STUDIES ON THE ACTIVE CENTER OF PANCREATIC AMYLASE

II. SMALL ANGLE X-RAY SCATTERING INVESTIGATIONS*

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Summary

1. The radius of gyration of α-amylase is 26.9 Å, as found by means of small angle X-ray scattering. This value decreases by 0.5 Å if amylase binds three moles of β-cyclodextrin per mole enzyme.

2. In case of partial saturation, the solution contains only saturated amylase-cyclodextrin complexes (amylase/cyclodextrin molar ratio 1:3) and amylase molecules free of cyclodextrin. The binding of β-cyclodextrin follows the all-or-none mechanism.

3. The specifically bound β-cyclodextrin molecules are accommodated in a trough of the amylase molecule. The plane of bound cyclodextrin is perpendicular to the longitudinal axis of the trough. It is suggested that the helical substrate, amylose, binds in this trough, too.

Experimental

Materials

Porcine pancreatic amylase was isolated and recrystallized three times according to HATFALUDI et al.2 All the experiments were conducted at 25°C in 10 mM Tris buffer, pH 7.5, containing 1.0 mM CaCl₂. Protein concentration varied between 150 and 400 μM.

Methods

The saturation of amylase was determined by preparative ultracentrifugation as described earlier1. Small angle X-ray scattering measurements were carried out with a Dron-I type (USSR) X-ray analytical instrument combined with a Shimadzu (Japan) small angle chamber. CuKα (λ = 1.54 Å) X-rays were used, the Kβ rays were removed by nickel filter and the
Fig. 1. Determination of the radius of gyration of amylase according to Guinier’s representation, at 400, 300 and 200 μM protein concentrations. Extrapolation to 0 concentration was carried out as seen in the insert.

The lengths of the arrows are proportional to the concentrations. The straight line connecting the points of arrows intersects at zero concentration with the straight line that connects the other end of the arrows.

continuous radiation was eliminated by a difference discriminator.

The radius of gyration of the amylase molecule was determined from the distribution of intensities extrapolated to zero concentration, from the so-called Guinier plot. Since the intensity of the scattered beam is approximately proportional to the concentration of dispersive solutions, to extrapolate to zero concentration the intensity distributions were divided by the intensity measured at 0.01 radian and extrapolated graphically as shown in fig. 1.

**Difference Method**

The changes in the radius of gyration caused by β-cyclodextrin binding were determined by our difference method described earlier. This method allows one to determine the change in the radius of gyration, ΔR₀, due to β-cyclodextrin binding, since the plot: logarithm of the ratio of intensity functions, obtained with amylase saturated with β-cyclodextrin and amylase free of cyclodextrin, vs. the square of the angle of scattering results in a straight line with a slope proportional to ΔR₀, the change in the radius of gyration.

Accordingly

\[ ΔR₀ = K \frac{m}{R₀} \]  

where in the case of CuKα beam K = −0.208 Å², R₀ is the radius of gyration of amylase, and m is the slope of the straight line.

The difference method practically eliminates the collimation error due to finite slit width and the interparticular error caused by finite concentrations, if the parameters of the instrument (slit width, slit distance etc.) and the protein concentration are kept constant during the measurements. At slit widths generally employed the error of Guinier plot is determined by the statistical fluctuation of impulses. If the slit width is increased, the number of impulses also increases, and thus the statistical error can be reduced. However, then a new source of error appears. By increasing the dimension of the slit, the collimation error due to finite slit values in enhanced. The Guinier plot more and more deviates from the straight line, in fact, to such an extent that the error of the radius of gyration cannot be diminished widening the slit. The situation is different in the case of difference method. This rendered it possible to work at large slit widths and high concentrations. The entrance slit was 60 μm, the detector slit was 500 μm (about 3 minutes) and the protein concentration was 400 μM (about 20 mg/ml). In this way a reasonable scattered intensity could be detected in the 20 minutes to 60 minutes interval. The large number of impulses is needed in order to sufficiently reduce the statistical error of points, σ, shown in the difference plot:

\[ σ = \frac{\sqrt{I₁} + \sqrt{I₀}}{I₁ - I₀} \]

Naturally, the error of the slope of difference plot is appreciably smaller than the error of individual points in the difference plot. The relative error of the