EFFECT OF UV IRRADIATION ON THE CATALYTIC AND ALLOSTERIC FUNCTIONS OF FRUCTOSE 1,6-DIPHOSPHATASE

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1. Introduction

A considerable amount of work has been carried out to elucidate the effect of UV irradiation on the catalytic activity of enzymes [1–4]. However, so far no studies seem to have been published on the effect of UV light on the regulatory functions of allosteric enzymes. The purpose of the present communication is to report on the effect of UV irradiation on the regulatory and catalytic functions of fructose 1,6-diphosphatase (E.C. 3.1.3.11).

Fructose diphosphatase is inhibited by the allosteric modifier AMP [5,6], and shows a complex response to modification of its sulfhydryl groups [7–9]. The enzyme contains approximately 20 SH groups, 5–6 of which possess a very high reactivity. Blocking of the highly reactive SH groups by mixed disulfide formation causes an increase in the catalytic activity by more than 400%, while blocking of the slow reacting SH groups causes inhibition. Studies of the effects of X-irradiation on the enzyme have shown [10] that its sensitivity to AMP inhibition is destroyed at approximately the same rate as the catalytic activity. On the other hand the ability of the enzyme to be stimulated by mixed disulfide formation is more than 10 times as sensitive to X-ray destruction as the catalytic function.

The results obtained by UV irradiation of fructose diphosphatase differ from those obtained by X-irradiation in several respects. Thus, UV irradiation does not affect the sensitivity of the native enzyme to the allosteric modifier AMP. Furthermore, the ability of the enzyme to be stimulated by mixed disulfide formation appears to be less sensitive than the catalytic activity. The most interesting finding is, however, that the presence of substrate during the UV irradiation decreased strongly the loss of the catalytic activity.

2. Materials and methods

Fructose-1,6-diphosphate, AMP, NADP, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) and glucose 6-phosphate isomerase were obtained from Sigma Chemical Co. (St. Louis, Mo.), and glucose 6-phosphate dehydrogenase from Boehringer & Soehne (Mannheim, Germany). Fructose 1,6-diphosphatase was prepared from rabbit liver, as described by Pontremoli et al. [11], with small modifications [9].

The enzyme activity was measured according to the method of Pontremoli et al. [11]. The assay mixture contained: 0.2 mM fructose diphosphate, 0.1 M glycine buffer, pH 9.4, 1 mM $MgCl_2$, together with a large excess of glucose 6-phosphate isomerase, glucose 6-phosphate dehydrogenase and NADP. The measurements were carried out at room temperature in a volume of 1 ml. The rate of NADP reduction was measured spectrophotometrically at 340 mμ. The native enzyme had a specific activity [9,11] of at least 120 units/mg. The ability of the enzyme to be stimulated by mixed disulfide formation was assayed by incubating the enzyme in 10 mM Tris buffer, pH 7.5, with a 500-fold molar excess of DTNB for 30 min, and comparing the

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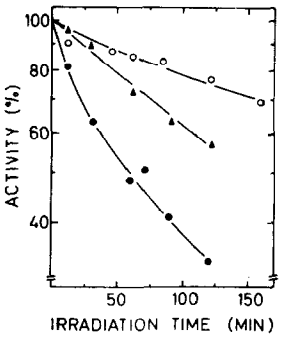

Fig. 1. Inactivation of fructose diphosphatase by UV irradiation. The enzyme was irradiated with UV light of 278 mμ. The ability of the enzyme to be stimulated by mixed disulfide formation was determined as described in Materials and Methods. All data are expressed in per cent of the activity of the unirradiated control. ●—● catalytic activity of enzyme irradiated in the absence of substrate; ○—○ catalytic activity of enzyme irradiated in the presence of 1 mM fructose diphosphate; ▲—▲ ability of the enzyme to be stimulated by mixed disulfide formation.

activity with that obtained in the absence of DTNB treatment. Protein concentration was determined by the method of Lowry et al. [12]; a molecular weight of 127,000 was used [7].

The enzyme was irradiated at a concentration of 0.2 mg protein/ml in 2.5 mM malonate buffer, pH 6.8, in a short flat bottomed tube. The radiation source was a Bausch & Lomb super Pressure Mercury lamp P-200 (HBO-200 W). The desired wavelength was obtained by using a monochromator (Grating 2,700 grooves/mm). The radiation was carried out at 278 mμ, which is an absorption maximum of the enzyme. The temperature was held at 0°C during the irradiation and the solution was gently stirred by blowing nitrogen onto the surface of the solution.

3. Results and discussion

The effect of UV irradiation on the catalytic function of fructose 1,6-diphosphatase is shown in fig. 1. Nearly linear dose response curves were obtained in a semilogarithmic plot. This is in contrast to the results obtained with X-irradiation where the enzyme showed a small, but definite stimulation by small doses of radiation [10]. The possible reason for this difference will be discussed below.

Irradiation of the enzyme in the presence of its substrate, fructose diphosphate, resulted in a considerable protection. Thus, in the presence of 1 mM fructose diphosphate a dose reduction factor of approximately 5 was obtained. This finding stands in strong contrast to most results obtained with UV irradiation of enzymes, where it has been found that the presence of substrate affords little or no protection of the catalytic function [1]. The protection observed cannot be explained simply by an altered absorption of energy by the enzyme-substrate complex since addition of fructose diphosphate did not significantly alter the absorbancy measured at 278 mμ. Probably, the protective effect of substrate may be related to induced changes in the structure of fructose diphosphatase [9].

It has been suggested [8] that the stimulation of the enzyme by mixed disulfide formation plays an important role in the regulation of the enzyme activity *in vivo*. It was therefore of interest to study the effect of UV irradiation on the sensitivity of the enzyme to stimulation by disulfides. It is apparent (fig. 1) that the ability of the enzyme to be stimulated by mixed disulfide formation is approximately two times less sensitive to UV light as is the catalytic activity. In contrast, in the case of X-ray inactivation, the sensitivity to disulfide stimulation was more than 10 times as sensitive as was the catalytic activity [10]. This difference in the response after X- and UV-irradiation may well give a clue to the understanding of the differences in the dose response curve for the catalytic activities mentioned above. When the enzyme is exposed to X-irradiation the destruction of the highly reactive sulfhydryl groups is approximately 20 times that of the less reactive sulfhydryl groups [10]. This selective destruction of the highly reactive sulfhydryl groups leads to a stimulation of the catalytic activity. When the enzyme was exposed to UV irradiation the ability of the enzyme to be stimulated was less sensitive than the catalytic activity. This indicates that the chemically most reactive sulfhydryl groups are not preferentially destroyed by UV, and that UV, in contrast to X-rays, oxidizes indiscriminately all types of SH groups in the enzyme. This view is further supported by SH titration of the UV-irradiated enzymes.

Studies of the effect of UV irradiation on enzyme samples that had been fully stimulated by blocking

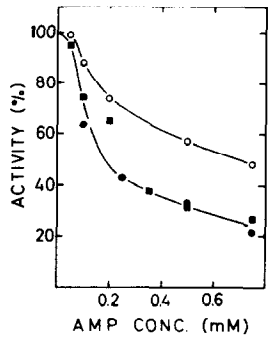

Fig. 2. Effect of UV irradiation on the inhibition of fructose diphosphatase by AMP. The effect of increasing concentration of AMP during the enzyme assay was measured after irradiation of the enzyme for 120 min in the absence and presence of substrate. All data are expressed in per cent of the activity found in the absence of AMP. ▪—▪ unirradiated enzyme; •—• irradiated enzyme; o—o enzyme irradiated in the presence of 1 mM fructose diphosphate.

of 5-6 SH groups by DTNB (DTNB-enzyme) indicate that the stimulated enzyme possesses approximately the same sensitivity to UV destruction as does the native enzyme. On the other hand, while addition of 1 mM fructose diphosphate to the native enzyme resulted in a dose reduction factor of approximately 5 it reduced the sensitivity of the DTNB enzyme only by a factor of approximately 2.

The effect of UV irradiation on the response of fructose diphosphatase to inhibition by the allosteric affector AMP is shown in fig. 2. No significant changes in the AMP inhibition curve was found in the UV-irradiated enzymes even though the catalytic activity was reduced by more than 70%. Similar results were found for the DTNB-stimulated enzyme (not shown). These results are in contrast to those obtained with X-rays where the inhibition by AMP was affected to about the same extent as the catalytic activity [10]. Surprisingly, it was found (fig. 2) that when the enzyme was irradiated in the presence of 1 mM fructose diphosphate the sensitivity to AMP was considerably reduced. Thus, the presence of substrate during irradiation protects the catalytic activity whereas the allosteric activity, as measured by loss of AMP inhibition, is sensitized. The possibility should be considered that the effects of substrate on the behaviour of the enzyme to UV irradiation is associated with structural changes induced in the enzyme by the substrate [9].

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