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Circular Dichroism Changes in Galactosyltransferase upon Substrate Binding†

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ABSTRACT: Circular dichroism studies with the galactosyltransferase isolated from bovine skim milk are described. Addition of UDP-galactose to the galactosyltransferase-Mn^{2+} complex causes a decrease in the negative mean residue ellipticity in the 205–220-nm range and positive increases in the 265- and 275–290-nm ellipticity. These data are consistent with the view that a conformation change involving aromatic amino acid residues occurs upon the binding of UDP-galactose to the galactosyltransferase-Mn^{2+} complex. No effects in the near-ultraviolet circular dichroism spectrum were observed upon the addition of UDP or glucose to the galactosyltransferase-Mn^{2+} complex.

Bovine skim milk galactosyltransferase (UDPgalactose: D-glucose 1-galactosyltransferase, EC 2.4.1.22) catalyzes the transfer of galactose from UDP-galactose to form β-1,4 linkages with either glucose (Fitzgerald et al., 1970a) or *N*-acetylglucosamine (Brew et al., 1968). α-Lactalbumin inhibits the reaction with *N*-acetylglucosamine but is required to obtain significant rates with glucose (Fitzgerald et al., 1970a). Magee and Ebner (1974) have shown that UDP-galactose and other uridine nucleotides give protection of a critical sulfhydryl residue in galactosyltransferase when Mn^{2+} is present. These same compounds also protect to varying degrees against inactivation of galactosyltransferase by trypsin (Magee and Ebner, 1974). These data indicated the possibility of a conformational change in the galactosyltransferase-Mn^{2+} complex upon addition of UDP-galactose.

Circular dichroism measurements have been used (D'Souza and Freisheim, 1972; Teichberg et al., 1970; Fretto and Strickland, 1971; Omenn et al., 1969) to investigate conformational changes in enzymes upon addition of substrates and/or inhibitors. Circular dichroism measurements were made on galactosyltransferase in the presence and absence of the substrates, UDP-galactose and glucose, and the product, UDP. The results showed that there was a change in the circular dichroism spectra only upon the addition of UDP-galactose.

Experimental Procedure

Tris, 2-mercaptoethanol, uridine diphosphate, and glucose were from Sigma; $MnCl_2$ and KCl were from J. T. Baker; UDP-galactose was from Calbiochem; and dioxane was from Fisher.

Galactosyltransferase was isolated from bovine milk by the procedure described by Magee et al. (1973). The specific activity of the enzyme as assayed by the method of Fitzgerald et al. (1970b) was 5.1 units/mg of protein. Protein was estimated by absorbance at 280 nm assuming an extinction coefficient of 1.0 for 1 mg ml^{-1} cm^{-1}.

Circular dichroism spectra were measured at 24° in a Cary Model 61 spectropolarimeter; 2-cm path-length cuvets were used in obtaining near-ultraviolet (250–310 nm) circular dichroism spectra while 1-mm cuvets were used for far-ultraviolet (200–250 nm) spectra. The buffer for all determinations was 10 m*M* Tris–100 m*M* KCl–0.5 m*M* 2-mercaptoethanol adjusted to pH 7.5. The mean residue weight of galactosyltransferase, 113, was calculated from the amino acid analysis of Trayer and Hill (1971). All spectra of galactosyltransferase represent the difference between solutions of enzyme, buffer, and added substrates and solutions of buffer and added substrates.

Results

No change is apparent in the far-ultraviolet circular di-

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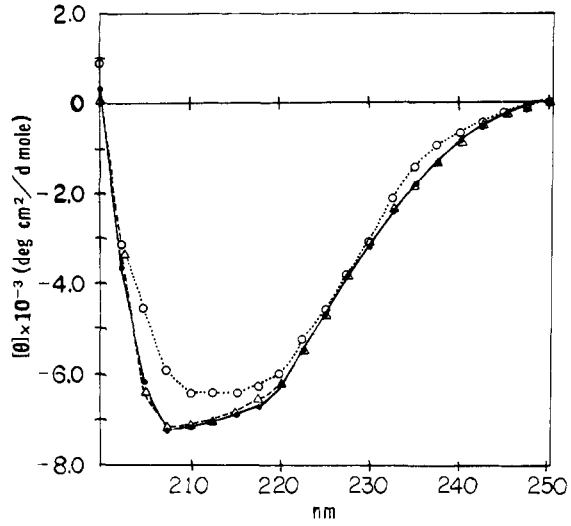

FIGURE 1: Circular dichroism of galactosyltransferase. Far circular dichroism spectra were obtained of enzyme (●——●); enzyme and 2 mM $MnCl_2$ (△---△); and enzyme, 2 mM $MnCl_2$, and 100 μM UDP-galactose (○···○). Galactosyltransferase concentration was 172 μg/ml.

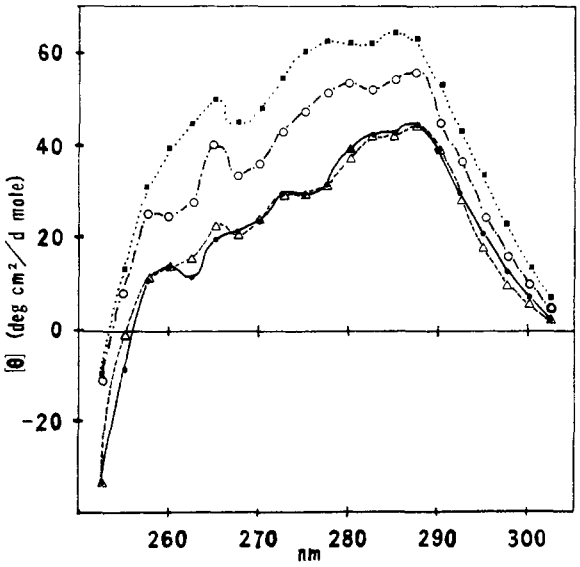

FIGURE 2: Near circular dichroism of galactosyltransferase. Spectra were obtained of enzyme (△---△); enzyme and 2 mM $MnCl_2$ (●——●); enzyme, 2 mM $MnCl_2$, and 25 μM UDP-galactose (○···○); and enzyme, 2 mM $MnCl_2$, and 50 μM UDP-galactose (■···■). Galactosyltransferase concentration was 858 μg/ml.

chroism spectrum of galactosyltransferase when $MnCl_2$ alone is added to the enzyme, but addition of 100 μM UDP-galactose to the enzyme-Mn^{2+} complex causes a decrease in the negative 200–220-nm ellipticity (Figure 1).

$MnCl_2$ also has no effect on the near-ultraviolet circular dichroism spectrum of galactosyltransferase, but again addition of 50 μM UDP-galactose to the enzyme-Mn^{2+} complex causes an increase in positive ellipticity in the 250–290-nm range as is shown in Figure 2. Figure 3 compares increases in observed ellipticity as a function of UDP-galactose concentration at 265 and 287.5 nm. These wavelengths were chosen as they represent wavelengths of maximum change upon addition of UDP-galactose. Double reciprocal plots of changes in ellipticity vs. UDP-galactose concentration (Figure 3) are linear at these two wavelengths. Addition of $MnCl_2$ to UDP-galactose causes no change in its circular dichroism spectrum. The observed positive ellipticity

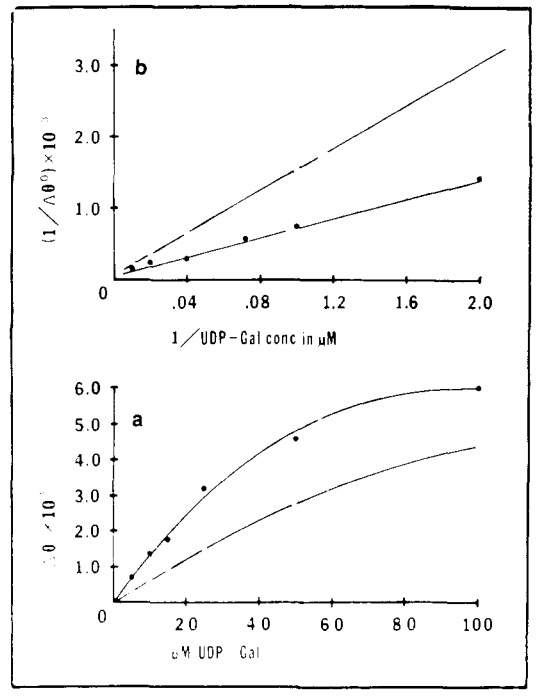

FIGURE 3: (a) Changes in observed ellipticity of galactosyltransferase-Mn^{2+} at 265 (●) and 287.5 nm (○) as a function of UDP-galactose concentration. (b) Double reciprocal plots of changes in observed ellipticity at 265 (●) and 287.5 nm (○) as a function of UDP-galactose concentration. Galactosyltransferase concentration was 858 μg/ml.

of UDP-galactose decreases slightly in the presence of 40% dioxane. Addition of 50 μM UDP or 100 mM glucose to galactosyltransferase-Mn^{2+} complex has no effect on its near-ultraviolet circular dichroism spectrum.

Discussion

The far-ultaviolet circular dichroism spectra of galactosyltransferase obtained in this study are similar to those reported by Klee and Klee (1972) but the changes observed by these authors upon the addition of $MnCl_2$ were not found. The present studies were done with at least a threefold higher concentration of galactosyltransferase and the enzyme was of higher specific activity. The far-ultraviolet circular dichroism spectrum of galactosyltransferase indicates about 10% α-helical content when calculated by the equation of Chen and Yang (1971) which is consistent with its relatively high proline content. The small change in ellipticity observed in the 200–220-nm region upon the addition of UDP-galactose was reproducible in a number of experiments using different enzyme preparations, but its origin is unclear.

The increase in mean residue ellipticity of the galactosyltransferase-Mn^{2+} complex in the near-ultraviolet circular dichroism spectrum upon the addition of UDP-galactose appears to be due to the binding of the UDP-galactose. The reciprocal plots (Figure 3b) indicate that the observed change is a saturable phenomena; but, unfortunately, concentrations of UDP-galactose above 100 μM could not be utilized to show complete saturation since UDP-galactose absorbs appreciably in the ultraviolet region. The possibility that the changes in the near-ultraviolet circular dichroism spectrum might arise from UDP-galactose are not likely since the locations of the major observed increases at 265 and 287.5 nm are different from UDP-galactose which shows a single positive band at approximately 270 nm. In

addition, the possibility that the observed change was due to UDP-galactose being positioned in a more hydrophobic environment was investigated by examining the circular dichroism spectrum of UDP-galactose in 40% dioxane, but the ellipticity decreased rather than increased and no significant shift occurred arguing against such an interpretation. The region of the circular dichroism spectrum (265, 287.5 nm) involved in the observed changes are areas generally considered to be attributed to aromatic amino acid residues (Adler et al., 1973). These data indicate the possibility that a conformational change involving aromatic amino acid residues occurs in galactosyltransferase-Mn^{2+} complex upon binding of UDP-galactose and are consistent with observations where Mn-UDP-galactose protects against sulfhydryl inactivation and susceptibility to trypsin (Magee and Ebner, 1974). The lack of change in the circular dichroism spectra when UDP was added to the enzyme-Mn^{2+} complex indicates that the carbohydrate moiety of the nucleotide is apparently necessary to elicit the change which is detectable by circular dichroism even though both UDP-galactose and UDP react kinetically with the same form of the enzyme (Morrison and Ebner, 1971).

The lack of change in the circular dichroism spectrum of the enzyme-Mn^{2+} complex upon the addition of 100 m*M* glucose indicates no observable conformation change even though galactosyltransferase-Mn^{2+} forms a dead-end complex with high levels of glucose (Mawal et al., 1971).

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