A Small-Angle X-Ray Scattering Study on Pre-Irradiated Malate Synthase. The Influence of Formate, Superoxide Dismutase, and Catalase on the X-Ray Induced Aggregation of the Enzyme

P. Zipper, R. Wilfing, M. Kriechbaum

Institut für Physikalische Chemie der Universität Graz, Heinrichstraße 28, A-8010 Graz, Austria

and

H. Durchschlag

Institut für Biophysik und Physikalische Biochemie der Universität Regensburg, Universitätsstraße 31, D-8400 Regensburg, Bundesrepublik Deutschland

Dedicated to Prof. Dr. J. Schurz *on the Occasion of His 60th Birthday*

The sulfhydryl enzyme malate synthase from baker's yeast was X-irradiated with 6 kGy in air-saturated aqueous solution (enzyme concentration: $\simeq$ 10 mg/ml; volume: 120 μl), in the absence or presence of the specific scavengers formate, superoxide dismutase, and catalase. After X-irradiation, a small aliquot of the irradiated solutions was tested for enzymic activity while the main portion was investigated by means of small-angle X-ray scattering. Additionally, an unirradiated sample without additives was investigated as a reference. Experiments yielded the following results:

1. X-irradiation in the absence of the mentioned scavengers caused considerable aggregation, fragmentation, and inactivation of the enzyme. The dose D_{37}^t for total (= repairable + nonrepairable) inactivation resulted as 4.4 kGy. The mean radius of gyration was found to be about 13 nm. The mean degree of aggregation was obtained as 5.7, without correction for fragmentation. An estimation based on the thickness factor revealed that about 19% of material might be strongly fragmented. When this amount of fragments was accordingly taken into account, a value of 7.1 was obtained as an upper limit for the mean degree of aggregation. The observed retention of the thickness factor and the finding of two different cross-section factors are in full accord with the two-dimensional aggregation model established previously (Zipper and Durchschlag, Radiat. Environ. Biophys. **18**, 99−121 (1980)).

2. The presence of catalytic amounts of superoxide dismutase and/or catalase, in the absence of formate, during X-irradiation reduced both aggregation and inactivation significantly.

3. The presence of formate (10 or 100 mM) during X-irradiation led to a strong decrease of aggregation and inactivation. This effect was more pronounced with the higher formate concentration or when superoxide dismutase and/or catalase were simultaneously present during X-irradiation. The presence of formate also reduced the amount of fragments significantly.

4. The results clearly show that the aggregation and inactivation of malate synthase upon X-irradiation in aqueous solution are mainly caused by $OH\cdot$; to a minor extent O_2^- and H_2O_2 are additionally involved in the damaging processes.

Introduction

Small-angle scattering has been proven to be the most powerful technique for determining size and shape of biopolymers in solution (*cf.* [1−3]). Recently small-angle X-ray scattering (SAXS) has been introduced by two of us as a potent tool for studying radiation damages of biopolymers [4−10]. In the case of malate synthase, SAXS was able to elucidate the X-ray induced aggregation of the enzyme; there-

by both the monitoring of the aggregation process in situ and the study of pre-irradiated samples were applied. For the early stages of the aggregation process a two-dimensional model of side-by-side association of enzyme particles could be convincingly established (*cf.* [5, 6]). Meanwhile a similar model could be derived by other authors by means of small-angle neutron scattering for superoxide dismutase (SOD) irradiated with ^{60}Co γ-rays [11].

Malate synthase from baker's yeast ($M_r = 185.000$) is a sulfhydryl enzyme, which suffers various pronounced damages upon X-irradiation in air-saturated aqueous solution. Besides aggregation also fragmen-

tation, partial unfolding, sulfhydryl loss, and inactivation could be established by SAXS and other techniques [4–8, 12]. Several substances, *viz.* the sulfhydryl reagent dithiothreitol (DTT), alcohol, the substrates acetyl-CoA and glyoxylate, and the substrate analogue pyruvate have been found to protect the enzyme during X-irradiation against aggregation and inactivation; DTT has also turned out to be able to repair enzymic activity.

In order to differentiate between the effects caused by OH·, O_2^-, and H_2O_2 (*cf.* [13, 14]) we investigated the radiation damage of malate synthase in the absence/presence of the specific scavengers formate, SOD, and catalase. The results from inactivation and repair experiments have already been described [15, 16]. It was found that the mentioned scavengers not only protect the enzyme against inactivation during X-irradiation, but also influence the post-irradiation inactivation as well as the reparability of enzymic activity by DTT.

The influence of formate, SOD, and catalase on the aggregation behaviour of X-irradiated malate synthase was investigated by SAXS. In order to allow statements on structure-function relationships of X-irradiated malate synthase, we parallelled the SAXS experiments with measurements of enzymic activity. The present paper reports on studies performed with malate synthase pre-irradiated in the absence/presence of the mentioned additives. For preliminary reports *cf.* [17, 18].

In conventional SAXS investigations on biopolymers, the occurrence of radiation damage of the samples during the SAXS experiments may be a hazard because it may lead to erroneous results. For investigations of very sensitive samples (*e.g.* sulfhydryl enzymes) special precautions against radiation damage may become necessary (*cf.* [4, 5, 8, 9, 24]), *e.g.* by addition of radioprotective substances. The question whether formate, SOD, and catalase might be used as radioprotectors in conventional SAXS experiments has stimulated the present study to some extent. In a subsequent paper (in preparation) this question will be discussed on the basis of additional experimental data in more detail.

Materials and Methods

Materials

Malate synthase (EC 4.1.3.2) was isolated from baker's yeast in electrophoretically pure form (*cf.*

[19]). Catalase (EC 1.11.1.6) from bovine liver and superoxide dismutase (SOD; EC 1.15.1.1) from bovine erythrocytes were obtained from Boehringer, Mannheim. All other reagents were of A-grade purity. Quartz-bidistilled water was used throughout.

Solutions

A 5 mM Tris/HCl buffer, pH 8.1, containing 10 mM $MgCl_2$, 1 mM MgK_2EDTA, and 0.2 mM DTT was used as a standard buffer. Malate synthase was dialyzed at 2 °C against this buffer. For experiments, carefully prepared stock solutions of malate synthase and additives (all pH 8.1) were mixed to give the following final concentrations: 50 μM (= 9.5 mg/ml) malate synthase, 0 or 0.4 μM SOD, 0 or 55 nM catalase, 0 or 10 or 100 mM formate. The final volume of the mixtures was 150 μl. A small aliquot (5 μl) of these solutions was diluted and used for enzymic tests, the main portion was applied to irradiation experiments.

X-Irradiation

Solutions were X-irradiated with the unfiltered radiation from a Philips PW 2253/11 X-ray tube (Cu, 50 kV, 30 mA) in the sealed microcell described previously [5, 12]. The volume of the cell was adjusted to 120 μl and the cell was thermostated to 4 °C. Using a dose rate of 290 Gy/min, as determined by means of a Fricke dosimeter, the samples were irradiated with 6 kGy. A small aliquot (10 μl) of the irradiated solutions was diluted and used for enzymic tests, the main portion of the irradiated solutions was investigated by SAXS.

Enzymic assay

The assay was performed at 20 °C as described previously [19, 20] by using a Zeiss PMQ II spectrophotometer. The irradiated solutions were tested shortly after stop of irradiation. Inactivation doses D_{37}^t for total inactivation were calculated from the ratio of activities of irradiated and unirradiated samples as in a previous paper [16].

Small-angle X-ray scattering

SAXS experiments were performed by means of a Kratky camera as in previous studies [5, 7]. The unfiltered radiation from a PW 2253/11 tube served as primary radiation. All samples were investigated at

4 °C in Mark capillaries of 1 mm diameter. The integral primary intensity of Cu K_α ($\lambda = 0.154$ nm) and K_β quanta amounted to 4.2×10^7 counts $s^{-1}cm^{-1}$. Scattering experiments were started shortly after finishing the pre-irradiation of the solutions. Each scattering curve was measured in the angular range $2.6 \leqslant 2\theta \leqslant 26$ mrad. The measurement of the most significant inner part of the scattering curves was completed in less than 2 hours; thus changes of the samples due to the primary radiation used in the SAXS experiments were negligible (*cf.* [5]). As is known from other SAXS experiments (unpublished) the presence of 10 or 100 mM formate or of catalytic amounts of SOD and/or catalase does not influence the scattering behaviour of unirradiated malate synthase to a significant extent.

The experimental data were evaluated on a UNI-VAC 1100/81 computer (*cf.* [21]). The method of indirect Fourier transformation (program ITP, [22]) was applied for the desmearing procedure and for the calculation of distance distribution functions $p(r)$. Since the maximum diameter of particles (aggregates) was a priori unknown, calculations with program ITP were performed in each case by using several different estimates for D_{max}. Only those solutions were accepted which satisfied the usual criteria for the optimum choice of the stabilization parameter (*cf.* [22]) and additionally led to $p(r)$ functions without oscillations or abrupt decays in the tail end. This procedure rendered possible a clear determination of the maximum diameter of aggregates in all but 4 cases (*cf.* Results). In those 4 exceptional cases

Table I. Aggregation, fragmentation, and inactivation of malate synthase ($c = 50$ μM) upon 6 kGy X-irradiation in the presence of specific additives.

Samples	Additives Formate [mM]	SOD [μM]	Catalase [nM]	$\overline{R}$ [a] [nm]	$\bar{x}$ [b]	Fragments [c] [%]	$\bar{x}_{max}$ [d]	D_{37}^t [e] [kGy]
1	–	–	–	12.5 / 13.3[f]	5.2 / 5.7[f]	18.9 / 19.4[f]	6.4 / 7.1[f]	4.4 ± 0.1
2	–	0.4	55	12.1 / 12.0[f]	3.8 / 3.7[f]	16.7 / 19.0[f]	4.6 / 4.6[f]	5.0 ± 0.2
3	–	0.4	–	10.0 / 9.6[f]	2.9 / 2.8[f]	12.4 / 16.0[f]	3.3 / 3.3[f]	5.0 ± 0.1
4	–	–	55	9.3 / 8.9[f]	2.4 / 2.3[f]	12.5 / 15.3[f]	2.7 / 2.7[f]	5.3 ± 0.1
5	10	–	–	9.1	1.6	11.1	1.8	6.3 ± 0.3
6	10	0.4	55	6.4	1.35	7.8	1.45	6.9 ± 0.3
7	10	0.4	–	5.2	1.2	6.5	1.3	7.1 ± 0.3
8	10	–	55	6.1	1.25	7.6	1.35	7.2 ± 0.2
9	100	–	–	6.5	1.2	6.3	1.3	9.7 ± 0.6
10	100	0.4	55	4.8	0.99	7.4	1.07	11.8 ± 0.7
11	100	0.4	–	4.7	1.0	4.2	1.04	11.0 ± 0.5
12	100	–	55	4.0	0.91	8.9	1.0	11.7 ± 1.0
13[g]	–	–	–	4.0	1.0			

[a] Mean radius of gyration, obtained directly by means of program ITP; accuracy about 5–10%.
[b] Mean degree of aggregation, obtained from the ratio of intensities at zero angle for the irradiated sample and the unirradiated reference (sample 13); accuracy about 5–10%.
[c] Relative amount of fragments (w/w), obtained from the $[I \times (2\theta)^2]_o$ values for the irradiated sample and the unirradiated reference (sample 13).
[d] Upper limit for the mean degree of aggregation, obtained from $\bar{x}$ by applying a correction for the presence of fragments.
[e] Inactivation dose for total (= repairable + non-repairable) inactivation, obtained from the ratio of activities before and after irradiation.
[f] Experimental scattering curve was extrapolated to smaller angles prior to the application of program ITP.
[g] Unirradiated reference.

D_{max} was assumed as 35 nm; in an alternative approach the experimental scattering curves were extrapolated from the innermost measuring points to smaller scattering angles according to Guinier's law, prior to the application of program ITP. The structural parameters determined from the desmeared scattering curves and from the $p(r)$ functions comprise mean values for the overall radius of gyration, $\bar{R}$, for the degree of aggregation, $\bar{x}$, for the radius of gyration of the cross-section, $\bar{R}_c$, and for the radius of gyration of the thickness, $\bar{R}_t$, (*cf.* [5]).

Results

X-irradiation of malate synthase was performed in the absence/presence of the specific additives formate and/or SOD and/or catalase. The composition of the mixtures used for the irradiation experiments is summarized in Table I (samples 1–12). Additionally an unirradiated solution without additives (sample 13) was used as a reference for the SAXS experiments.

Small-angle X-ray scattering

The experimental scattering curves of the 12 pre-irradiated samples and of the unirradiated reference are shown in a semi-logarithmic plot in Fig. 1 (the tail end region of the curves has been omitted for the benefit of a better presentation of the course at small angles). The scattering curve for the unirradiated reference (sample 13) obviously is of Gaussian type; the same is true for the scattering curves obtained for the samples containing 10 or 100 mM formate (samples 5–12). The scattering curves of the irradiated samples without formate (samples 1–4) show a quite different behaviour; the intensities in the innermost portion of the scattering curves are considerably higher than typical of a Gaussian course. This clearly reflects the occurrence of strong aggregation upon X-irradiation in the absence of formate.

The desmearing of the scattering curves was performed as described in Materials and Methods. It offered no problems, except with the scattering curves of the strongly aggregated samples 1–4. Obviously the problems with these samples arose from the presence of aggregates the size of which exceeded the limit of about 30 nm imposed by the SAXS experiment (this is clear from the sampling theorem, *cf.* [23]).
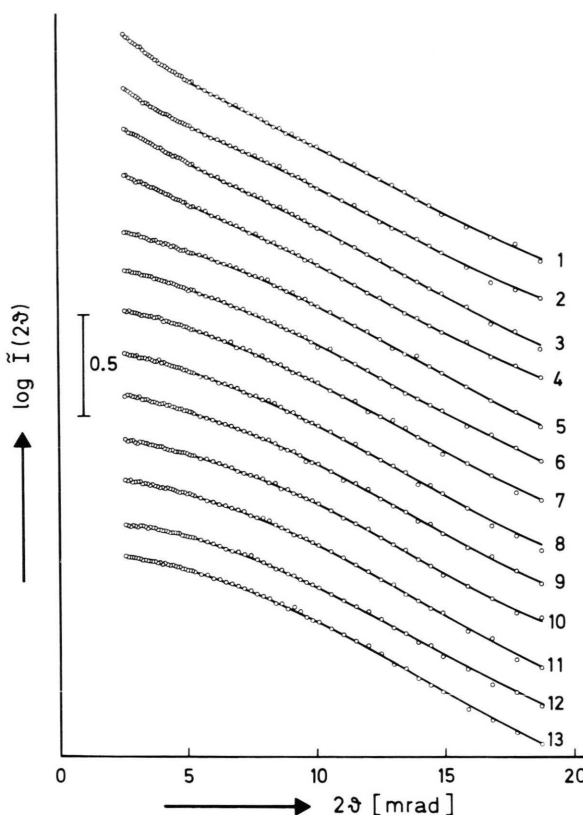

Fig. 1. Plots of log $\tilde{I}(2\theta)$ *vs.* 2θ of the experimental slit-smeared scattering curves of samples 1–13 (composition *cf.* Table I). The symbols show the raw data, the solid lines represent the approximation calculated by program ITP. For convenience, the curves are shifted by multiples of 0.2 logarithmic units on the ordinate.

Guinier plots of the inner portions of the desmeared scattering curves are shown in Fig. 2. The Fourier transforms of the scattering curves, the distance distribution functions $p(r)$, are presented in Fig. 3. In the case of samples 1–4, the distance distribution functions which were obtained from extrapolated experimental scattering curves are shown by dashed lines.

According to the theory of SAXS, the areas under the $p(r)$ functions are proportional to the mean molecular weights of the particles, and the distances at which the functions approach zero represent the maximum extension of the particles. The initial slopes of the curves shown in Fig. 2 are also related to the size of the particles. Therefore, both Fig. 2 and Fig. 3 convincingly demonstrate the X-ray induced
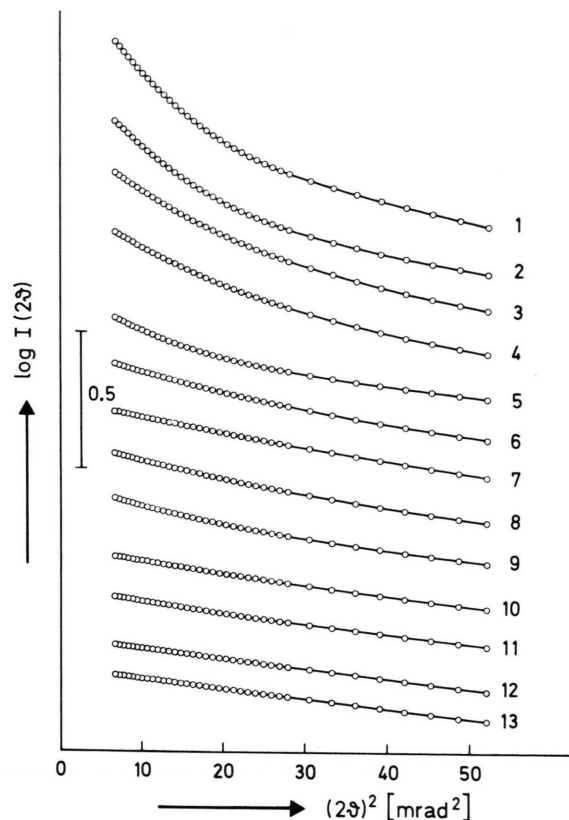
Fig. 2. Guinier plots of the desmeared scattering curves of samples 1−13. The curves are shifted by multiples of 0.15 logarithmic units on the ordinate.
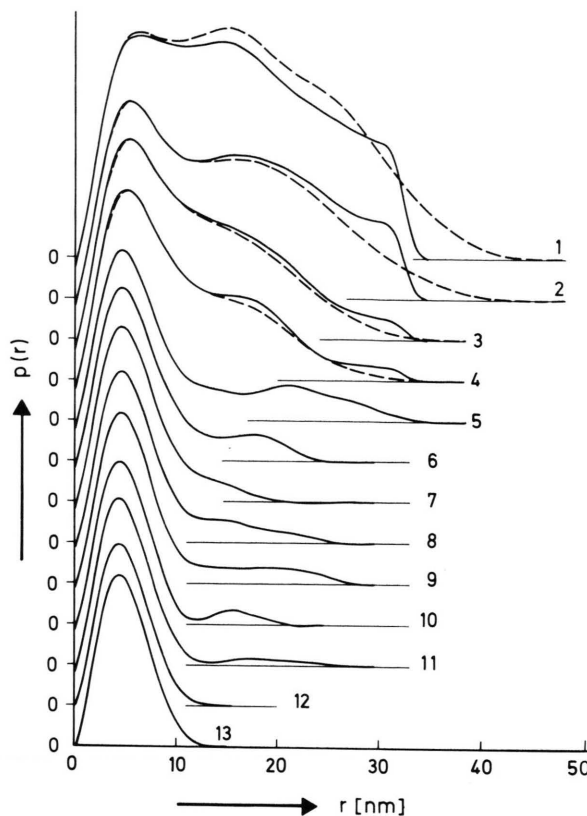

Fig. 3. Distance distribution functions $p(r)$ of samples 1−13. For convenience, the curves are shifted on the ordinate. In the case of samples 1−4, curves shown by solid lines were calculated on the assumption of $D_{max} = 35$ nm; curves shown by dashed lines were obtained after extrapolation of the inner part of the scattering curves towards smaller scattering angles (*cf.* text).

aggregation of malate synthase as a function of additives. The results are quantified in terms of the radii of gyration, $\overline{R}$, and degrees of aggregation, $\bar{x}$, listed in Table I.

As follows evidently from a comparison of the two distance distribution functions calculated for sample 1, the $p(r)$ function obtained from the extrapolated scattering curve corresponds to higher values for $\overline{R}$ and $\bar{x}$ than the $p(r)$ function derived from the original scattering curve (*cf.* Table I). It seems reasonable to give preference to the higher values. Contrary to sample 1, the extrapolation of the experimental scattering curves in the case of samples 2−4 reduced both the radii of gyration and the degrees of aggregation slightly (*cf.* Table I). Apart from these differences, it is obvious that the principal features of the

$p(r)$ functions of samples 1−4 at distances of $r < 30$ nm do not depend much on the way of calculation. This finding supports the reliability of the data derived from these $p(r)$ functions. The accuracy of the $\overline{R}$ and $\bar{x}$ values of samples 1−4 may be estimated to be about 5−10%. For samples 5−12, the accuracy of $\bar{x}$ values is about 5%, while the $\overline{R}$ values are certainly less accurate because they depend to a higher degree on the tails of the $p(r)$ functions than the $\bar{x}$ values.

The comparison of the $p(r)$ functions in Fig. 3 and of the $\overline{R}$ and $\bar{x}$ values in Table I clearly shows that formate is the predominant factor influencing the extent of X-ray induced aggregation of malate synthase. But evidently also SOD and/or catalase influence the aggregation behaviour, however, to a minor extent.
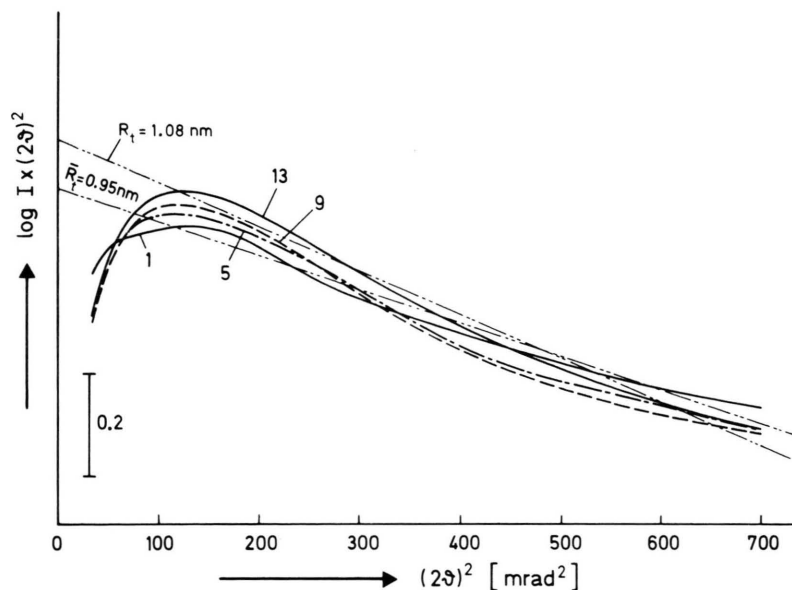
Fig. 4. Thickness Guinier plots of selected scattering curves (samples 1, 5, 9, 13). The drawn straight lines show the Gaussian approximation for the thickness factors of sample 1 ($R_t = 0.95$ nm) and sample 13 ($R_t = 1.08$ nm), representing the two limiting cases.

Since native malate synthase is of oblate shape, a Guinier plot of the thickness factor allows the determination of a radius of gyration of the thickness (*cf.* [24]). Fig. 4 shows a comparison of Guinier plots of the thickness factors for selected irradiated samples (samples 1, 5, 9) and for the unirradiated reference (sample 13). The radii of gyration of the thickness which are derived from the slopes of the drawn straight lines differ only slightly; the thickness radii of gyration of samples 2–12 fall between the two limiting cases of 0.95 and 1.08 nm (samples 1 and 13). This indicates the retention of the particle thickness despite aggregation.

Cross-section Guinier plots of the scattering curves for samples 1–13 are shown in Fig. 5. The curves for the samples containing formate (samples 5–12) are similar to the curve obtained for the unirradiated reference (sample 13). They correspond to a cross-sectional radius of gyration of about 2.4 nm. The samples irradiated in the absence of formate (samples 1–4) show different cross-section plots. Especially the curves for samples 1 and 2 exhibit a pronounced increase of intensity towards zero angle; the limiting case, sample 1, delivers two cross-sectional radii of gyration of different size (5.6 and 2.3 nm). The behaviour observed for samples 1 and 2 clearly reflects an increase of the lateral dimension during aggregation.
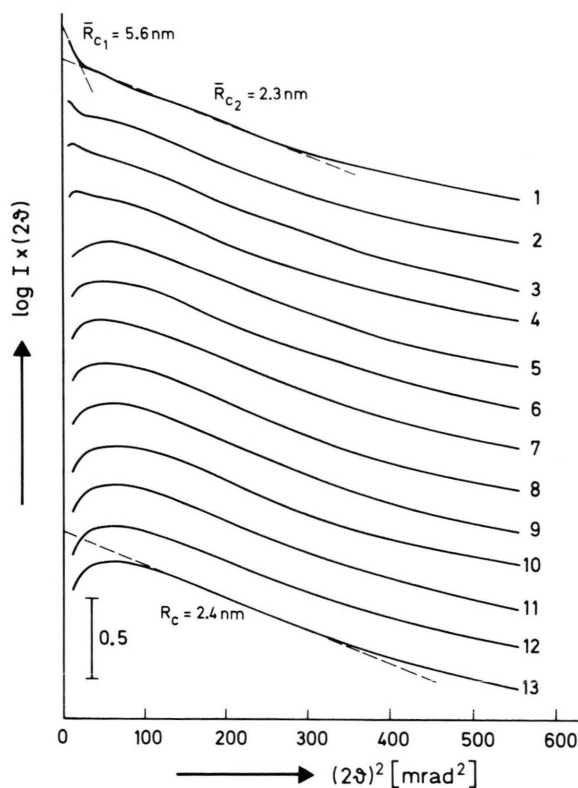

Fig. 5. Cross-section Guinier plots of the scattering curves of samples 1–13. The curves are shifted by multiples of 0.25 logarithmic units on the ordinate.

Enzymic tests

The ratios of enzymic activities of samples 1–12, measured before and shortly after X-irradiation, yielded the inactivation doses D_{37}^{i} outlined in Table I. An examination of these values clearly demonstrates the strong influence of formate on the X-ray induced inactivation. The effects caused by SOD and/or catalase are less pronounced.

Discussion and Conclusions

Aggregation in the absence of additives

The results found for the sample of malate synthase irradiated in the absence of additives (sample 1) are in good accord with previous findings [5]. The enzyme obviously aggregates as a consequence of X-irradiation. The observed increase of the overall radius of gyration, the finding of two different cross-sections, and the observed retention of the particle thickness corroborate the previously established two-dimensional model for the early steps of aggregation (*cf.* [6]).

It can be taken for granted that the maximum size of aggregates in sample 1 is greater than 35 nm. This follows from the abrupt decay of the $p(r)$ function at distances slightly smaller than the assumed D_{max} of 35 nm (*cf.* Fig. 3: sample 1, solid line). The $p(r)$ function derived from the extrapolated scattering curve suggests the maximum size of aggregates to be about 45 nm (*cf.* Fig. 3: sample 1, dashed line). Of course, this value can only be an estimation. A satisfying improvement might be achieved in future experiments by extending the measurements down to smaller scattering angles in order to increase the information content of the experimental scattering curves with regard to large aggregates.

The occurrence of a second peak in the $p(r)$ functions for sample 1 around $r \simeq 15$ nm (*cf.* Fig. 3: sample 1) can be related to the two-dimensional side-by-side association of enzyme particles; the same explanation may hold for the additional ripple around $r \simeq 25$ nm in the $p(r)$ function shown by the dashed line (*cf.* also [6]).

Both the thickness radius of gyration for sample 1 and the corresponding limiting value of $[I \times (2\theta)^2]_0$ of the thickness factor at zero angle, obtained by extrapolation, are smaller than the values derived for the unirradiated sample 13 (*cf.* Fig. 4). Both observations indicate that some fragmentation must have

taken place. Based on the ratio of the $[I \times (2\theta)^2]_0$ values for samples 1 and 13, the relative amount of strongly fragmented material may be estimated as about 19% (*cf.* Table I). This result, which rests on the assumption that the fragments do not contribute to the $[I \times (2\theta)^2]_0$ value significantly, implies that actually the mean degree of aggregation might be higher than the value found for $\bar{x}$. An estimation of the upper limit for the mean degree of aggregation can be made quite easily by assuming that the fragments which were split off from the enzyme particles do not contribute significantly to $\bar{x}$. In this way the upper limit is obtained as $\bar{x}_{max} = 7.1$ (*cf.* Table I). It should be noted that the mean degree of aggregation would be only slightly smaller than $\bar{x}_{max}$ (*viz.* by about 5%) if the mean molecular weights of the fragments amounted to 20% of the molecular weight of the native enzyme particle ($M_r = 185\,000$; *cf.* [24, 25]). The X-ray induced formation of considerable amounts of fragments of the enzyme subunit, with definite molecular weights from 14\,000 to 54\,000, has been proven by polyacrylamide gel disc electrophoreses in the presence of sodium dodecyl sulfate [12].

Aggregation in the presence of additives

As has been convincingly demonstrated above (*cf.* Fig. 3 and Table I), all used additives impede the X-ray induced aggregation of malate synthase, but to a quite different extent.

The additives also influence the extent of fragmentation as may be concluded from an analysis of the $[I \times (2\theta)^2]_0$ values, performed as outlined above for sample 1. The estimation of relative amounts of fragments (*cf.* Table I) yields 15 ± 3% in the absence of formate, and 8 ± 2% or 7 ± 2% in the presence of 10 or 100 mM formate. The corresponding $\bar{x}_{max}$ values for samples 2–12 are also listed in Table I.

An analysis of the $\bar{x}_{max}$ values shows that catalytic amounts of the additives SOD and/or catalase reduce the extent of aggregation by about 35 to 62% when no formate is present (*cf.* samples 2–4 with sample 1). The presence of the OH· scavenger formate, in the absence of other additives, reduces the extent of aggregation by about 75 to 82%, depending on the concentration of formate (10 or 100 mM; cf. samples 5 and 9 with sample 1). An additional diminution of aggregation in the presence of formate by about 18–30% is provided by the simultaneous presence of catalytic amounts of SOD and/or catalase (*cf.* sam-

ples 5−8 and 9−12). When 100 mM formate and the additive catalase are simultaneously present the aggregation is suppressed completely, while fragmentation still occurs (*cf.* sample 12 with sample 13; *cf.* also the $p(r)$ functions in Fig. 3). The data also show (*cf.* Table I and Fig. 3) that the combination of SOD and catalase does not improve the protection against aggregation, as compared to the single effects of these additives.

The observed inhibition of aggregation by formate, SOD, and catalase gives clear evidence that primarily $OH^{\cdot}$ radicals, but also $O_2^{\bar{\cdot}}$ and H_2O_2, are involved in the X-ray induced aggregation process of malate synthase.

Inactivation in the absence or presence of additives

An inspection of the D_{37}^t values given in Table I clearly reveals the protective effects of the additives formate, SOD, and catalase against the X-ray induced inactivation of malate synthase. Again the effectiveness of formate is predominant. A previous study [16], which used concentrations of malate synthase lower by a factor of about 20, led to similar conclusions. The D_{37}^t values obtained now are all higher than those found in the previous study, in accord with the indirect effect of radiation. A quantitative comparison of the D_{37}^t values in the present and in the earlier study yields a mean value for dD_{37}^t/dc of 0.51 ± 0.06 kGy ml mg^{-1}. This value is in

agreement with previous results of 0.53 kGy ml mg^{-1} [8].

Correlation between aggregation and inactivation

Our experiments have established that each of the additives formate, SOD, and catalase is effective as a radioprotector, in a similar manner against aggregation and inactivation of malate synthase. This finding is in accord with the well-known action of the mentioned additives as scavengers for $OH^{\cdot}$, $O_2^{\bar{\cdot}}$, and H_2O_2. Our data, however, do not give indications for a radioprotection due to a specific interaction between these additives and malate synthase. This can be shown *e.g.* in a plot of D_{37}^t *vs.* $\bar{x}$ (Fig. 6). This plot demonstrates a high degree of correlation between the inactivation dose and the degree of aggregation.

On the other side, a radioprotection due to a specific interaction between additive and malate synthase has been inferred in an earlier study from the different effectiveness of the substrate glyoxylate and its analogue pyruvate against aggregation and inactivation [5]. An involvement of enzyme sulfhydryls both in X-ray induced aggregation and inactivation has been demonstrated by electrophoretic and chemical studies after blocking the sulfhydryls [12].

Some implications for small-angle X-ray scattering

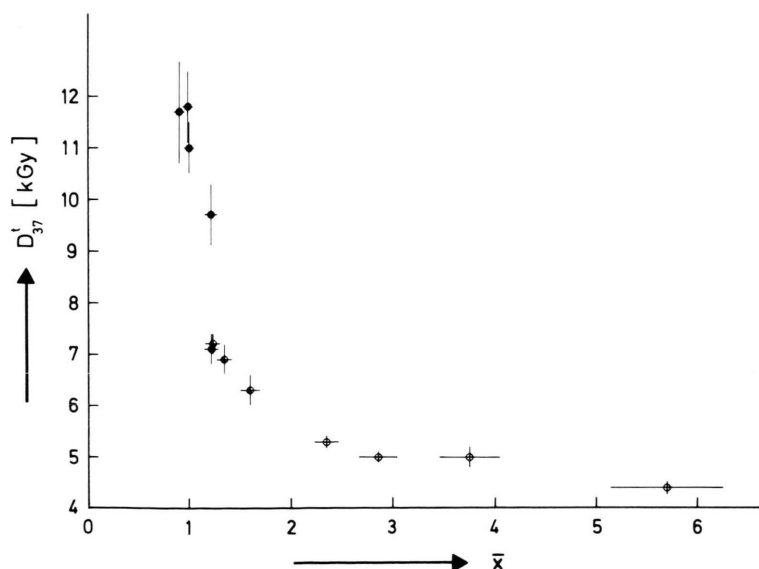It has been shown by our experiments that the presence of formate, SOD, and catalase may dimin-

Fig. 6. Plot of inactivation dose, D_{37}^t, *vs.* mean degree of aggregation, $\bar{x}$, of samples 1−12 (*cf.* Table I). The concentrations of formate (0, 10, 100 mM) are indicated by the symbols (empty, half-full, full).

ish the radiation damage of malate synthase to a high degree. It is plausible that this result may also hold for other biopolymers. This suggests a suitable way to avoid radiation damages in conventional SAXS experiments on biopolymers. The presence of formate (*e.g.* 10 mM) and/or of SOD or catalase (catalytic amounts, *e.g.* 0.01 mg/ml) may suppress unwanted radiation effects sufficiently (*cf.* [17]).

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