Small-Angle X-Ray Scattering on Malate Synthase from Baker's Yeast

Considerations on the Effects of Bound Ligands

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Binding of the substrates to the anisometric enzyme causes slight changes of some molecular parameters (Zipper and Durchschlag, Eur. J. Biochem., 1978). Estimations based on several plausible assumptions allow a separation of the experimentally observed effects into effects caused by the substrates or by the enzyme in the enzyme-substrate complexes or by binding of buffer molecules. The results show that the observed changes of molecular parameters are primarily due to structural changes in the enzyme molecule. From the changes in the radius of gyration upon substrate binding, the binding sites of the substrates may be localized to be at a radial distance of 5.3 nm from the centre of the enzyme particle. Binding of one or both of the substrates induces different structural changes of the enzyme particle. On formation of the [enzyme·acetyl-CoA], [enzyme·glyoxylate], or [enzyme·pyruvate] complexes, an increase of the short axis by $4.5 \pm 1\%$ occurs, while the formation of the [enzyme·acetyl-CoA·pyruvate] complex does not change this axis significantly. At the same time, binding of the substrates leads to a decrease of the long axes of the enzyme particle by $2.0 \pm 0.2\%$, independent on the kind of the complex formed. These changes of the axes correspond to an increase of the axial ratio by $6.7 \pm 1\%$ on formation of the [enzyme·acetyl-CoA], [enzyme·glyoxylate], or [enzyme·pyruvate] complexes and by 2.7% on formation of the [enzyme·acetyl-CoA·pyruvate] complex, *i. e.* in all cases to a decrease of anisometry of the enzyme particle.

Introduction

Malate synthase (EC 4.1.3.2) catalyzes the Mg^{2+}-dependent synthesis of malate from acetyl-CoA and glyoxylate [1, 2] according to the reaction:

$$\mathrm{HOOC\text{-}CHO + CH_3COSCoA + H_2O \rightleftharpoons HOOC\text{-}CH(OH)CH_2\text{-}COOH + CoA\text{-}SH}\,.$$

The enzyme from baker's yeast has been characterized by enzymological [3–5] and physico-chemical [3, 5–9] investigations. Evidence for the occurrence of structural changes of the yeast enzyme upon substrate binding was obtained from previous spectroscopic, hydrodynamic and X-ray studies [6–9].

The molecular structure of the native substrate-free enzyme and of enzyme-substrate complexes was described by using the small-angle X-ray scattering technique [8, 9]. The enzyme was shown to have a slit-smeared radius of gyration $\tilde{R} = 3.82_5 \pm 0.01$ nm, a radius of gyration $R = 3.95_5 \pm 0.02$ nm, a maximum particle diameter $D = 11.2 \pm 0.6$ nm, a radius of gyration of the thickness $R_t = 1.04 \pm 0.04$ nm, a molecular weight $M_r = 187\,000 \pm 3000$, and a correlation volume $V_c = 338 \pm 5$ nm^3. The comparison of the experimental scattering curve with theoretical scattering curves of various models showed an oblate ellipsoid of revolution (semiaxes $a = b = 6.06$ nm and $c = 2.21$ nm, axial ratio $v = 0.364$) to be the best fit. Binding of the substrates (acetyl-CoA, glyoxylate) or of the substrate analogue pyruvate caused slight changes of some molecular parameters. If no corrections for the bound substrates were applied, these changes imply an increase of the axial ratio by 2.2–6.9%, *i. e.* substrate binding induces a decrease of anisometry.

Relevant statements concerning structural changes of the enzyme in the various complexes, however, need separation of the experimentally observed effects into effects due to ligand binding and due to structural effects of the enzyme itself. The present paper reports on a procedure how the con-

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tributions of the bound ligands can be estimated and taken into account for the calculation of the dimensions of the enzyme particle in the enzyme-substrate complexes. This makes possible the establishment of a schematic model for the structural changes of the enzyme upon substrate binding.

Materials and Methods

Materials, preparation and handling of the enzyme, experimental techniques and evaluation procedures as well as a short survey of the theoretical background has been described in a preceding paper [9]. The basic theory of small-angle X-ray scattering has been published in detail elsewhere [10 – 12].

A few experimental details, necessary for understanding of the following considerations, have to be recapitulated. X-ray experiments [9] were performed at 4 °C in 5 mM Tris-HCl buffer, pH 8.1, containing 10 mM $MgCl_2$, 1 mM MgK_2EDTA and 0.2 or 2 mM dithiothreitol, at protein concentrations $2.5 \leqq c \leqq 57$ mg/cm³. The following enzyme-ligand complexes were investigated: [enzyme·Mg^{2+}], [enzyme·Mg^{2+}·glyoxylate], [enzyme·Mg^{2+}·pyruvate], [enzyme·Mg^{2+}·acetyl-CoA], [enzyme·Mg^{2+}·acetyl-CoA·pyruvate]. (The ligand Mg^{2+} has been omitted in the following formulae of the enzyme-substrate complexes; Mg^{2+} does not cause significant structural changes, as follows from previous CD-experiments [6]). The concentration of these ligands amounted to 10 mM Mg^{2+}, 50 or 90 mM sodium glyoxylate, 50 or 90 mM sodium pyruvate, 2 or 6 mM acetyl-CoA; the use of the higher concentrations of these ligands did not lead to an additional significant change of the molecular parameters investigated by the X-ray study. Considerations on the effects of bound ligands, therefore, have to take into account the possible influence of the substrates glyoxylate and acetyl-CoA and of the substrate-analogue pyruvate and of buffer molecules, preferably of the ions sodium, magnesium and chloride.

Results and Discussion

Contributions of bound ligands

An analysis of the results of the previous X-ray study [9] on the substrate-free enzyme and on enzyme-substrate complexes showed the following

Table I. Molecular parameters and models of the substrate-free enzyme, of the enzyme-substrate complexes and of the enzyme in the enzyme-substrate complexes.

	Molecular parameter					Model (oblate ellipsoid)			
	$\tilde{R}$ [nm][a]	R [nm][a]	R_a [nm][b]	D [nm][a]	V_c [nm³][a]	$a=b$ [nm]	c [nm]	v	V [nm³][c]
Substrate-free enzyme	3.825	3.955	0.99	11.2	338	6.06	2.21	0.364	340
enzyme-substrate complexes[d]									
acetyl-CoA	3.815	3.93	1.04	10.9	358	5.99	2.33	0.389	350[e]
acetyl-CoA+pyruvate	3.80	3.92	1.00	11.0	344	5.99	2.23	0.372	336[e]
pyruvate; glyoxylate	3.79	3.905	1.025	10.7	338	5.96	2.29	0.384	341
		$R_{E,L}$ [nm]	$R_{aE,L}$ [nm]	d [nm]	z [nm]				
enzyme in the complexes[f]									
a) $R_{E,P} = R_{E,A}$									
acetyl-CoA		3.90	1.04	5.3	1.14	5.94	2.33	0.392	344
acetyl-CoA+pyruvate		3.88	0.99	5.3	1.07	5.93	2.22	0.374	327
pyruvate; glyoxylate		3.90	1.025	5.3	1.13	5.95	2.29	0.385	340
b) $R_{E,P} = R_{E,AP}$									
acetyl-CoA		3.915	1.03	4.6	1.59	5.97	2.30	0.385	343
acetyl-CoA+pyruvate		3.90	0.98	4.6	1.50	5.97	2.19	0.367	327
pyruvate; glyoxylate		3.90	1.02	4.6	1.57	5.95	2.28	0.384	338

[a] Taken from ref. 9.
[b] Axial radius of gyration $R_a = R_t \sqrt{9/10}$ (cf. ref. 9)
[c] Calculated from a and c.
[d] Dimensions of the enzyme-substrate complexes, calculated directly from the experimental molecular parameters R and R_a.
[e] The volume of the enzyme in the complex is smaller by 3 nm³.
[f] Molecular parameters and dimensions of the enzyme in the enzyme-substrate complexes and radial distance d and z-coordinates of the binding sites, calculated as described in the text.

changes of molecular parameters upon substrate binding (*cf.* Table I):

a) the overall radius of gyration decreases, as has been established both with the slit-smeared and the desmeared curves;
b) the maximum particle diameter decreases;
c) the radius of gyration of the thickness increases;
d) on binding of acetyl-CoA alone or together with pyruvate the correlation volume increases, while it appears to be unchanged on binding glyoxylate or its analogue pyruvate.

The principal question is whether these observed changes of molecular parameters do actually reflect changes of the enzyme structure or whether they may be explained by other factors. To answer this question we have to take into account all effects which may influence these parameters.

Radius of gyration

The radius of gyration of a dissolved particle is defined by the relation (*cf.* ref. [13])

$$R^2 = \frac{\int_V \Delta\varrho(\boldsymbol{r})\, r^2\, d\boldsymbol{r}}{\int_V \Delta\varrho(\boldsymbol{r})\, d\boldsymbol{r}}, \qquad (1)$$

where $\Delta\varrho(\boldsymbol{r})$ is the local difference between the electron density of the particle and the surrounding solvent, $\boldsymbol{r}$ is measured from the electronic centre of gravity, and the integrals are taken over the entire particle volume.

According to this definition a change of the radius of gyration may be caused by three different factors:

1) in the case of an inhomogeneous particle by raising or lowering the electron density of the solvent,
2) by the attachment of ligands to the particle or by removal of ligands from it,
3) by a structural change leading to a decrease or increase of the overall size of the particle.

In principle, two or three of these factors may superimpose; this may lead to an enhancement or compensation of the effects.

In the case of malate synthase, the first mentioned possibility, the change of the electron density contrast, is highly unlikely as the enzyme was shown to be quite homogeneous [9] and moreover the differences between the electron densities of pure buffer and the various buffer-substrate mixtures are very small.

To estimate the sign and size of possible effects due to attachment of ligands to the particle or removal of ligands from it, we will consider mainly the following limiting cases: scattering masses located in the electronic centre of gravity; scattering masses located on the enzyme surface, either at the smallest possible distance from the centre (*i. e.* 2.21 nm, corresponding to the short semiaxis) or at the largest possible distance from the centre (*i. e.* 6.06 nm, corresponding to the long semiaxes).

In principle, a decrease of the radius of gyration could be caused by attachment of additional scattering masses (substrates or analogues, buffer molecules) to the particle at distances smaller than R, or by removal of scattering masses (buffer molecules) from the particle at distances larger than R. The maximum effect upon attachment of ligands would occur when the additional masses were located in the electronic centre of gravity, though this case is very unlikely; the maximum effect upon removal of ligands would occur when the masses were removed from the poles of the long axes.

By our scattering experiments, the maximum decrease of the radius of gyration was found when pyruvate or glyoxylate were bound to the enzyme. Thus we may ask whether *e. g.* 4 pyruvate molecules (*cf.* refs. [6, 9]) brought into the centre of the enzyme particle will change the radius of gyration to a detectable extent. Since the enzyme has about 23 000 net electrons and four pyruvate molecules have only about 110, the effect on the radius of gyration turns out to be less than 0.01 nm. The same result is obtained when the estimation is made for glyoxylate. Thus even this extreme location of four small ligands cannot account for the observed decrease of the radius of gyration.

However, there remains the possibility that due to unspecific binding the number of these ligand molecules, pyruvate or glyoxylate, is a little higher than assumed above. It would be of interest therefore to estimate how many molecules of pyruvate, when bound to the enzyme surface in the smallest possible distance from the centre (*i. e.* at 2.21 nm), are necessary in order to produce a decrease of the radius of gyration by 0.05 nm. This estimation leads to a number of 31 pyruvate molecules. Such a large number of ligand molecules would have shown up in the experimental determination of m_L,

the mass of bound ligands [9]. Apart from this, the location of 31 pyruvates around the poles of the short axis of the enzyme and nowhere else is highly unrealistic. A more realistic basis would be the assumption of a rather uniform distribution of unspecifically bound ligand molecules over the entire surface, but this would give rise to an increase of the radius of gyration rather than to a decrease.

Due to the larger number of net electrons contributed by acetyl-CoA (about 134 per molecule), the binding of four molecules acetyl-CoA at a distance of 2.21 nm from the centre would, indeed, be able to reduce the overall radius of gyration in about the observed extent.

It remains to discuss the question whether the observed decrease of the radius of gyration may be caused by changing the distribution of buffer molecules, preferably of ions, on the enzyme surface. It should be noted that by addition of pyruvate or glyoxylate to the enzyme solution the concentration of ions is greatly enhanced. This, of course, favours the binding of buffer ions to the enzyme rather than the removal of ions from the enzyme.

90 – 100 sodium, magnesium or chloride ions, when binding to the enzyme surface at the minimum possible distance from the centre (*i. e.* at 2.21 nm) could explain a decrease of R by 0.05 nm. The more realistic case of uniform ion binding on the enzyme surface would result in an increase of R, as has already been pointed out above. An estimation made for a hypothetical removal of ions from the enzyme surface at the maximum possible distance from the centre (*i. e.* at 6.06 nm) shows that 44 – 49 sodium, magnesium or chloride ions must be removed in order to produce a decrease of R by 0.05 nm.

The change of ions in the surroundings of a particle might change its hydration too. But, assuming similar densities for water involved in hydration and the bulk water, we may neglect these additional effects.

Maximum particle diameter

The same factors as mentioned above for a change of the radius of gyration can cause a change of the maximum particle diameter. For the reasons discussed before, the experimentally observed decrease of the maximum particle diameter can hardly be explained by changing the electron density contrast. Since the net electron densities of the enzyme and of possible ligands (substrates or analogues, buffer molecules) are all positive, the attachment of the ligands to the enzyme surface can only lead to an enhancement or retention of the value of the maximum particle diameter. The removal of buffer molecules from the extreme positions at the poles of the long semiaxes could lead to a decrease of this parameter.

Radius of gyration of the thickness

Changes of the radius of gyration of the thickness can be treated in a similar way as changes of the overall radius of gyration. This implies that a change of the electronic density contrast can be ruled out. The mere attachment of 4 pyruvate or glyoxylate molecules at the enzyme surface at the poles of the short axis would increase the radius of gyration of the thickness by only 0.01 nm, which is small as compared to the experimentally found increase. The binding of 4 molecules acetyl-CoA at the same positions would enlarge the thickness radius of gyration by 0.04 nm. An increase of R_t by 0.074 nm would result from the binding of 31 pyruvate molecules or 90 – 100 sodium, magnesium or chloride ions at 2.21 nm. On the contrary, by removing 44 – 49 buffer ions from the extreme positions at 6.06 nm, the radius of gyration of the thickness would increase only by 0.01 nm. Removing the ions from less extreme positions would lead to a still smaller increase or even to a decrease of R_t.

Evidence for structural changes

Summarizing the above results for the limiting cases we can state, that the binding of 4 molecules acetyl-CoA or of 31 molecules pyruvate or glyoxylate or of 90 – 100 buffer ions to the enzyme surface at the smallest possible distance from the centre could account for the observed decrease of the overall radius of gyration R and increase of the radius of gyration R_t of the thickness, but not for the observed decrease of the particle diameter D. Similarly, the unlikely removal of 44 – 49 buffer ions from the enzyme surface at the maximum possible distance from the centre could account for the observed decrease of R and perhaps also for the decrease of D, but not for the observed increase of R_t.

The only explanation which holds for the observed changes of all three parameters is the as-

sumption that the enzyme itself undergoes structural changes upon substrate binding. This principal result is not affected by the circumstance that the changes of the molecular parameters are relatively small and subject to errors.

It should be noted, that the increase of uniform binding of buffer molecules, which would result in an increase of all three molecular parameters, could compensate a decrease of the radius of gyration and of the particle diameter. When unspecific binding of ions to the particle takes place, the actual decrease of the radius of gyration and of the particle diameter might be still larger than the experimentally found decrease.

Models for the enzyme particle in the enzyme-substrate complexes

In order to estimate the extent of the structural changes of the enzyme particle in the enzyme-substrate complexes we have to correct the experimental molecular parameters for the contributions due to the bound ligands. An unequivocal correction would require the knowledge of the position of the ligand molecules. Since such an a priori information is lacking in the case of malate synthase we can perform only estimations based on more or less reasonable assumptions.

General assumptions and relations

For biochemical reasons we can assume that the binding site for glyoxylate and hence also for pyruvate is near to that for acetyl-CoA. Thus the assumption that all binding sites have the same radial distance d from the particle centre is not unrealistic.

We have investigated three different kinds of complexes of the enzyme [9], namely complexes with a) glyoxylate or pyruvate, b) acetyl-CoA and c) acetyl-CoA plus pyruvate. For our estimation of structural changes, it is necessary to assume that in two of the three different kinds of complexes the enzyme has undergone about the same changes of structure. (In the following we shall see that this assumption is in accord with the results of the model calculations.) This assumption implies that the overall radius of gyration of the enzyme in both complexes would be the same. With $R_{E,P}$, $R_{E,A}$ and $R_{E,AP}$ designating the hypothetical radii of gyration of the enzyme in the [enzyme·pyruvate], [enzyme·acetyl-CoA] and [enzyme·acetyl-CoA·pyruvate] complex, respectively, three different cases are possible:

1) $R_{E,P} = R_{E,A}$
2) $R_{E,P} = R_{E,AP}$
3) $R_{E,A} = R_{E,AP}$.

The following equations relate the experimental radii of gyration of the complexes, R_P, R_A, R_{AP}, with the hypothetical radii of gyration of the enzyme in the various complexes:

$$R_P^2 = \frac{Z_E R_{E,P}^2 + n Z_P d^2}{Z_E + n Z_P} \tag{2a}$$

$$R_A^2 = \frac{Z_E R_{E,A}^2 + n Z_A d^2}{Z_E + n Z_A} \tag{2b}$$

$$R_{AP}^2 = \frac{Z_E R_{E,AP}^2 + n(Z_A + Z_P) d^2}{Z_E + n(Z_A + Z_P)} \tag{2c}$$

where Z_E, Z_P and Z_A are the respective numbers of net electrons of enzyme, pyruvate and acetyl-CoA, n is the number of ligand molecules or pairs of ligand molecules per enzyme particle, and d is the radial distance of the binding sites from the particle centre. Relations analogous to Eqns (2a–c) connect also the experimental axial radii of gyration $R_{a,L}$ of the complexes ($R_a = R_t\sqrt{9/10}$, *cf.* ref. [9]; L designates any of the three ligands A, P, or AP) with the hypothetical axial radii of gyration $R_{aE,L}$ of the enzyme in the complexes and the z-coordinates of the binding sites.

Calculation of dimensions

The above relations together with the formerly mentioned assumptions establish the following procedure for estimating molecular parameters and dimensions of the enzyme in the various complexes. By combining two of the Eqns (2a–c), we obtain an equation which can be solved for the radial distance d of the binding sites by inserting the respective experimental values for the radii of gyration of the complexes. The knowledge of d allows the calculation of the radii of gyration $R_{E,L}$ of the enzyme in the various complexes and, assuming the binding sites to be located on the surface of the particle, the estimation of the z-coordinate of the binding sites (by using in first approximation the dimensions of the substrate-free enzyme). Then from the z-coordinate of the binding sites and from the experimental axial radii of gyration of the complexes, the axial radii of gyration $R_{aE,L}$ and the short semiaxes of the enzyme in the different

complexes can be calculated. Finally, the long semiaxes are calculated from $R_{E,L}$ and $R_{aE,L}$. By estimating the z-coordinate of the binding sites on the basis of the dimensions of the enzyme in the complexes and by repeating the second part of the above procedure, the estimated dimensions may be further refined.

This procedure could be applied to only two of the above mentioned cases (namely $R_{E,P} = R_{E,A}$ and $R_{E,P} = R_{E,AP}$), since in the third case ($R_{E,A} = R_{E,AP}$) an imaginary number resulted for the radial distance d. The number n of ligands was always chosen as 3 or 4, the results thus obtained differed only slightly and could be averaged. A summary of results is presented in Table I.

It should be noted that the results are not only subject to errors of indefinite size due to the applied procedure and its idealized assumptions and approximations, but may be affected also by errors of the experimental values used in the various computations. The influence of the experimental errors can easily be estimated. On the assumption that the overall radii of gyration of the complexes are accurate to ± 0.01 nm and the axial radii of gyration to ± 0.02 nm (these limits of error correspond to the standard deviations of the mean values), we obtain the following limits of error: ± 0.6 nm for the radial distance of the binding sites and ± 0.4 nm for their z-coordinate, ± 0.04 nm for the long semiaxes and ± 0.05 nm for the short semiaxis of the particle, ± 9 nm^3 for the particle volume and ± 0.009 for the axial ratio.

As a comparison of the data obtained for the two different cases ($R_{E,P} = R_{E,A}$ and $R_{E,P} = R_{E,AP}$) shows, the results obviously do not depend much on the choice of the basic assumptions. A more detailed analysis of the dimensions of the models of the enzyme in the enzyme-substrate complexes (*cf.* Table I) reveals that the results clearly contradict the basic idea of the second case that the structures of the enzyme in the [enzyme·pyruvate] and [enzyme ·acetyl-CoA·pyruvate] complexes should be about the same. While the dimensions and axial ratios for the enzyme in the complexes with pyruvate and acetyl-CoA plus pyruvate differ considerably, the differences between the data for the [enzyme·pyruvate] and [enzyme·acetyl-CoA] complexes are well within the limits of error. These findings are in good agreement with the assumption that $R_{E,P}$ equals $R_{E,A}$, based on the idea that the structures of the enzyme in the [enzyme·pyruvate] and [enzyme ·acetyl-CoA] complexes are about the same.

Conclusions on structural changes of the enzyme particle

On this basis, ligand binding would be accompanied by the following structural transitions of the enzyme (*cf.* Table I and Fig. 1): Binding of pyruvate (or glyoxylate) or acetyl-CoA at a radial distance of $d = 5.3$ nm would result in a decrease of the long semiaxes of the enzyme particle by about 0.115 ± 0.005 nm (*i. e.* by $1.9 \pm 0.1\%$) and

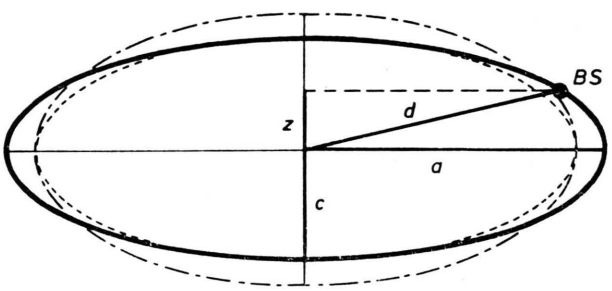

Fig. 1. Schematic drawing of the cross-section of the models of the substrate-free enzyme (———) and of the enzyme in the [enzyme·glyoxylate] or [enzyme·pyruvate] or [enzyme·acetyl-CoA] complexes (·—·—·) and in the [enzyme·acetyl-CoA·pyruvate] complex (·····). *a*, *c*, semiaxes of the oblate ellipsoids (*b* equals *a* and is perpendicular to *a* and *c*); *d*, *z*, radial distance from particle centre and z-coordinate of the binding site BS; the values for *a*, *c*, *d*, and *z* are given in Table I. In the drawing the changes of axial dimensions are enlarged by a factor of 5.

an increase of the short semiaxis by about 0.10 ± 0.02 nm (*i. e.* by $4.5 \pm 1\%$). This implies an increase of the axial ratio by $6.7 \pm 1\%$. Formation of the [enzyme·acetyl-CoA·pyruvate] complex, on the other hand, would still lead to a decrease of the long semiaxes by about 0.13 nm (*i. e.* by 2.1%), however, the short axis of the enzyme particle would practically not change. Thus the increase of the axial ratio as compared to the substrate-free enzyme would amount to only about 2.7%.

It is interesting to note, that almost the same changes of axial ratio, namely increases by $6.2 \pm 0.7\%$ and 2.2%, follow also from the dimensions of the complexes as derived directly from the experimental data without the corrections for the contributions due to the substrates (*cf.* Table I).

A final estimation shows, that the specific binding of the substrates on the enzyme surface at a radial distance of 5.3 nm without accompanying

structural changes of the enzyme would increase the radius of gyration by 0.007 – 0.043 nm and the radius of gyration of the thickness by less than 0.005 nm. A decrease of the overall radius of gyration in the experimentally observed extent could be achieved by additionally removing 90 – 130 buffer ions from the binding sites of the substrates, however, this would also decrease the radius of gyration of the thickness to a value being considerably smaller than the corresponding quantity of the substrate-free enzyme. The observed changes of experimental molecular parameters can therefore be interpreted as to reflect unequivocally structural changes of the enzyme particle, namely a decrease of anisometry.

In order to compare the particle volumes (*cf.* Table I) calculated on the basis of the dimensions of substrate-free enzyme, of the various enzyme-substrate complexes, and of the enzyme in the complexes, the volumes of the complexes may be corrected for the ligands by subtracting 3 nm^3 for acetyl-CoA and 0.3 nm^3 for pyruvate or glyoxylate, as may be estimated from their molecular weights and specific volumes (*cf.* ref. [9]).

The following conclusions can be drawn from the data on the particle volumes: Binding of pyruvate or glyoxylate obviously has no valuable effect on the enzyme volume. Binding of acetyl-CoA alone seems to increase slightly the volume of the enzyme, whereas on binding of acetyl-CoA plus pyruvate the enzyme volume decreases significantly. These findings do not fully agree with the behaviour of the experimentally determined correlation volume on ligand binding; however, this may be explained by the low accuracy of volume determinations as mentioned earlier [9]. It should be noted, however, that the difference between the volumes calculated for the enzyme in the complexes with acetyl-CoA and acetyl-CoA plus pyruvate is about the same as the difference between the experimentally determined correlation volumes of these complexes.

The X-ray small-angle scattering technique proved to be able to unveil several small changes of some molecular parameters of malate synthase on substrate binding [9]. We present clear evidence that these changes can be attributed primarily to structural changes in the enzyme particle. Though the models for the enzyme-substrate complexes described above provide only an approximate description of the overall enzyme structure, statements on the structural changes can be made.

Binding of the substrates acetyl-CoA or glyoxylate (or its analogue pyruvate) induce similar structural effects concerning the changes of axial ratio and particle volume (*cf.* Table I and Fig. 1). This behaviour is also in accord with the kinetic data, which suggest a random mechanism for the binding of the two substrates to malate synthase [5]. The simultaneous binding of acetyl-CoA and pyruvate causes a structural change different from that induced by only one ligand. Obviously, only the binding of both ligands forces a structural change which favours the first step of the catalytic action of the enzyme, *i. e.* the enolization of acetyl-CoA.

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