Chiral Recognition of Prochiral Centres and General Acid–Base Catalysis

NECESSARILY INTERRELATED MANIFESTATIONS OF ACTIVE-SITE STRUCTURE

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(Received 7 November 1975)

On the basis of Ogston's [(1948) *Nature (London)* 162, 963] argument, the following conclusions are indicated by the stereochemistry of the reversible oxidation of glycolate by lactate dehydrogenase: (1) general acid–base catalysis is involved in the reaction; (2) the transformation of enzyme-bound (tetrahedral) substrate into enzyme-bound (trigonal) product involves a conformational transition of the enzyme–coenzyme complex.

Ogston (1948) in his now classic paper has explicitly stated two conditions that have to be fulfilled for the chiral recognition of a prochiral centre by an enzyme. It appears that Ogston's (1948) postulate is, as the rule, fulfilled by enzyme reactions (Alworth, 1972). This recognition has since led to a most spectacular development of stereochemistry, in which a very important role is played by such reactants as [(*S*)-2-³H]glycolate (Fig. 1), where chirality is based on the stereospecific substitution of hydrogen atom(s) by deuterium or tritium (Robinson & Cornforth, 1974). I want to show here that, from a refinement of the requirements stated by Ogston (1948), a strong indication can be obtained for the occurrence of general acid–base catalysis in enzymic reactions. The presented considerations specifically refer to the lactate dehydrogenase-catalysed reversible oxidation of glycolate.

Lactate dehydrogenase is known to oxidize, not only L(+)-lactate to pyruvate, but also glycolate to glyoxylate. It is firmly established (Johnson *et al.*, 1965; Lüthy *et al.*, 1969) that the prochiral C-2 atom of glycolate is chirally recognized by the enzyme, it being the pro-*R*-hydrogen atom that is specifically eliminated when glycolate is reversibly oxidized to glyoxylate. As shown in Fig. 1, this means that the methyl group of L(+)-lactate and the pro-*S*-hydrogen atom of glycolate occupy the same steric position.

The 'three points' in a prochiral substrate that, according to Ogston's (1948) argument, are necessarily involved in its chiral recognition by the enzyme must involve at least three of the four substituents of a tetrahedral centre (e.g. the α -carbon atom of glycolate in Fig. 1). Accordingly, it is quite reasonable to investigate whether the chemical nature of the reaction and the nature of the four substituents enable us to select the three essential points of contact by eliminating the fourth as non-essential. For the lactate dehydrogenase reaction the arguments are as follows. (1) The carboxyl group must be one of the essential points of contact, not only because the substrate specificity of lactate dehydrogenase shows a quite stringent requirement for an α -carboxyl group, but also because of the high chemical reactivity of a carboxyl group compared with the other three substituents of the central carbon atom (Fig. 1). (2) The carbon-bound hydrogen atom that is eliminated in the reaction is removed directly and stereospecifically to the re-face of the nicotinamide ring of enzyme-bound coenzyme (Cornforth *et al.*, 1966). This characteristic of the reaction does require a direct contact in the enzyme–coenzyme–substrate 'pre-complex' between coenzyme and substrate. (3) Accordingly, we are left with the following alternatives for the identity of a third essential point of contact: either it involves the alcoholic hydroxyl group, which

Fig. 1. Steric orientation of the four substituents of the α -carbon atom of some reduced substrates of lactate dehydrogenase

is identical in lactate and glycollate (Fig. 1); or both the methyl group of L(+)-lactate and the pro-*S*-hydrogen atom of glycollate are able to establish the missing third essential contact with a third binding subsite of the enzyme. Of these alternatives, the possibility that the pro-*S*-hydrogen atom may be involved in the chiral recognition of glycollate is automatically eliminated, since one simply cannot think of any 'steric' or 'reactivity' model (Popják, 1970, pp. 124–125) that would suggest otherwise. (4) Therefore the alcoholic hydroxyl group must be directly involved in the chiral recognition of glycollate by the enzyme-coenzyme complex.

This conclusion is illustrated in Fig. 2(a). In Fig. 2(b) the argument is by analogy extended to the chiral recognition of glyoxylate in the reverse reaction. On the basis of the X-ray-crystallographic findings obtained by Adams *et al.* (1973), Arg-171 and His-195 are indicated in Fig. 2 as providing two of the required 'contact points' in both ternary enzyme-coenzyme-substrate complexes. However, it should be noted that the presented considerations are completely independent of these identifications.

Conclusions

(1) The ('absolute') stereospecificity of the reversible oxidation of glycollate very strongly indicates that general base-general acid catalysis is involved in this reaction. With reference to Fig. 2, this stereochemical argument can be outlined as follows. For the reaction to take place, the enzyme-coenzyme complex must interact with both the hydroxyl substituent of glycollate (Fig. 2a) and the carbonyl oxygen atom of glyoxylate (Fig. 2b). Microscopic reversibility requires that the enzymic site participating in this interaction be identical for the forward (Fig. 2a) and reverse reactions (Fig. 2b). Secondly, conversion of enzyme-bound glycollate into enzyme-bound glyoxylate involves a reversible deprotonation reaction. Therefore the conjugate base and acid forms of some functional group of the enzyme (e.g. His-195; Fig. 2) are by far the most likely candidates for this 'point of contact', whereby the corresponding interactions were naturally defined as hydrogen bridges. Accord-

ingly, stereospecificity and general acid-base catalytic action of lactate dehydrogenase appear to be two interrelated manifestations of the unique three-dimensional structure of its active site, each of which can hardly be explained without involving the other.

That this type of relationship between (substrate) stereochemistry and proton transfer (within enzyme-substrate complexes) might be the common feature of a great number of enzyme reactions was perhaps first noted by Wang (1968), and remarkably amplified in a recent review by Bender & Kézdy (1975). Although there is an enormous literature that indicates or implies in diverse ways the involvement of general acid-base catalysis in enzyme reactions, it appears (see Bender & Kézdy, 1975) that no single piece of experimental evidence yields more support to this idea than the chiral recognition by lactate dehydrogenase of both glycollate and glyoxylate, as discussed above. When considering the strength of this evidence, one should note that lactate dehydrogenase does not contain co-ordinated heavy metal, and therefore 'Lewis acid catalysis' [as suggested, e.g., by Dunn *et al.* (1975) for alcohol dehydrogenase] can hardly be an alternative to general catalysis by the conjugate acid/base pair of a Brønsted acid function (e.g. ImH^+/Im ; Fig. 2).

(2) A second conclusion that can be drawn from Fig. 2 concerns the three-dimensional structure of the active site. Fig. 2 demonstrates that there must be a significant difference in the steric orientation of those not necessarily identified, but necessarily identical, 'contact points' by which glycollate and glyoxylate are chirally recognized by the enzyme. This naturally follows from the rearrangement of the prochiral centre in the reaction, since it is tetrahedral in glycollate (Fig. 2a) and trigonal in glyoxylate (Fig. 2b). With reference to Emil Fischer's (1894) classical complementarity model, this suggestion is equivalent to defining the unknown structure of the 'lock' through the familiar structure of the 'key'. Fig. 2 accordingly involves a dynamic concept of the 'lock', i.e. that its structure is gradually changing as the 'key' is turned over.

Fig. 2 implies that this dynamic system can be described with reference to the unique three-

Fig. 2. Approximate three-dimensional orientation (and tentative identification; Adams *et al.*, 1973) of the three essential 'points of contact' through which (^3H -labelled) glycollate and glyoxylate are chirally recognized by lactate dehydrogenase

dimensional structures of two 'orientation complexes' (in the sense used, e.g., by Huisgen & Mayr, 1975) and the frequency with which these two complexes are interconverting. The physical reality of this concept is quite strongly supported by the results of a pre-steady-state kinetic analysis of the lactate dehydrogenase reaction (Südi, 1974*a,b*).

On the other hand, two complete sets of three-dimensional co-ordinates have not yet been obtained by X-ray crystallography for any interconverting enzyme-substrate $\rightleftharpoons$ enzyme-product system comparable with that shown in Fig. 2. Therefore it is noteworthy that some recent observations made in quite simple non-enzymic systems provide instructive models for these structural aspects of Fig. 2 (see, e.g., Vögtle & Goldschmitt, 1974; Kanters *et al.*, 1975). Both the usefulness and the limitations of these simple models obviously depend on the analogy between 'crystal environment' (in the sense used, e.g., by Bürgi, 1975) and 'macromolecular environment'.

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