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# DIOL DEHYDRATASE: N-TERMINAL AMINO ACID SEQUENCES AND SUBUNIT STOICHIOMETRY*

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ABSTRACT: N-terminal sequence analysis of diol dehydratase and its constituent subunits shows that the ratio of the 60K:51K:29K:15K subunits in the native enzyme is 2:1:2:2. From the amino acid compositions of the individual subunits diol dehydratase appears to be a peripheral membrane protein.

INTRODUCTION: Diol dehydratase (EC 4.2.1.28) from Klebsiella pneumoniae (ATCC 8724) uses AdoCbl as a specific cofactor in catalyzing the conversion of (RS)-1,2-propanediol to propionaldehyde (1, 2). AdoCbl-dependent enzymes occur ubiquitously in nature (3); nevertheless, the amino acid compositions only for ribonucleotide reductase (4) and for one of the subunits (60K) of diol dehydratase (5) have been reported.

Recent improvements in procedure have allowed us to reproducibly isolate from the bacterial membrane enzyme composed of four different subunits having molecular weights of 60K, 51K, 29K, and 15K daltons (5). The availability of such protein allows determination of the amino acid composition and average hydrophobicities of the various subunits which can help to establish whether diol dehydratase is an integral or peripheral enzyme (6, 7) and further, allows speculation about the membrane-associated topography of the enzyme (8). Finally, comparison of the sequences of the individual subunits with the amino acid composition of the steps in a sequence determination on the entire multi-subunited enzyme complex allows determination of the relative subunit stoichiometry of the functional complex (9).

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ABBREVIATIONS: AdoCbl, adenosylcobalamin; HPLC, high pressure liquid chromatography; Pth, phenylthiohydantoin; SDS, sodium dodecyl sulfate.

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MATERIALS AND METHODS: Enzyme preparation. Diol dehydratase was isolated as previously described (5). In order to remove substances which would interfere with the sequence analysis of "native" enzyme, reagent grade acetone was used to precipitate freshly isolated enzyme from solution. The precipitate was pelleted by centrifugation. The pellet was washed with acetone, dried under nitrogen and then dissolved in 0.1% SDS (Bio-Rad electrophoresis grade, recrystallized from ethanol) and 0.05 M $(NH_4)HCO_3$. This sample was frozen at -70° C until it was to be used.

Preparation of subunits. The subunits of diol dehydratase were separated by preparative gel electrophoresis (5,10). After electroeluting the subunits from the gel, they were dialysed to remove excess salts and detergent, and then lyophilized. After lyophilization aliquots were re-electrophoresed to confirm their purity.

Amino acid analysis. Protein samples were hydrolyzed in vacuo with 6N HCl or 3 M mercaptoethanesulfonic acid for 12, 24, 48 or 72 hours. After hydrolysis, the samples were stored at -70° C until the analyses could be performed on a Durrum D-500 amino acid analyzer.

N-terminal sequence analysis. Automated Edman degradation of the subunits was done with a spinning cup sequenator (11), using a previously described computer program (12). Pth-amino acids were identified by HPLC (11, 13).

Determination of subunit stoichiometry. The protein sequenator was allowed to proceed through several cycles on samples of "native" diol dehydratase. The relative amounts of Pth-amino acids released in each cycle were determined.

Each HPLC sample contained dansylglutamine as an internal standard as did a sample containing 250 pmol of each Pth-amino acid. Background levels of Pth-amino acids were determined in the cycle preceding the cycle of interest and subtracted to give the actual nanomolar yield. The actual yield was then corrected for repetitive yield (96% per cycle) and for the Pth-amino acid recovery percentages reported elsewhere (12). Residues selected for comparison have recovery percentages of at least 85%.

RESULTS AND DISCUSSION: The different amino acid compositions (Table I) and and N-terminal sequences (Table II) of the four types of subunits show the smaller subunits are unlikely to be proteolytic derivatives of the large ones. Though the available sequence data is relatively limited, some striking homologies are apparent among the 15K, 29K and 51K subunits (Figure 1). In contrast, the 60K subunits shows no apparent homologies with the other three subunits.

The discriminating function (Ž) for diol dehydratase and its constituent subunits was calculated according to Barrantes (6). The 29K and 60K subunits are the most hydrophobic (Ž = 0.24 and 0.22, respectively). These values are similar to that of subunit 2 of cytochrome c oxidase (Ž = 0.25) which is only partially accessible for protein modification by lactoperoxidase iodination (14, 15). This suggests that either, or both, of the 60K and 29K subunits serve to anchor the enzyme to the membrane. This supports the previous proposal that the enzyme is a peripheral membrane protein with perhaps limited contact with the lipid bilayer (5).

Table I: Amino Acid Composition of Diol Dehydratase

| Amino Acid | Native Diol Dehydratase (mol%)[a] | Subunits (mol%) 60K | 51K | 29K | 15K | Subunits (residues/subunit)[b] 60K | 51K | 29K | 15K |
|---|---|---|---|---|---|---|---|---|---|
| Asx | 10.3 | 11.4 | 9.7 | 7.5 | 12.8 | 63 | 47 | 20 | 18 |
| Thr[c] | 5.0 | 4.8 | 5.9 | 4.5 | 5.8 | 26 | 28 | 12 | 8 |
| Ser[c] | 6.3 | 6.1 | 8.2 | 6.7 | 6.5 | 34 | 40 | 18 | 9 |
| Glx | 12.2 | 11.5 | 11.2 | 14.5 | 11.0 | 64 | 54 | 40 | 16 |
| Pro | 5.4 | 5.5 | 5.2 | 7.1 | 5.1 | 30 | 25 | 19 | 7 |
| Gly | 9.5 | 9.1 | 12.6 | 9.6 | 5.6 | 50 | 61 | 26 | 8 |
| Ala | 10.7 | 10.5 | 10.2 | 9.9 | 14.2 | 58 | 49 | 27 | 20 |
| Cys[e] | 0.3 | 0.5 | 0.3 | 0.0 | 0.0 | 3 | 2 | 0 | 0 |
| Val | 6.7 | 7.8 | 5.5 | 6.2 | 5.3 | 43 | 27 | 17 | 8 |
| Met | 2.6 | 3.7 | 1.8 | 1.1 | 2.5 | 20 | 9 | 3 | 4 |
| Ile[d] | 5.2 | 5.1 | 5.0 | 7.1 | 3.8 | 28 | 24 | 19 | 5 |
| Leu[d] | 8.0 | 7.0 | 8.6 | 8.3 | 9.4 | 39 | 41 | 23 | 14 |
| Tyr | 2.4 | 2.7 | 2.0 | 2.0 | 2.6 | 15 | 10 | 6 | 4 |
| Phe | 2.9 | 3.2 | 3.0 | 2.9 | 1.9 | 18 | 14 | 8 | 3 |
| His | 1.5 | 1.4 | 1.8 | 1.7 | 0.9 | 8 | 9 | 5 | 1 |
| Lys | 4.9 | 4.4 | 4.8 | 5.9 | 5.7 | 24 | 23 | 16 | 8 |
| Arg | 5.9 | 4.9 | 4.1 | 4.6 | 6.6 | 27 | 20 | 13 | 10 |
| Trp[d] | 0.7 | 0.4 | 0.6 | 1.0 | 1.3 | 2 | 3 | 3 | 2 |

[a]Calculated from the subunit stoichiometry and the compositions of the individual subunits.

[b]Calculated on the basis of the given molecular weights and mole percentages and rounded to the nearest integral value.

[c]Mole percent values were obtained by linear extrapolation to zero time from values obtained after 12, 24, 48, and 72 hours of hydrolysis.

[d]Maximum value taken.

[e]Determined after performic acid oxidation (20).

The average ratios of the subunits obtained from two different sequenator runs on "native" enzyme yielded a stoichiometry of $(60K)_2$ $(51K)_1$ $(29K)_2$ $(15K)_2$ (± 10%). This suggests a molecular weight for native enzyme of 259K which agrees well with the value found for active enzyme in the presence of detergents which prevent aggregation (5). This composition can be compared with a previous report (16) showing that two protein components are necessary to tightly bind AdoCbl. Component S has a molecular weight of 200K and is, itself, composed of four different subunits; component F has a molecular weight of 26K (17). A single component F (26K) with component S (200K) would then account for their total observed molecular weight of about 230K. This suggests that the 51K

Table II: N-Terminal Sequences for the Diol Dehydratase Subunits[a]

| Subunit | 5 10 15 20 25 30 35 40 |
|---|---|
| 60K | M R S K R F E A L A K R P V N Q D G F V K E W I E E G F I A M E S P N D X X(K)X |
| 51K | M N T S E L E T L I R T I L S E G L T P G S T P V G P G G K G I F G(S)V P E A I |
| 29K | M E I N E K L L R Q I I E D V L S E M K G S D K P V S F N A P A A S A A P Q A S |
| 15K | M N T D A I E S M V R D V L S R M N S L Q G E A P A A A P A A G G A S R S A R V |

[a]Parentheses ( ) denote uncertainty of a specific residue. The letter "X" signifies an undetermined residue.

subunit, of which there is one copy per molecule of enzyme, gives rise by proteolysis to component F and one of the subunits of component S. Further support for the possibility that components F and S are generated by proteolysis comes from our inability to separate the enzyme isolated by our procedure (5) into these two components.

That the intact enzyme contains two subunits of 60K, 29K and 15K and a single subunit of 51K daltons suggests a particularly important role for the 51K subunit in the binding of AdoCbl and catalysis, because the intact enzyme binds a single coenzyme molecule per 250K daltons (18) and incorporation of a single molecule of 2,3-butanedione is sufficient to inactivate the holoenzyme (19)

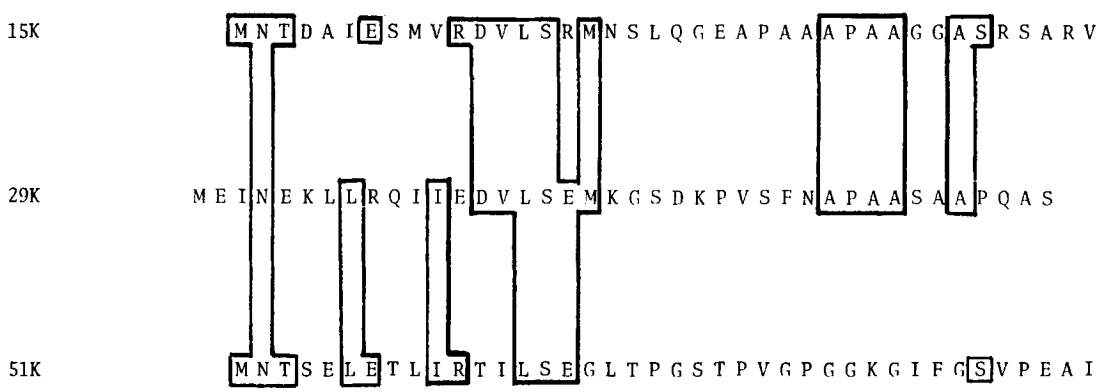


Figure 1: Comparison of sequences for 15K, 29K and 51K subunits. The 29K subunit is offset two residues to maximize homology. The boxed residues are the same in at least two of the subunits.

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