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Concerted Reduction of Yeast Uridine Diphosphate Galactose 4-Epimerase*

A. U. Bertland II, Y. Seyama, and H. M. Kalckar†

ABSTRACT: A concerted reduction of yeast UDPGal 4-epimerase by 5′-UMP and specific carbohydrates results in a seven- to tenfold increase in the 450-mμ fluorescence emission, with a concomitant loss in catalytic activity. During the reduction approximately 1 mole of 5′-UMP is bound per mole of epimerase dimer. Sodium borohydride will bring about the same type of reduction, again provided that 5′-UMP is also present. The inactive reduced epimerase can be rendered active by dilution of the reduced epimerase and subsequent storage at 4°. The bound NAD group of epimerase or the NADH group formed after the concerted reduction of the epimerase can be isolated by titration of the available sulfhydryl groups in the epimerases, using *p*-chloromercuribenzoic acid and subsequent chromatography on Sephadex.

UDPGal 4-epimerase[1] from yeast and *Escherichia coli* can undergo a modification, which has been called concerted reductive inactivation, since the bound pyridine nucleotide is reduced by a specific sugar in the presence of 5′-UMP, and the catalytic activity decreases as a function of the degree of reduction (Kalckar *et al.*, 1970). Since the fluorescence characteristics (Bertland, 1970) and the alterations in the ordered backbone peptide structure (Bertland and Kalckar, 1968), which accompanies such a reductive inactivation, have largely been studied in the yeast epimerase the detailed description of the reductive modifications will mainly center around the operations carried out on the yeast enzyme.

The process seems to be a concerted reaction, even in the presence of specific deoxy sugars, since any extensive reduction of the prosthetic group of the intact epimerase requires not only the addition of specific sugars but also the presence of small amounts of 5′-UMP. The reduced inactive epimerase can be reactivated.

The present investigation describes the systematic investigation of the concerted reductive process.

Materials

UDPGlc dehydrogenase, NAD, NADH, 5′-UMP, galactose, and D- and L-fucose were purchased from Sigma Chemical Company; L-arabinose and UDPGal from Calbiochem; [2-^{14}C]5′-UMP from Schwarz BioResearch; *p*-chloromercuribenzoic acid was purchased from Mann Research Laboratories; sodium borohydride was obtained from Matheson Coleman and Bell; D-mannose, D-ribose, 2-deoxy-D-galactose, and 2-deoxy-D-ribose were obtained from Pfanstiehl; 3-

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[1] Abbreviations used are: UDPGal, uridine 5′-(α-D-galactopyranosyl pyrophosphate); UDPGal 4-epimerase (UDPGlc 4-epimerase, EC 5.1.3.2).

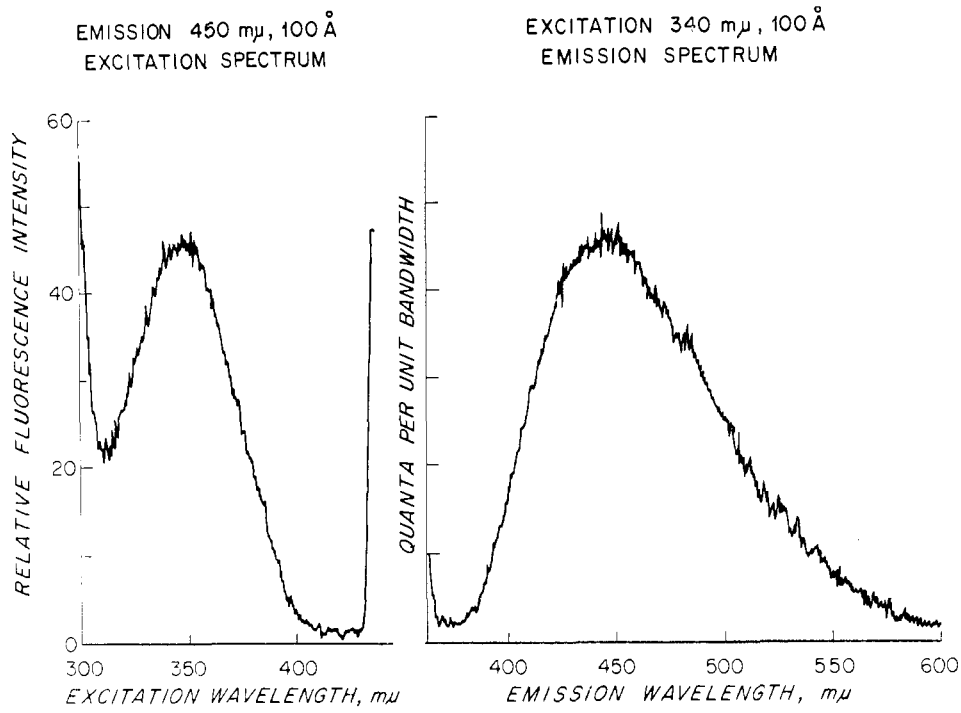

FIGURE 1: Direct tracing of the fluorescence excitation spectrum and fluorescence emission spectrum of native UDPGal 4-epimerase in 0.01 M Tris-HCl buffer (pH 7.4), 10^{-4} M EDTA, and 10^{-4} M 2-mercaptoethanol at 25°.

deoxy-D-glucose was a gift of Dr. N. Richtmeyer, National Institutes of Health.

Methods

Radioactivity measurements were determined by a Nuclear-Chicago planchet counter.

Protein was determined by 260- to 280-mμ absorption or by the biuret method using bovine serum albumin as standard.

Reduction of native UDPGal 4-epimerase was performed in 0.1 M Tris-HCl buffer, pH 7.5, containing 10^{-3} M 2-mercaptoethanol and 10^{-4} EDTA. For the development of the reduction the optimum concentration of sugar is 10^{-2} M and 5′-UMP is between 10^{-3} M and 10^{-4} M. The reaction mixture was incubated at 27°, or incubated overnight at 4°.

Catalytic activity of epimerase is measured by method of Maxwell *et al.* (1958) and Darrow and Rodstrom (1966). The catalytic activity is expressed as micromoles of UDPGlc formed from UDPGal per mg of protein per minute at 25°.

Fluorescence emission and excitation spectra were measured with a Turner Model 210 Spectro absolute spectrofluorometer. The instrument and methods of correction are described by Turner (1964). Quartz cells (1 × 1 cm path length) were used. Fluorescence units were standardized with quinine sulfate, 2.21 μg/ml in 0.1 M H_2SO_4, with 100-Å excitation and emission bandwidth. The per cent transmission is adjusted to give 50 fluorescent emission units at 455 mμ with excitation at 350 mμ. The resolution in all figures presented is 100 Å for both excitation and emission wavelength. All measurements were performed at 25°.

Titrations of epimerase with p-mercuribenzoic acid were performed in 0.01–0.1 M Tris-HCl buffer at 25°. Complete titrations have been previously described (Creveling *et al.*, 1965).

Sodium Borohydride Reduction. Sodium borohydride was prepared in 0.25 M concentration in 0.2 M glycine buffer at pH 11. Epimerase (0.125 mg/ml in 0.01 M Tris-HCl, pH 7.4, 10^{-3} M 2-mercaptoethanol, and 10^{-4} M EDTA) is treated with 10^{-4} M 5′-UMP and 2.5×10^{-3} M $NaBH_4$. The reaction is allowed to proceed in the dark at 25° and can be followed by an increase in 450-mμ fluorescence emission, produced by excitation at 340 mμ, and a decrease in catalytic activity.

Spectrophotometric measurements were made in the Cary 14, using the sensitive slide-wire or, in some cases, in the Zeiss PMQ II spectrophotometer. Semimicro quartz cuvets (1-cm path length) were used in either case.

Results

Reduction, Fluorescence Emission, and Inactivation of Purified Yeast UDPGal 4-Epimerase. If purified epimerase is incubated with L-arabinose no changes in catalytic activity or in the fluorescence emission at 450 mμ are discernable. However, if 5′-UMP is also added, the blue fluorescence emission at 450 mμ is increased seven- to tenfold, accompanied by a marked drop in catalytic activity. The most rapid development of the increase in fluorescence emission is observed with an L-arabinose concentration of 10^{-2} M and 5′-UMP at 10^{-3} to 10^{-4} M concentration. Preliminary data on the specificity of nucleotides and sugars required for the fluorescence increase have been presented (Bertland *et al.*, 1966). As seen in Figure 1, the fluorescence emission maximum is at 440–450 mμ, with an excitation maximum at 340–350 mμ. The rapid and extensive increase in the blue emission by the concerted effect of L-arabinose and 5′-UMP serves as a simple and highly sensitive test for this reaction. The reaction can also be followed by measurement of catalytic activity. From Figure 2, it can be seen that the catalytic activity of epimerase decreases proportionally to the increase in fluorescence. This type of concerted reaction is largely due to a reduction of the bound NAD of epimerase to bound NADH, as will be described in the present article.

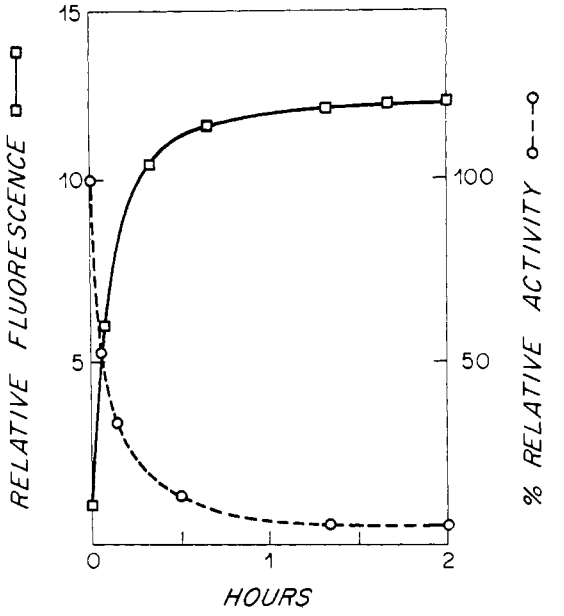

FIGURE 2: Time-dependent increase of fluorescence emission at 450 mμ and decrease of enzymatic activity of UDPGal 4-epimerase, in 0.1 M Tris-HCl buffer, pH 7.4, 10^{-4} M EDTA, and 10^{-3} M 2-mercaptoethanol at 25°. Epimerase (0.08–20 mg per ml) was treated with 10^{-2} M L-arabinose and 10^{-4} M 5′-UMP at zero time.

Specificity of the Concerted Reaction. All the sugars which are active depend on the presence of 5′-UMP with the exception of 2-deoxy and 3-deoxy sugars, as seen in Figure 3. These sugars, in the absence of 5′-UMP, bring about a limited reaction as followed by fluorescence emission of 450 mμ, the fluorescence increases 1.4- to 1.7-fold, and the reaction is complete in 30 sec. Even in these cases, addition of 5′-UMP will cause the reaction to proceed much further. In the presence of 5′-UMP a number of 2-deoxy and 3-deoxy sugars are active in the reaction, showing fluorescence emission increases of 2- to 6-fold, as seen in Figures 4 and 5. In all cases, the subsequent addition of L-arabinose, the most effective sugar, brings the level of final enhancement of fluorescence to the near maximal of 10- to 12-fold, as shown in Figure 2 with L-arabinose.

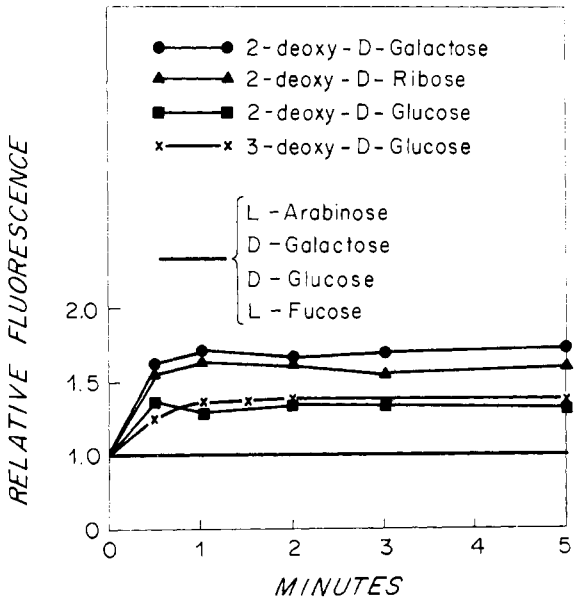

FIGURE 3: Formation of fluorescent epimerase in the absence of 5′-UMP. Fluorescence emission increase of epimerase at 450 mμ with time. All sugar concentrations were 10^{-2} M; the reaction mixture was as in Figure 2 without 5′-UMP at 25°.

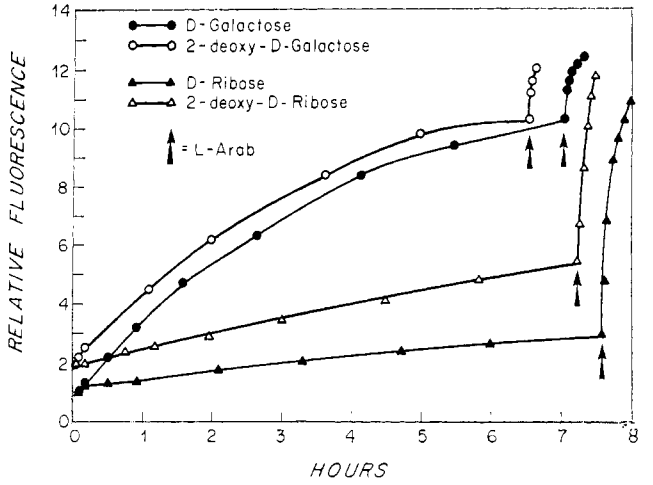

FIGURE 4: Formation of fluorescent epimerase in the presence of 5′-UMP. Fluorescence emission increase at 450 mμ in the presence of 2-deoxy sugars, concentration 10^{-2} M, in the presence of 10^{-4} M 5′-UMP.

Sodium Borohydride Reduction of Epimerase also Requires 5′-UMP. Reduction of the epimerase bound NAD by sodium borohydride also requires the presence to 5′-UMP (see Table I). The fluorescence emission increase is seven- to tenfold at 450 mμ and is complete in 1 hr at 27°. The catalytic activity of epimerase decreases to about 3% of original activity similarly as the reduction by L-arabinose and 5′-UMP. The concerted $NaBH_4$–5′-UMP reduction is, however, less stable in maintaining the high fluorescence emission, as well as the low catalytic activity (see section on reactivation). In 24 hr at 4° about 30% of the fluorescence is lost. A sample of $NaBH_4$–5′-UMP-reduced epimerase was incubated for 3 hr at 27° and then passed through a Sephadex G-50 column, equilibrated

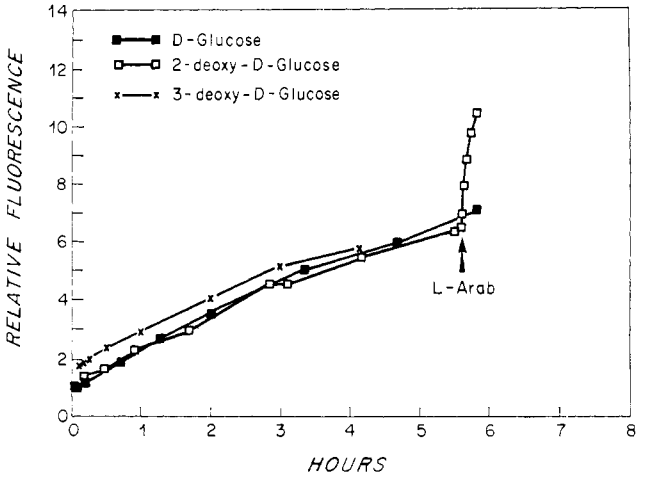

FIGURE 5: Formation of fluorescent epimerase in the presence of 5′-UMP. Fluorescence emission increase at 450 mμ in the presence of 2- and 3-deoxy sugars, 10^{-2} M concentration, and 10^{-4} M 5′-UMP. At the time indicated by the arrow L-arabinose was added to a final concentration of 10^{-2} M.

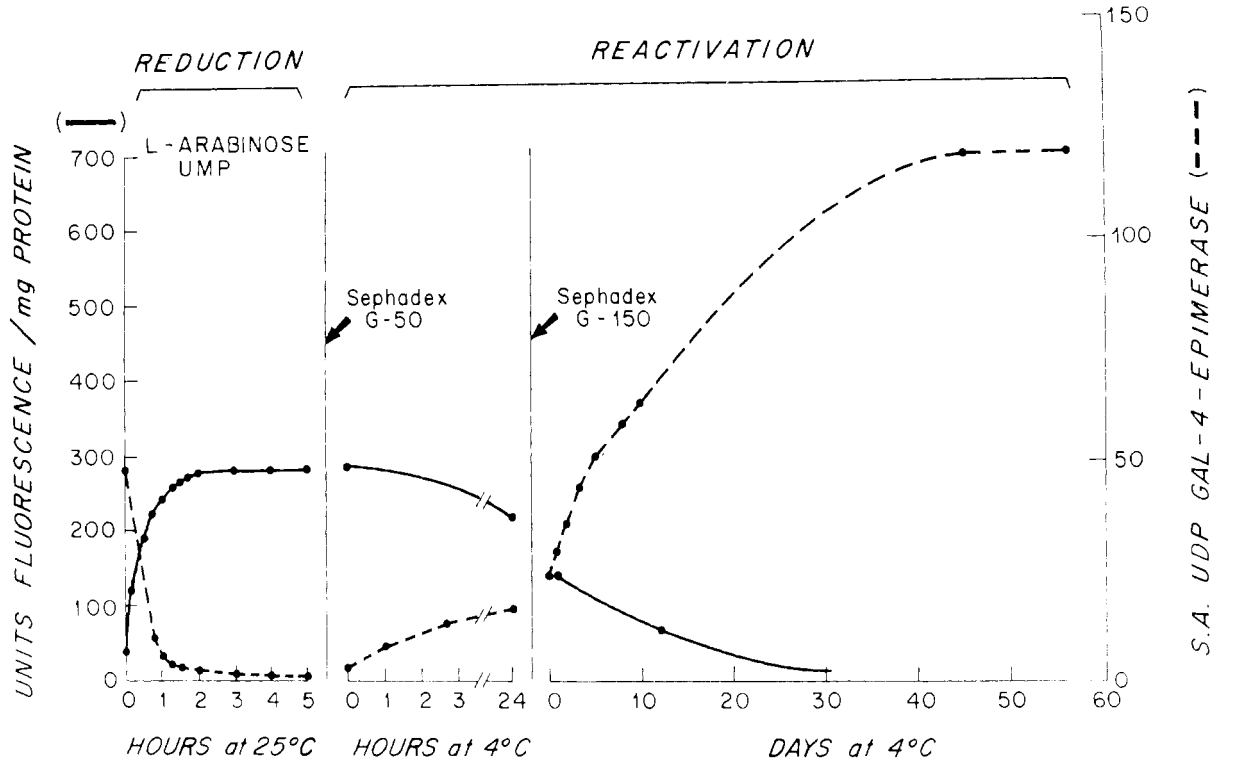

FIGURE 6: Reactivation of reduced epimerase in 0.1 M Tris-HCl buffer containing 10^{-3} M 2-mercaptoethanol and 10^{-4} M EDTA. Sephadex G-50 column chromatography (1.9 × 16 cm) and Sephadex G-150 (.9 × 25 cm) equilibrated with the above buffer; fluorescence 450-mμ emission with 350-mμ excitation (———); specific catalytic activity (---).

with 0.01 M Tris-HCl, pH 7.4, 10^{-3} 2-mercaptoethanol, and 10^{-4} M EDTA at 27°. After the column chromatography there was an immediate increase in catalytic activity to give approximately 30% of the original activity.

Substrate Effect on Reduction. One of the proposed mechanisms of UDPGal 4-epimerization invokes the reversible oxidation and reduction of the pyridine nucleotide. Incubation of yeast epimerase with the substrates UDPGal or UDPGlc, however, has never shown any effect on the fluorescence of the NAD nucleotide of yeast epimerase, in spite of the fact that UDPGlc, at least, is able to elicit a distinct, although small, reduction of the bound NAD (A. Bhaduri and H. M. Kalckar, unpublished). It can also be stated briefly that the development of the concerted reduction is not altered by the addition of UDPGal.

If the kinetics of the catalytic conversion of UDPGal is studied after the concerted reduction, the little catalytic activity left does not show any abberant features. For instance, the K_m with respect to UDPGal appears to be the same as that of the native enzyme. The remnant catalytical activity is probably due exclusively to the small fraction of epimerase remaining in the native form.

Reactivation of Reduced Epimerase. An epimerase reduced by L-arabinose and UMP showed a reactivation feature as follows. If dilute reduced epimerase solutions (0.1–0.2 mg per ml) were stored at 4°, catalytic activity returned gradually. In 5–6 days, on an average, reactivation occurred to give more than 50% of the original activity of the native enzyme; this reactivation is accompanied by a slow loss of fluorescence at the 450-mμ band. The sample was chromatographed also on a Sephadex 150 column to check on gross molecular weight changes; however, the columns are not necessary; dilution is sufficient for reactivation. The finally reactivated epimerase in both sample, if stored up to 20 days at 4°, more than doubled its specific activity over that of the initial starting material (see data, Figure 6). The dilute reactivated epimerase remains stable over several weeks. Fluorescence of the 450-mμ emission is difficult to follow at the later stages in these highly diluted epimerase samples.

After reduction by $NaBH_4$ and 5′-UMP, followed by column chromatography there was a relatively rapid rise in activity to give up to 30% of the activity of the native epimerase. It was not possible by storage of dilute samples to accomplish any further rise in activity. This is in contrast to the epimerase reduced by specific sugars.

TABLE 1: Effect of 5′-UMP on the BH_4 Reduction of Epimerase.[a]

		Fluorescence (Arbitrary Units)		
		Prior to Addn of BH_4	Incubation	
			0.5 hr	16 hr
1	+UMP	60	512	422
2	−UMP	60	110	53

[a] E^{red}: 10 nmoles would contain at least 8 nmoles of NADH; 160 μl of epimerase solution (11.9 mg/ml) corresponding to 10 nmoles of dimer ± 10 μl of 10^{-2} M 5′-UMP; 10 μl of BH_4 (5 mg in 0.05 ml of 0.1 M glycine buffer, pH 11), final volume 1 ml. Left for 16–18 hr at 4° until practically all the excess hydrogen gas has been released.

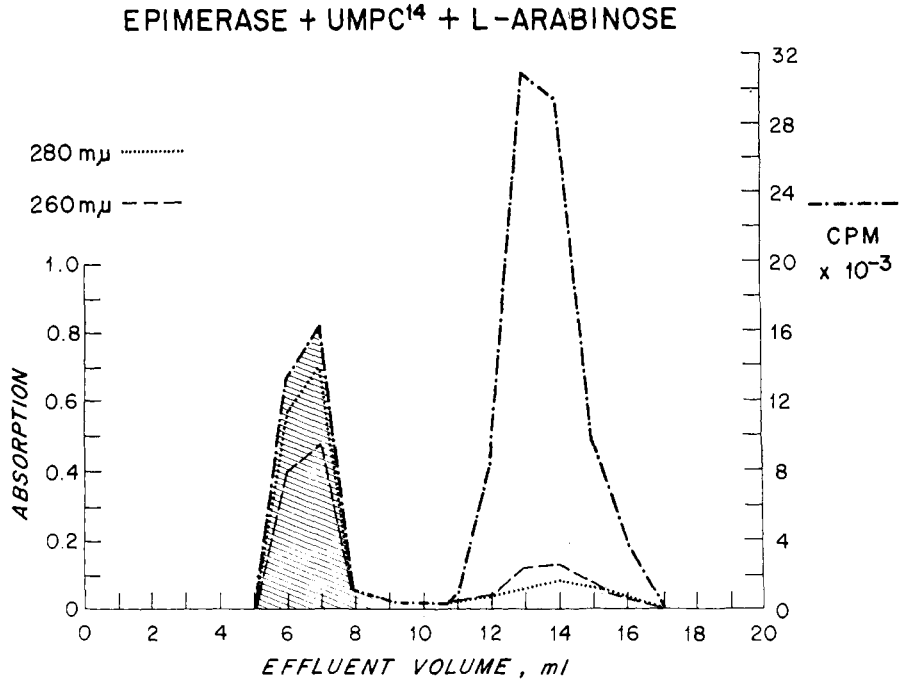

FIGURE 7: Chromatography of yeast epimerase on Sephadex G-50 (0.9 × 10 cm) equilibrated with 0.1 M Tris-HCl buffer, pH 7.4, 10^{-3} M 2-mercaptoethanol, and 10^{-3} M EDTA. Epimerase (2.3 mg), 1.1 × 10^{-4} M [2-^{14}C]5′-UMP, and 10^{-2} M L-arabinose were present in a final volume of 1 ml.

Binding of [2-^{14}C]*5′-UMP During the Concerted Reduction.* The concerted reduction of the NAD bound to epimerase by incubation with 5′-UMP and an active sugar results in the binding of 5′-UMP to the protein, but neither the sugar nor its products are bound. The controls which include epimerase and 5′-UMP in the absence of sugar, or in the presence of an inactive sugar, showed much less binding of 5′-UMP. Each of the reaction mixtures after 2- to 3-hr incubation at 27° was dialyzed 24 hr at 4°, against 1 l. of buffer, 0.1 M Tris-HCl, pH 7.4, containing 10^{-3} M 2-mercaptoethanol and 10^{-4} M EDTA. The samples then were passed through a Sephadex G-50 column, as described in the legend to Figure 6. In the protein peak in Figure 7, tubes 6 and 7 contained 0.55 mole of [2-^{14}C]5′-UMP per mole of epimerase dimer, respectively, or close to 0.8 mole per mole of newly reduced epimerase dimer. In the second peak the free [2-^{14}C]5′-UMP and sugar are recovered. Overall recovery of protein and radioactivity after dialysis and chromatography was 75–80%.

Binding studies show that for L-arabinose, D-arabinose, and no sugar the equivalent of [2-^{14}C]5′-UMP per equivalent of epimerase is, respectively, 0.55–0.80, 0.12–0.14, and 0.13–0.14. The average nonspecific binding in both cases was 0.14 mole of [2-^{14}C]5′-UMP binding per mole of epimerase dimer.[2]

Removal of Bound Cofactor and 5′-UMP from Epimerase. It was shown by Maxwell *et al.* (1958) that epimerase-bound NAD or NADH, when treated with *p*-mercuribenzoic acid loses the characteristic 450-mμ fluorescence emission band. Darrow and Rodstrom (1966) have further shown that the *p*-mercuribenzoic acid treated epimerase dissociates into two subunits (*cf.* also 1970). Under the conditions described the binding of 5′-UMP to reduced epimerase, although not covalent, persists after passage through five Sephadex G-50 columns. However, the bound 5′-UMP can be removed readily, together with the bound NADH, by titration of the available SH group with *p*-mercuribenzoic acid. The resulting apoepimerase will not bind 5′-UMP anymore.

As appears from the titration of a reduced epimerase which has bound approximately 1 mole of [2-^{14}C]5′-UMP per reduced dimer, the 5′-UMP is released together with the reduced NAD group when the *p*-mercuribenzoic acid subunits are chromatographed on a Sephadex G-50 column.

The experimental details are described in Table II. The incubation mixture was passed through a Sephadex G-50 column equilibrated with 0.01 M Tris-HCl buffer, pH 7.5. The protein tubes contain the epimerase subunits, the fluorescence emission at 450 mμ, and epimerase-bound [2-^{14}C]5′-UMP (which is 0.8 mole/mole of newly reduced epimerase). Five nmoles of this epimerase preparation, most of which is in the reduced form, was treated with 80 nmoles of *p*-mercuribenzoic acid (estimating 16 titrable sulfhydryl groups per mole of enzyme dimer). When this sample was then passed through a second Sephadex G-50 column (equilibrated with 0.1 M Tris-HCl buffer, pH 7.5, containing 10^{-3} M 2-mercaptoethanol and 10^{-4} M EDTA), protein tubes show that there is no longer any [2-^{14}C]5′-UMP or any fluorescence bound to the protein. The recovery of [2-^{14}C]5′-UMP from the reduced enzyme was 85% and, as judged by the fluorescence, we can account for practically all the reduced NADH. Fluorescence was determined quantitatively by comparing it to free NADH fluorescence and absorption at 340 mμ as shown in Table II. From 5 nmoles of epimerase we obtained 4.6 mmoles of NADH, determined by fluorescence and by 340-mμ absorp-

[2] Three samples of 2.3 mg of epimerase each, in 0.1 M Tris-HCl buffer, pH 7.4, 10^{-3} M 2-mercaptoethanol, and 10^{-3} M EDTA (1-ml vol) were incubated for 4 hr at 25° with 1.1 × 10^{-4} M [2-^{14}C]5′-UMP in the presence and absence of specific sugars at 10^{-2} M concentration. The samples were chromatographed on Sephadex G-50 columns (0.9 × 16 cm) equilibrated with the above buffer at 25°. Samples (1 ml) were collected, and protein and protein-bound [2-^{14}C]5′-UMP were determined. Protein and [2-^{14}C]5′-UMP recoveries amounted to 75–85%. The range in equivalents of binding represents several experiments.

TABLE II[a]

Column	Protein peak	Effluent peak cofactor + PMB
1. Epimerase (holo)	9.1 nmoles	
[2-^{14}C]5′-UMP	7.3 nmoles	
2. Epimerase (apo)	5 nmoles	
[2-^{14}C]5′-UMP		3.3 nmoles
NADH fluorescence (340/460 mμ)		4.5 nmoles
NADH absorptions (340 mμ)		4.5 nmoles

[a] An incubation mixture of epimerase, [2-^{14}C]5′-UMP, and L-arabinose was passed through a Sephadex G-50 (0.9 × 16 cm) column, equilibrated with 0.01 M Tris-HCl, pH 7.5. Upon elution the protein tubes showed that 0.8 equiv of [2-^{14}C]5′-UMP was bound per equiv of protein; 5 nmoles of this protein was treated with 80 nmoles of *p*-mercuribenzoic acid and passed through a Sephadex G-50 column (0.9 × 16 cm) equilibrated with 0.1 M Tris-HCl buffer, pH 7.5, 10^{-3} M 2-mercaptoethanol, and 10^{-4} M EDTA.

tion, and 3.3 nmoles of 5′-UMP, as determined by ^{14}C counts. The fluorescence of the released NADH group showed no significantly higher quantum yield of emission as compared with free NADH. The released NADH group reacted almost quantitatively with excess pyruvate in the presence of catalytical amounts of crystalline lactic acid dehydrogenase.

Discussion

The concerted reduction of epimerase shows broad specificity with respect to the carbohydrates, although L-arabinose is by far the most effective. Reduction of epimerase by sodium borohydride does not proceed very far unless 5′-UMP is present at the same time. Subsequent to Sephadex chromatography, either type of reduced epimerase can be reactivated by storing the reduced fluorescent epimerase at 4° in a highly dilute solution. Catalytic activity of epimerase increases upon storage, but if L-arabinose was used as a reductant, the reactivation usually proceeded much further (100–200% of the catalytic activity of the native epimerase). The importance of epimerase concentration as a function of reactivation during storage is under further study. The much greater effectiveness of storage of dilute epimerase solutions than of concentrated ones could be interpreted in various ways. It could be due to an improved dissociation of bound 5′-UMP (or other bound substances), or another factor in the failure of effective reactivation of more concentrated solutions of reduced epimerase may be interaction with neighboring reduced epimerase molecules; this interaction may interfere with the conformational changes needed for reactivation (Kalckar *et al.*, 1969). The effect of epimerase concentrations on the reoxidation of the prosthetic group should also be considered. A reactivation of reduced epimerase by 2-ketoglucose preparations has also been observed (Kalckar *et al.*, 1969), but it is not clear in this case whether more than one factor is involved or whether 2-ketoglucose is the effective reactivator (Y. Seyama and H. M. Kalckar, unpublished, 1970).

The prosthetic group of native or reduced epimerase can be liberated from the protein by *p*-mercuribenzoic acid reaction of the sulfhydryl groups. Since epimerase contains only one prosthetic group per dimer and the *p*-mercuribenzoic acid treatment dissociates the epimerase dimer into subunits of a molecular weight of 60,000 (*cf.* Darrow and Rodstrom, 1970; Kalckar *et al.*, 1969), it suggests that the cofactor is bound by the dimer. This question is under further investigation (L. H. Bertland and A. U. Bertland, II, in preparation, 1970).

Rapid titration of the available sulfhydryl groups by *p*-mercuribenzoic acid (using even a small excess) gives a better yield of stable apo enzyme (A. U. Bertland, 1970, unpublished) as well as of the prosthetic group (reduced or unreduced).

The identity of the released prosthetic group, either from native or reduced epimerase, is not fully clear. The released group from the native epimerase did not respond to UDPGlc dehydrogenase whatsoever, nor was the response to an alcohol dehydrogenase a quantitative one (Kalckar *et al.*, 1969).

The reduced prosthetic group was practically completely reoxidized by a fivefold excess of pyruvate in the presence of lactate dehydrogenase, much like NADH. Clearly the prosthetic group of yeast UDPGal 4-epimerase is closely related to NAD. However, two observations made in our laboratory raise the problem of identity with NAD, particularly clearly. The first one is the lack of response to UDPGlc and its dehydrogenase mentioned above. This is clearly in contrast to observations made on UDPGal 4-epimerase from *E. coli* (Wilson and Hogness, 1964). The other observation, which raises a problem as to the identity of the prosthetic group from the yeast epimerase, is the fact that it moves much more slowly on polyethylenimine thin-layer chromatography than NAD (A. U. and L. H. Bertland, unpublished, 1970). Another contrast between the *E. coli* epimerase and that of yeast epimerase concerns the ability of the substrate to reduce the bound NAD group. In *E. coli*, UDPGlc added in excess is able to raise the level of reduced epimerase distinctly (Wilson and Hogness, 1964), and UDPGal is as effective if not more so (Kalckar *et al.*, 1970). Addition (even a large excess) of UDPGlc to yeast epimerase gives only a minute increase in the level of reduced epimerase and UDPGal is not able to bring about reduction (A. Bhaduri and H. M. Kalckar, unpublished, 1967). It is clear that the NAD group of yeast epimerase behaves differently from that of the *E. coli* epimerase. The identity of the complex of the NAD group be it from *Saccharomyces fragilis* or *E. coli* is not as yet clear.

The stoichiometry of 5′-UMP binding during the concerted reduction was not one to one. However, since the native epimerase has already 10–15% of its cofactor in the reduced from (Creveling *et al.*, 1965), the stoichiometry should be based on the newly reduced cofactor. On this basis the stoichiometry is nearly one to one. The nature of nonspecific binding found is not as yet clear.

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Cell Wall Polymers and Phage Lysis of *Lactobacillus plantarum**

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ABSTRACT: Two bacteriophages virulent for strains of *Lactobacillus plantarum* were isolated. Phage 1 lysed strain 8014 which has a ribitol teichoic acid and a phosphorylated polysaccharide (molecular ratio P:Rha:Glc:Gal:Glc_{NH_2}, 1:6:1:1:1) in its cell wall, but not strain 10241 which has a wall teichoic acid but no polysaccharide. Phage 2 lysed both strains. Mutants of strain 8014 resistant to phage 1 possessed a cell wall polysaccharide of different composition from that found in wild type cells; in most cases rhamnose and galactose were absent or present only in small amounts. Isolated cell walls of one of these mutants (M1) did not inactivate phage 1. A mutant of strain 10241 resistant to phage 2 differed from the parent organism in the glucose content of its ribitol teichoic acid (P:Glc in teichoic acid, mutant = 1:0.04 and parent = 1:1.04). These results indicate that the cell wall polysaccharide is essential for infection by phage 1 whereas the glucose moiety of ribitol teichoic acid is an important determinant of phage 2 infection.

The cell wall of *Lactobacillus plantarum* ATCC 8014 comprises, in addition to peptidoglycan, a ribitol teichoic acid with glucose substituents (Armstrong *et al.*, 1958) and a rhamnose-containing, phosphorylated polysaccharide (Ikawa and Snell, 1960; Anderson *et al.*, 1969). The detailed structure of the teichoic acid is well established (Archibald *et al.*, 1961), and it has been identified as the antigenic determinant of group D *Lactobacillus* (Sharpe *et al.*, 1964). Little information, however, is available on the chemical composition of the wall polysaccharide.

Other strains of *L. plantarum* show considerable variation in wall composition (Adams *et al.*, 1969). The polysaccharide component, for example, may be completely absent, or the ribitol teichoic acid may be replaced by a polyglycerol phosphate polymer, as in *L. plantarum* N.I.R.D. Cα106.

Teichoic acids are known to be involved in the phage receptor sites of certain Gram-positive bacteria. The glucose moiety of ribitol or glycerol teichoic acid is essential for phage adsorption in strains of *Bacillus subtilis* (Glaser *et al.*, 1966; Young, 1967). Similarly the *N*-acetylglucosamine residue of ribitol teichoic acid is necessary for phage fixation in *Staphylococcus aureus* (Coyette and Ghuysen, 1968; Chatterjee, 1969).

The present communication describes an attempt to correlate phage sensitivity with differences in wall composition of various strains of *L. plantarum*. In addition, the wall compositions of several phage-resistant mutants were examined in order to gain more specific information about the nature of the phage receptor sites.

Materials and Methods

Growth of Bacteria. L. plantarum Cα106 was a gift from M. E. Sharpe; all other strains were obtained from the American Type Culture Collection. Stock cultures were maintained on Brewer's thioglycollate agar (Difco). The medium of DeMan *et al.* (1960) was used for large-scale cultivation. Batches (20 l.) of medium were inoculated with an overnight culture (1 l.) of the organism and incubated at 28° for 20 hr. Cells were harvested with a Sharples refrigerated centrifuge and washed twice with 0.6% NaCl.

Bacteriophage Isolation, Propagation, and Assay. Phage 1 was isolated from corn silage and phage 2 from anaerobic sewage sludge after enrichment on *L. plantarum* ATCC 8014. The methods of Adams (1959) were used in enrichment, plaque isolation, and phage assay procedures. The growth, plating, and dilution medium was Brewer's thioglycollate broth (BT) or agar.

Phage propagation was accomplished by inoculating logarithmically growing cultures (10 ml) of *L. plantarum* ATCC 8014 with 0.1 ml of a phage suspension in BT prepared from a single plaque. After overnight incubation at 28°, the lysates were filtered through membrane filters (Millipore type HA, 0.45 μ). The filtrates (10^8–10^9 plaque-forming units (pfu)/ml) were stored at 4°.

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