binds to the apoenzyme conformation [Eklund, H., Nordström, B., Zeppezauer, E., Söderlund, G., Ohlsson, I., Boiwe, T., Söderberg, B.-O., Tapia, O., Bränden, C.-I., & Åkesson, Å. (1976) *J. Mol. Biol. 102*, 27–57] and a triclinic complex with coenzyme and substrate bound to the holoenzyme conformation [Eklund, H., Samama, J.-P., Wallén, L., Brändén, C.-I., Åkesson, Å., & Jones, T. A. (1981) *J. Mol. Biol. 146*, 561–587]. The interpretation of difference electron density maps for both crystal modifications is based on calculations where the phase angles have been derived from refined enzyme models. H_2NADH binds to the coenzyme binding domain in an extended conformation. The tetrahydronicotinamide ring is situated within the active site region in both enzyme conformations, but the orientation of the ring plane in relation to the active site zinc atom is very different. In the triclinic LADH–H_2NADH–aldehyde complex the coenzyme analogue binds in a similar way as NADH to a ternary inhibited complex. The combined effect of the presence of H_2NADH and the large DACA substrate triggers the gross conformational change of the protein, which involves the movement of the catalytic domains in the dimeric molecule. In contrast, only local structural changes in the orthorhombic LADH–H_2NADH complex are observed upon coenzyme analogue binding. A network of hydrogen bonds between water molecules, the coenzyme, and side chains from the catalytic domain bridges the cleft between the domains in the orthorhombic complex. Since the conformational change narrows the cleft, no such water arrangement is found in the triclinic substrate complex. In the orthorhombic complex the substrate binding pocket is occupied by a methylpentanediol (MPD) molecule used as the precipitating agent during crystallization. MPD binds within hydrogen-bonding distance to zinc-bound water. The X-ray analysis shows that the aldehyde molecule is directly liganded to the metal atom in the triclinic LADH–H_2NADH–DACA complex. The binding of the chromophoric aldehyde substrate within the single crystals used for diffraction experiments was monitored by microspectrophotometric measurements.

Alcohol dehydrogenase from horse liver (LADH)[1] is known to undergo conformational changes upon coenzyme and substrate binding. These changes result in a rotation of the catalytic domains with respect to a central core, the coenzyme binding domains in the dimeric molecule (Eklund et al., 1981). This core structure, however, is virtually identical with that of the unliganded enzyme structure. One functionally important role of the conformational difference is that the coenzyme and substrate clefts become narrower and less accessible to solvent. Our interest has been to investigate the factors that influence this conformational transition. The importance of the proper positioning of the nicotinamide ring has been demonstrated in earlier investigations. Thus the protein remains in the apoenzyme conformation in the complex with pyridine adenine dinucleotide, where the carboxamide group is absent (Samama et al., 1977). There is a similar conformation in the complex with the inactive analogue 5-methylpyridine adenine dinucleotide, where steric repulsion between the methyl group and the protein prevents proper positioning of the nicotinamide ring (Samama et al., 1981). In both these complexes the bound coenzyme analogues are folded in a nonproductive binding manner such that the pyridinium ring is located 20 Å from the catalytic zinc atom; this is called the "surface fold".

Other studies have shown the importance of the environment of the catalytic zinc atom. Thus the active coenzyme analogue 3-iodopyridine adenine dinucleotide binds with the surface fold when the aldehyde competitive inhibitor imidazole is bound to the catalytic zinc atom. The corresponding complex with the inhibitor dimethyl sulfoxide (Me_2SO) bound to zinc gives

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[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; MPD, 2-methyl-2,4-pentanediol; DACA, *trans*-4-(*N*,*N*-dimethylamino)cinnamaldehyde; H_2NADH, 1,4,5,6-tetrahydronicotinamide adenine dinucleotide; Me_2SO, dimethyl sulfoxide; enzyme, horse liver alcohol dehydrogenase (LADH; EC 1.1.1.1).

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