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Correlation Studies Based on Enzyme Structure and Kinetic Results: Deduction of Productive Substrate Orientation in the Active-Site Pocket of Horse Liver Alcohol Dehydrogenase

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Kinetic information on the reduction of alkylated cyclohexanones catalyzed by horse liver alcohol dehydrogenase was correlated with the three-dimensional structure of the enzyme. The substrates investigated were: 2-, 3-, and 4-alkylcyclohexanones with alkyl groups methyl, ethyl, i-propyl, and t-butyl. Kinetic studies establishing at which position of the cyclohexanone ring an alkyl group leads to fast, slow, or no reduction and at which position an increase in the size of the alkyl group leads to a decrease of the rate of reduction, allows one to deduce at which position an alkyl group leads to favorable or unfavorable interactions with groups of the enzyme. On the basis of the X-ray structure of the enzyme and on plausible assumptions regarding the arrangement of the reacting atoms, models were built of the enzyme–NAD^+–alkylcyclohexanol complexes formed during reduction. These models were analyzed with respect to favorable and unfavorable interactions. By changing the orientation of the cyclohexanol molecules in the complex it was possible to arrive at a structure in which the interactions observed in the model correlated extremely well with those deduced from kinetic analysis. As a result, a probable structure of the enzyme–coenzyme substrate complex with productive substrate orientation was obtained. In this orientation the oxygen of the substrate appears to be directly bound to the active-site zinc. In addition the excellent correlation between the kinetic and the structural information demonstrates that the method of kinetically deducing the occurrence of interactions between groups of the substrate and the enzyme can be used to obtain information about the topography of the active site.

INTRODUCTION

NAD^+-dependent horse liver alcohol dehydrogenase catalyzes the oxidation of primary and secondary alcohols and the reduction of aldehydes and ketones with relatively broad specificity and, as such, is one of the most extensively investigated enzymes, both from the point of view of structure and kinetics (*1–4*). The exact structure of the enzyme and of the complex between the enzyme and the ADP–ribose part of the coenzyme is provided by crystallographic investigations. It is, however, not possible to use this direct experimental approach to produce the same kind of information about enzyme–coenzyme–substrate complexes, since these ternary complexes represent transient species. To overcome this difficulty an indirect method needed to be developed, which does not require that the species to be investigated be stable and isolable. We think that for studying

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enzyme–coenzyme–substrate complexes such a method is at hand, when results of crystallographic investigations are correlated with results of kinetic analysis. In addition these correlation studies are expected to provide new insight into functional aspects normally not obtainable when structural and kinetic results are evaluated separately. Clearly the impact of these correlation studies depends strongly on the number and suitability of the substrates used. In this respect liver alcohol dehydrogenase represents a very favorable case, since it has a sufficiently broad specificity (*5*). Among the substrates which had been analyzed kinetically, the alkylated cyclohexanones play a particularly important role, since their structure can be varied systematically within very wide limits and their conformations can be defined by well-documented theoretical considerations (*6*). Liver alcohol dehydrogenase belongs to a group of oxidoreductases which have been investigated kinetically using a large number of alicyclic ketones and whose active-site topography had been specified by typical "diamond lattice sections" (*7*).

In this paper we report on correlation studies carried out by building models of the enzyme–NAD^+–alcohol complexes formed during reduction. The correlation studies are based on a crystal-structure analysis carried out in Uppsala (*1*) and on kinetic investigations pursued in Zürich (*8, 9*). Preliminary accounts of this work have been published elsewhere (*10–12*).

MODEL BUILDING

The active-site pocket of horse liver alcohol dehydrogenase including the coenzyme and one of each of the studied alkylated cyclohexanol molecules was built with Kendrew-type models (Fig. 1). The atomic coordinates of the amino acids, the zinc atom, and the oxygen atom of the zinc-bound water in the apoenzyme were directly available from an electron density map at 2.4 Å resolution of the orthorhombic apoenzyme crystals (*1*). The position of the coenzyme in the active-site pocket was obtained by combining the information from a study at 2.9 Å resolution of ADP–ribose binding to the enzyme in the orthorhombic crystals (*13*) and from a recently calculated electron density map at 4.5 Å resolution of triclinic crystals of an enzyme–NADH–dimethylsulfoxide complex (Eklund and Brändén, unpublished results). The electron density corresponding to the coenzyme molecule was transferred from the triclinic to the orthorhombic map of the enzyme–ADP–ribose complex by using the positions of the active-site zinc atom as a common origin. After minor adjustments of its position the ADP part led to a good fit, but major changes of the position of the terminal ribose were required in order to fit the nicotinamide–ribose part into the density.

The cyclohexanol molecules, which carried methyl, ethyl, i-propyl, or t-butyl groups in 2-, 3-, and 4-position, were built into the model of the enzyme–NAD complex assuming that the hydroxyl oxygen occupies the position of the oxygen atom of the zinc-bound water and that the spatial arrangement

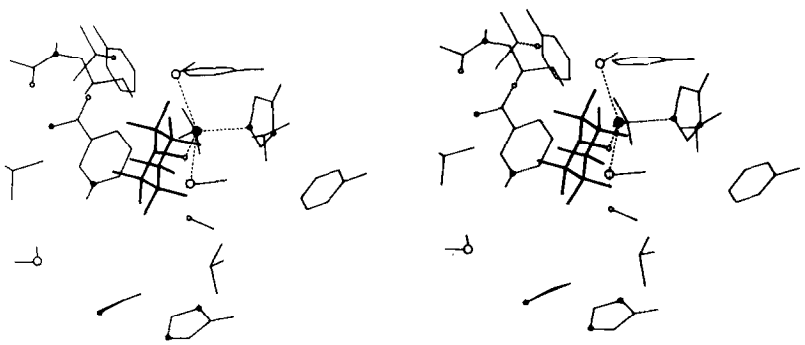


FIG. 1. Stereo diagram of the active-site pocket containing the nicotinamide moiety of the coenzyme and the cyclohexanol ring in the deduced orientation. The 10 positions occupied by the alkyl groups of the various alkylated cyclohexanols are indicated. O, Sulfur atom; o, oxygen atom; ●, zinc atom; •, nitrogen atom. (---) Bonds to the ligands of zinc; (·····), hydrogen bridges and assumed path of the hydrogen transfer.

of the nicotinamide ring, the reacting hydrogen atom at C-1 and the atoms C-1 and oxygen of the cyclohexanol molecule is the same for all substrates considered. The reacting hydrogen atom and C-1 were positioned such that the atoms C-1, hydrogen, and C-4 of nicotinamide are on a straight line which, together with the N-1–C-4 axis of the nicotinamide ring, is located in a plain perpendicular to the nicotinamide ring and forms an angle of 105° with the N-1–C-4 axis (Fig. 1). The angle of 105° was chosen to minimize molecular motion and overlap with the C-4 hydrogen. Furthermore the distance between C-1 of cyclohexanol and C-4 of nicotinamide was adjusted to 3.5 Å. In the resulting model the C–O bond of cyclohexanol and the N-1–C-4 axis are skewed with an angle of 90°. The distance from the zinc atom to the center of the nicotinamide ring is 4.5 Å and to C-1 of cyclohexanol 3.3 Å. The nicotinamide ring allows the oxygen of its carboxyamide group to form a hydrogen bond to the side-chain oxygen of Thr-178 while the nitrogen points toward the main-chain oxygen of Ala-317. Of particular interest is the consequence that the nicotinamide ring appears in the orientation with the A side facing the zinc atom and the B side facing the protein surface, in accordance with the known stereochemistry of the hydrogen transfer with liver alcohol dehydrogenase (*14*). The opposite orientation of the ring with the B side facing zinc can definitively be ruled out due to steric interference between the carboxyamide group and the side chain of Cys-46.

Of the two chair conformations of cyclohexanol, whose relative stability depends on the size and the position of the substituting alkyl group (*6*), only one was found to fit into the active site. When the position of the reacting oxygen and hydrogen is kept fixed, the carbon skeleton of the cyclohexanol molecules in the two chair conformations occupy quite different positions with respect to the enzyme. It was manifest that the cyclohexanol molecules could not bind in the conformation with axial reacting hydrogen due to overlap with the carboxyamide group of the coenzyme, whereas nothing prevented their binding in the conformation with equatorial reacting hydrogen (Fig. 1). Fur-

TABLE 1

SUMMARY OF THE MEASURED RATE CONSTANTS FOR PRODUCT FORMATION AND DEDUCED INTERACTIONS OF THE ALKYL GROUPS WITH THE ACTIVE-SITE POCKET[a]

Configuration of the 2-, 3-, and 4-alkyl-cyclohexanol products	Rate constants for product formation with alkyl groups of increasing size (s^{-1})				Alkyl-group positions[b]	Interactions of the alkyl groups with the active-site pocket
	Methyl	Ethyl	i-Propyl	t-Butyl		
(1*R*,2*R*)	0	0	0	0	2_a	Unfavorable
(1*R*,2*S*)	0	0	0	0	2_e	Unfavorable
(1*S*,2*S*)	1.3	0.8	0	0	$2'_a$	Balanced[c]
(1*S*,2*R*)	0	0	0	0	$2'_e$	Unfavorable
(1*R*,3*S*)	0.18	0	0	0	3_a	Balanced[c]
(1*R*,3*R*)	0.1	0	0	0	3_e	Unfavorable
(1*S*,3*R*)	0.18	0.1	<0.05	<0.05	$3'_a$	Balanced[c]
(1*S*,3*S*)	12.9	13.3	12.0	7.0	$3'_e$	Balanced[c]
trans-1,4	10.6	10.6	10.7	—	4_a	Favorable
cis-1,4	12.8	13.0	12.0	—	4_e	Balanced[c]

[a] Racemic alkylcyclohexanones were reduced with recycling coenzyme using the coupled enzyme system with aldolase and glyceraldehyde 3-phosphate dehydrogenase (*5, 8*) at pH 8 and 25°C. Concentrations: substrate, 40 m*M;* NADH, 1.33 m*M;* enzyme (EE-isoenzyme), 0.4 μM. Rate constants were calculated from product-analysis data obtained after different reaction times and refer to the initial phase of the reaction. For cyclohexanone this rate constant is 24 s^{-1}. Details on the experimental conditions, product analysis, and synthesis of the substrates will be described elsewhere (*9*). The arguments used for deducing the conformation of cyclohexanol and the interactions of the alkyl groups are to be found in the text.

[b] Based on cyclohexanol in the conformation for equatorial hydrogen transfer.

[c] No interactions or compensated unfavorable and favorable interactions.

thermore in this conformation the C-4 end of the cyclohexanol ring appears in an ideal position to create hydrophobic interactions with the side chains of the amino acids Leu-57, Leu-141, Phe-93, and Ile-318 which, with the exception of Ile-318, are all located on the side of the ring facing the active-site zinc atom (Fig. 1). For all alkylated cyclohexanols considered, the cyclohexanol ring was thus fixed in the chair conformation with equatorial reacting hydrogen.

Within the freedom of motion allowed by the geometric constraints of the model the alkylated cyclohexanol molecules were allowed to change their orientation. In analogy to the view developed on the pathway of nucleophilic addition to a carbonyl group (*15*) we assumed that there is a strongly preferred arrangement of the reacting centers for the reaction to occur at high rate. The cyclohexanol molecules considered in the different orientations with respect to the active-site pocket were therefore fixed such that their cyclohexanol rings all appeared in the same orientation with respect to each other. At the 10 possible positions of substitution in the cyclohexanol ring the occurrence of favorable or unfavorable interactions between the alkyl groups and groups of the active-site pocket were examined. This information obtained from model building was then correlated with equivalent information available from the kinetic results summarized in

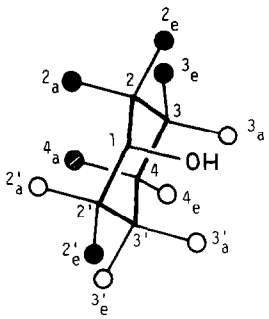


FIG. 2. Schematic diagram summarizing the kinetic results. The cyclohexanol rings of all alkylated cyclohexanols are superimposed. Alkyl groups which create unfavorable or favorable interactions are represented by ● and ◎, respectively; those which create no interactions or lead to a compensation of unfavorable and favorable interactions (indicated as balanced in Table 1) are represented by ○. The orientation of the cyclohexanol ring is the same as in Fig. 1.

Table 1 and in Fig. 2. Among the structurally possible orientations the one was selected which showed the best correlation between model and kinetic results.

RESULTS AND DISCUSSION

The active site of liver alcohol dehydrogenase can be considered as a pocket with the top open to the environment and the bottom closed by groups of the active site and the nicotinamide moiety of the coenzyme (Fig. 1). Since all of the polar groups involved in the catalysis are located in the bottom, this part of the pocket is quite hydrophylic (Fig. 3). It can be divided into the following three parts: (i) The zinc atom with its coordination sphere. The ligands of the zinc atom are the sulfur atoms of Cys-174 and Cys-46, the ϵ-nitrogen atom of His-67, and, as it resulted from the work described in this paper (see below), the oxygen atom of the substrate. The sulfur atom of Cys-46 is on the outside of the pocket and therefore never comes into contact with the substrate. (ii) The nicotinamide moiety of the coenzyme whose carboxyamide group is coplanar with the pyridine ring. Model building revealed that the nicotinamide ring is positioned such that the oxygen of its carboxyamide group forms a hydrogen bridge to the side-chain oxygen of Thr-178, and the nitrogen points toward the main-chain carbonyl oxygen of Ala-317. (iii) The general acid–base system consisting of the hydroxyl group of Ser-48 and the imidazole group of His-51. The oxygen atom (as the donor) of the substrate forms a hydrogen bridge to the serine oxygen which itself is hydrogen bridged to the imidazole δ-nitrogen. The imidazole ϵ-nitrogen is situated on the surface of the enzyme molecule and thus is in close contact with the surrounding solution.

The amino acid side chains which were found to interact with the C-4 end of the substrate form a compact hydrophobic belt around the pocket (Fig. 4). The part of the belt closer to the interior of the enzyme molecule is composed of two rows of three side chains each, those of Ile-318, Phe-93, and Phe-140, closer to the bottom,

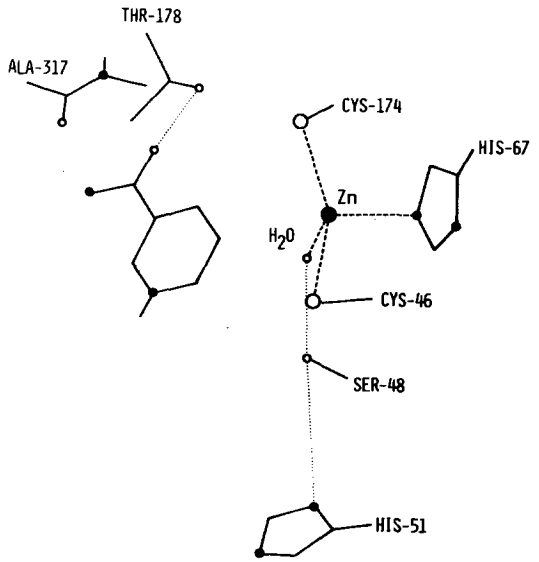


FIG. 3. Schematic diagram of the hydrophilic bottom of the active-site pocket. Symbols and orientation as in Fig. 1.

and those of Phe-110, Leu-116, and Leu-141, further away from the bottom. The part of the belt closer to the outside of the enzyme molecule consists of a single row of four hydrophobic side chains, those of Leu-57, Pro-296, Met-306, and Leu-309 (the last two belong to the second subunit). All of these side chains face the

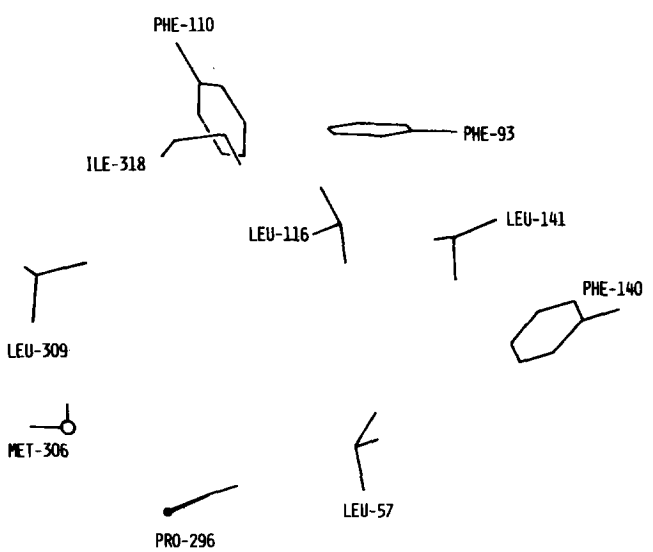


FIG. 4. Schematic diagram of the hydrophobic belt of the active-site pocket. Symbols and orientation as in Fig. 1.

interior of the pocket and are properly oriented for contacting the hydrophobic parts of the substrate.

Between the bottom and the narrow part of the hydrophobic belt there is a hole in the wall of the pocket. The diameter of this hole, which is lined mostly by polar side chains, is too small to permit substrate to pass through; however, it is very likely that water molecules can leave or enter the pocket in concert with the in-and-out movement of the substrate at the top.

At the bottom of the pocket, near the interface between the hydrophilic bottom and the hydrophobic belt, the positioning of hydrophobic alkyl groups of the substrate in the deduced orientation is expected to be very critical and it is not astonishing that only in one 2-position, in $2'_a$, a small alkyl group such as methyl can be located without suffering severe repulsion. In the light of this disposition of hydrophobic and hydrophilic parts in the active-site pocket (Fig. 5a), the results derived from model building are in very good agreement with the kinetic results obtained for the reduction of 2-alkylcyclohexanones (*8, 9*), in which case the stereospecificity is so high that predominantly one product, *trans*-(1*S*,2*S*)-2-alkylcyclohexanol, was formed (Table 1). The rate of formation of this product was found to be very low relative to that of cyclohexanol and when the size of the alkyl group was increased the rate decreased, with ethyl only slightly and with i-propyl and t-butyl so much that no product could be detected. From this kinetic behavior of the 2-alkylcyclohexanones it was concluded that for the formation of the (1*S*,2*S*)-alcohol the cyclohexanone ring has to acquire the conformation in which the alkyl group is axial and the reacting hydrogen equatorial (*9–12*).

The reasoning behind this conclusion is that an alkyl group in the axial position is less stable than in the equatorial position and that this relative instability increases with increasing size of the alkyl group (*6*). For formation of the product under consideration a substrate which in its more stable conformation (alkyl group equatorial) would have to be reduced with axial hydrogen transfer, in order to be reduced with equatorial hydrogen transfer, must at some stage during the reaction change from the more stable to the less stable conformation (alkyl group axial). Under these conditions the rate of reduction depends on the energy difference between the two conformers and decreases with increasing energy difference. Therefore the finding that it is the (1*S*,2*S*)-alcohols which are formed at low and decreasing rates with increasing size of the alkyl group strongly suggested that the reduction occurs with axial alkyl group and equatorial reacting hydrogen.

Clearly, the considered intramolecular interactions cannot be made responsible for the other three products not being formed, so that the further conclusion was drawn that at the 2_a-, 2_e-, and $2'_e$-positions intermolecular interactions with groups of the active site are operative and that they are preventing the formation of the other products.

The same unfavorable intermolecular interactions, which lead to the repulsion of an alkyl group in the 2_a-position are obviously also responsible for preventing the cyclohexanone ring to adopt the conformation for axial hydrogen transfer. In this conformation C-2 of the cyclohexanone ring would appear in the 2_a-position so that the substrate cannot bind in a productive mode. This is very convincingly confirmed by the finding that adamantanone (*16*) is reduced at an extremely low

rate and the two bridged bicyclic ketones bicyclo[3.2.1]octan-2-one (*5*) and bicyclo[3.3.1]nonan-2-one (*10*) at rates comparable to that of cyclohexanone. In the case of adamantanone the 2_a-position is occupied by a methylene group (*12*). For the bridged bicyclic ketones it was predicted (*10*) that they are reduced in the orientation in which the critical 2_a-position is free and only the $2'_a$-position is occupied by a methylene group (*12*). This was recently confirmed to be true in the case of bicyclo[3.2.1]octan-2-one (*5*).

Furthermore from the high rate of reduction of the bridged bicyclic ketone it can be concluded that the $2'_a$-position is not sterically hindered by groups in the bottom

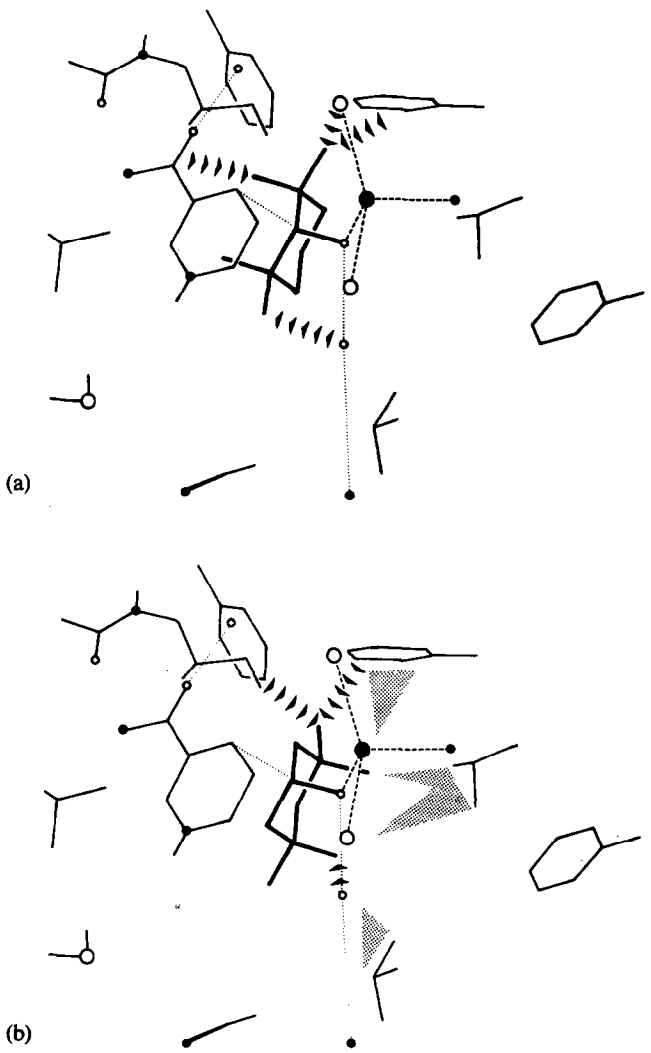
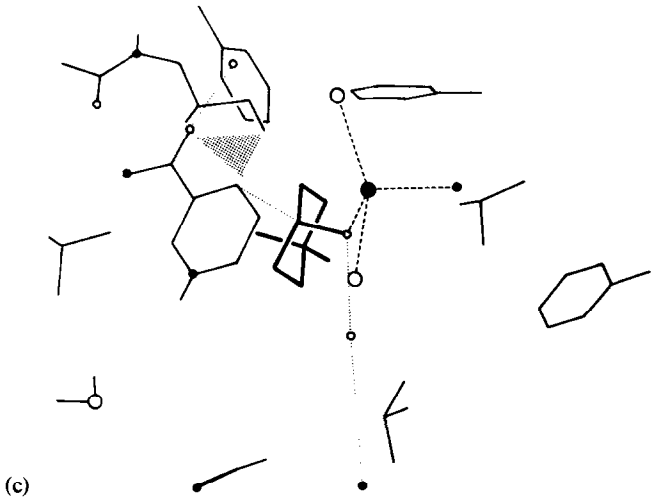
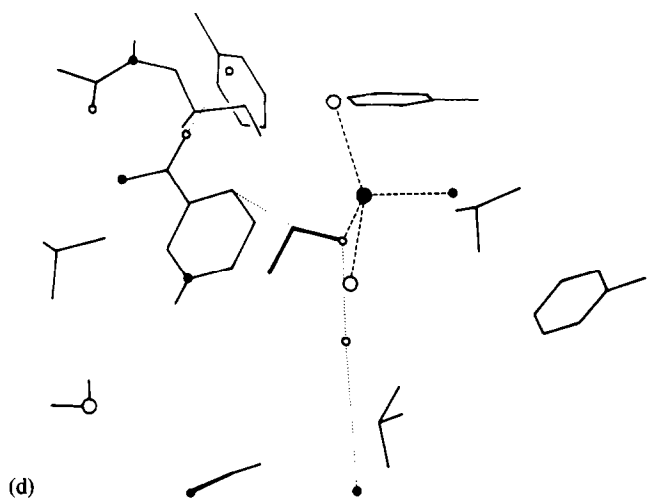


FIG. 5. Schematic diagram of the orientation of the alkylated cyclohexanols and of ethanol and the interactions created by the various alkyl groups. (a) 2-Alkyl groups; (b) 3-alkyl groups; (c) 4-alkyl groups; (d) ethanol. The arrows signify unfavorable and the shaded areas favorable interactions. Symbols and orientation as in Fig. 1.



(c)



(d)

of the pocket and hence that the low rate of formation of the (1*S*,2*S*)-alcohol is really due to intramolecular interactions with the alkyl group. This latter conclusion is of great importance, since the coming into effect of intramolecular interactions constitutes a central piece of evidence in the line of arguments used for deducing that the cyclohexanone ring has to adopt the conformation with axial alkyl group and hence with equatorial reacting hydrogen.

In the hydrophobic part of the active site pocket, there is much more space for alkyl groups to be positioned than in the bottom part (Figs. 5b, c). This steric situation is very clearly reflected by the kinetic behavior of the 3- and 4-alkylcyclohexanones (Table 1). These substrates were reduced with a considerably lower stereospecificity than the 2-alkylcyclohexanones; of all the possible 3-

alkylcyclohexanols only (1*R*,3*R*)-3-alkylcyclohexanols were not found as products (*8, 9*). Formation of this alcohol with equatorial hydrogen transfer would require the alkyl group to be in the highly hindered 3_e-position. The 3-alkylcyclohexanols, for which the equatorial position $3'_e$ is accessible without intermolecular interaction, were formed at a high rate, which is comparable to that of cyclohexanone and is little dependent on the size of the alkyl group; even the 3-t-butyl homologue was formed at such high rate. Taking into consideration under what steric conditions intramolecular interactions slow down the rate, as discussed in the case of the 2-alkylcyclohexanones, this kinetic behavior strongly indicates that alkyl groups in this position are not hindered by intramolecular interactions and hence appear in the equatorial position. Such a perfect correlation of the kinetic results with the model provides strong evidence that for all of these substrates the cyclohexanone ring adopts the same orientation and conformation as in the case of the 2-alkylcyclohexanones, and that it is therefore allowed to compare substrates with 2-,3-, and 4-alkyl groups on the basis of a single model in which all of the cyclohexanol rings are superimposed.

The rates of formation of the two 3-alkylcyclohexanols, which require the alkyl group to be axial, were found to be low and decreasing with increasing size of the alkyl group. In view of the effects created by intramolecular interactions, these results are in good agreement with the observation made with an alkyl group appearing in the $2'_a$-position. We are facing here, however, a more complicated situation: the model shows that an alkyl group in the $3'_a$-position is in addition hindered by the side chain of Ser-48, and consideration of the intramolecular interactions reveals that 1,3-diaxial interactions involving alkyl and hydroxyl (*6*) add to the relative instability of both 3-alkylcyclohexanols with axial alkyl: in the 3-alkyl case the energy difference between the conformers with axial and equatorial alkyl is larger than in the 2-alkyl case. Since, as the model shows (Fig. 5b), these destabilizing effects are partly compensated by favorable interactions occurring with groups of the hydrophobic belt, the overall effect turns out to be similar to that observed with the 2-alkylcyclohexanones and is in full agreement with the picture developed about the factors determining the rate of reduction.

An exceptional behavior, but one which is fitting extremely well, was observed with the 4-alkylcyclohexanones (*8, 9*). The formation of the *trans*-alcohols, for which the alkyl group must acquire the axial position when the cyclohexanone ring is in the deduced conformation, occurs at high rates (Table 1). These rates were comparable to those of the formation of the *cis*-alcohols, which correspondingly place the alkyl group into the equatorial position, and remained nearly constant when the alkyl group was changed from methyl to i-propyl. This observation leads to the conclusion that an alkyl group in the 4_a-position has to be stabilized by strong hydrophobic interactions with groups of the active site, a disposition, which was unambiguously verified by inspection of the model. An alkyl group in this position is ideally located to form interactions with the hydrophobic belt in the vicinity of Ile-116 (Fig. 5c).

From these comparative studies we have learned how substrates, which give rise to interactions in several different positions, are oriented in the active-site pocket. Since the positioning of such a large number of alkyl groups, all attached

to the same cyclohexane ring, is very critical, the resulting disposition of the reacting centers can be expected to be quite representative, also for substrates which are fixed by much fewer interactions. In this context it is very interesting to consider the stereospecificity observed for the oxidation of ethanol, since this substrate is solely oriented by interactions near the interface between the hydrophilic bottom and the hydrophobic belt. Model building reveals that the position of the ethanol methyl group corresponding to C-2 of the cyclohexanol ring is closer to the hydrophilic part of the interface than that corresponding to C-2′, suggesting that the latter is the less hindered position. Without knowledge, however, of the discrimination of alkyl groups in the alkylated cyclohexanones, e.g., that $2'_a$ is the only 2-position not creating unfavorable interactions, it would not be possible to rationalize the productive mode of binding of ethanol, since the observable differences for ethanol are rather small.

When ethanol is oriented such that the Re-hydrogen is transferred to the nicotinamide, as is required to explain the observed stereospecificity (*14*), its methyl group appears at the less hindered position corresponding to C-2′ (Fig. 5d). The high stereospecificity for ethanol oxidation then can be considered as a result of the very unfavorable interactions occurring at the position corresponding to C-2, a conclusion which is well supported by the extremely low reactivity of i-propanol. On the other hand this strong repulsion at C-2 brings up the problem as to why a cyclic compound such as cyclohexanol, which reacts at a rate comparable to that of ethanol, is a substrate at all. Both from the kinetic results and from model building it follows that the C-4 end of the cyclohexanol ring creates strong interaction with the hydrophobic belt, and it seems very likely that these interactions are prominent enough to overcome the repulsive forces at C-2. Although this compensation phenomenon provides a good explanation for the case under discussion, it points out how careful in general one has to be if substrates as different as cyclohexanol and ethanol are compared.

The results obtained by model building and kinetic mapping, which cover purely qualitative but very marked effects (work on more quantitative aspects is in progress), were found to complement and mutually confirm each other so well that they allow us to draw a number of conclusions, which are of general interest in enzymology. On the basis of these results it is possible to establish the importance of the orientation effect as a factor determining the reaction rate (*17*). The orientation of the cyclohexanone ring of the substrates, which react at high rates as compared to cyclohexanone, defines a reference arrangement of the reacting centers. In the case, for example, of the formation of (1*R*,3*R*)-3-methylcyclohexanol (see above) which occurs at an extremely low rate, the methyl group in the highly hindered 3-position, forces the C-4 end of the cyclohexanone ring away from the hydrophobic belt by about 1.5 Å. If this displacement results in a pivoting of the cyclohexanone ring around the carbonyl oxygen the arrangement of the reacting centers is changed and the angle at which the hydrogen approaches C-1 of the cyclohexanone ring differs by about 18° from that of the reference arrangement. According to the views developed about the strong orientational preference in nucleophilic addition to a carbonyl group (*15*), this change in geometry is sufficient to account for the observed change in rate.

The results also provide new evidence that the proposed mechanism of action for liver alcohol dehydrogenase (*2*) is correct. There is no doubt, at least in the case of the investigated small substrates, that a water molecule inserted between the substrate oxygen and the zinc atom, as suggested recently (*18*), would displace the substrate molecule too much from the deduced orientation to be compatible with both the kinetic results and model building. Furthermore, they demonstrate very clearly that the structure of functionally important regions of an enzyme molecule are the same in the crystalline state as in solution and that the crystallographically determined three-dimensional structure of an enzyme can serve as an appropriate model on which deductions concerning the dynamics of enzyme catalysis can be based. Finally it is demonstrated that the kinetic mapping method which was applied in these correlation studies and had previously been used to predict the three-dimensional picture of the active site (*7–10*), now found to be correct, represents a valuable tool in enzymology. It seems very likely that it can analogously be applied to study the binding sites of other biologically interesting macromolecules, for example of receptor proteins, which are barely accessible to more direct methods of investigation.

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