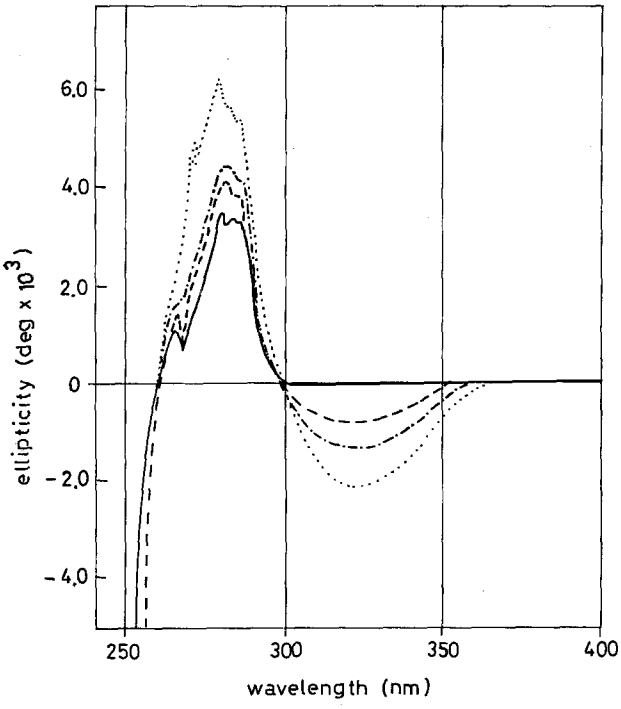
shown in the present communication that the measurement of the intensity of the new CD-band around 320 nm can be used for the determination of the binding constant.

Materials and methods. Crystalline apotransketolase (specific activity 16–21 units/mg protein) was obtained from Sigma Chemical Co. (St. Louis, USA). Transketolase activity was measured as described previously[2]. Transketolase protein was determined spectrophotometrically at 280 nm using $E^{1\%}_{1\,cm} = 14.5$[3].

Circular dichroism measurements were done on a Cary model 60 spectropolarimeter with Mod 6003 CD-attachment using cylindrical quartz cells of 0.5 cm pathlength.



Circular dichroism spectra of transketolase in 20 mM sodium phosphate buffer containing 10 mM Na_2SO_4, pH 7.2, 0.5 cm cuvettes were used. The reconstitution was carried out at 25° ± 0.1 °C. Conditions were: $7.9 \times 10^{-6} M$ apotransketolase, $3.7 \times 10^{-3} M$ $MgCl_2$ and $4 \times 10^{-6} M$ (---), $8 \times 10^{-6} M$ (-·-·), $24 \times 10^{-6} M$ (...) thiamine diphosphate.

Results and discussion. The Figure shows the CD-spectra obtained when apotransketolase/Mg^{++} is titrated with its coenzyme thiamine diphosphate. A CD-band around 320 nm is generated upon holotransketolase formation, which is due to charge transfer complex interaction between tryptophan as electron donor and thiamine diphosphate as electron acceptor. Assuming that all thiamine diphosphate molecules which bind to the tryptophan residues of apotransketolase give the same contribution to the intensity of the CD-band at 320 nm, it is possible to determine the apparent association constant for thiamine diphosphate from the following equation:

$$K_{app} = \frac{[\text{Holo-TK}]}{\{[\text{Apo-TK}] - [\text{Holo-TK}]\}\,\{[\text{TPP}] - [\text{Holo-TK}]\}}.$$

The initial concentrations of apotransketolase = [Apo-TK] and thiamine diphosphate = [TPP] are known, the concentration of holotransketolase = [Holo-TK] is obtained from the magnitude of the CD-band at 320 nm. The relation between the magnitude of the CD-band and the holotransketolase concentration is derived from the fact that the CD-band increases with increasing amounts of thiamine diphosphate up to a point where saturation is reached. Further increase of thiamine diphosphate concentration does not affect the intensity of the CD-band any more. We assume that at this point all apotransketolase has been converted to holoenzyme. An apparent association constant of $0.73 \times 10^6\ M^{-1}$ is obtained for thiamine diphosphate, which is in good agreement with the values obtained by other methods[2]. From activity and fluorescence quenching measurements $1.0 \times 10^6\ M^{-1}$ was found.

Zusammenfassung. Mit Hilfe des Circulardichroismus wurde die Bindungskonstante von Thiamindiphosphat in der Transketolase der Bäckerhefe bestimmt.

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The Number of Coenzyme Binding Sites in Transketolase from Baker's Yeast

Yeast transketolase has been found to consist of two identical subunits[1] suggesting two catalytic sites. In the present communication we report on the number of coenzyme binding sites.

Materials and methods. Crystalline apotransketolase (specific activity 16–21 U/mg protein) was obtained from Sigma Chemical Co. (St. Louis, USA). Transketolase activity was measured as described previously[2]. Transketolase protein was determined spectro-photometrically at 280 nm using $E^{1\%}_{1cm}$ 14.5[3]. The diphosphate of C^{14}-labelled thiamine (thiazole-2-C^{14}; specific activity 18.9 μC/μmole, obtained from the Radiochemical Centre, Amersham, England) was synthesized as previously described[2]. The enzyme preparation showed only a single band in the analytical acrylamide gel electrophoresis.

Results and discussion. For the determination of the number of coenzyme binding sites apotransketolase was incubated with an excess of Mg^{++} and C^{14}-thiamine diphosphate for 30 min at 25 °C. The reconstituted enzyme was gel-filtered over Sephadex G-25, the fractions were assayed for transketolase activity and pooled. Aliquots were taken for the determination of protein, thiamine diphosphate and transketolase activity. From the Table

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Determination of the number of binding sites per mole of transketolase

Transketolase		Thiamine diphosphate		Moles thiamine diphosphate/mole transketolase	
mg/ml	nmoles/ml	dpm/ml	nmoles/ml		Corrected
0.029	0.21	4633	0.33	1.59	1.80

In a total volume of 200 µl, 0.17 mg apotransketolase in 0.1 *M* glycylglycine buffer, pH 7.3, were incubated with 5 m*M* $MgCl_2$ and 0.3 m*M* C^{14}-thiamine diphosphate (specific activity 6.3 µC/µmole). After a recombination time of 30 min at 25 °C, the incubation mixture was applied to a Sephadex G-25 column (1 × 55 cm), equilibrated with 20 m*M* sodium phosphate buffer, pH 5.7. The flow rate was 11.5 ml/h, fraction volume was 2.0 ml. The fractions were analyzed for transketolase activity and pooled (5.7 ml). Aliquots were taken for the determination of protein, thiamine diphosphate and transketolase activity. Protein was determined by the method of LOWRY, using a calibration curve which had been obtained with a transketolase solution standardized by the Biuret method[3]. Thiamine diphosphate was determined by radioactivity measurements. The counting efficiency was 82% when 0.5 ml of the transketolase pool were added to the scintillation liquid (4 g PBD/l ethanol/toluene, 1:1). The transketolase assay was carried out in the presence and absence of added thiamine diphosphate/Mg^{++}. A stimulation of 13% of enzymatic activity was found when the coenzyme was added, indicating a loss of bound coenzyme upon gel filtration. Assuming a linear relationship between enzymatic activity and thiamine diphosphate molecules bound to apotransketolase, we have corrected the value found for thiamine diphosphate by these 13%.

it can be seen that 1.8 moles of thiamine diphosphate were bound per mole of transketolase (MW 140 000). This result agrees well with the findings of KOCHETOV et al.[4], although these authors have used a pH of 7.6 for gel filtration, where we get a complete removal of thiamine diphosphate[2]. Furthermore, these authors determined protein according to LOWRY, but no calibration is mentioned. This is essential, since transketolase has a high tyrosine content[5].

Zusammenfassung. Nachweis, dass 2 Mole Thiamindiphosphat pro Mol Apotransketolase gebunden werden.

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Metabolic Evaluation of Sexual Dimorphism. IV. Metabolic Differences Related to the Oxidative Metabolism

It has been demonstrated that the metabolic differences of mice submaxillary glands are related to the different behaviour of some regulatory enzymes of the energy metabolism[1,2]. Moreover the glucose oxidation by slices of submaxillary glands, both males and females, is stimulated by epinephrine and dibutyryl-cyclic AMP[3]. Their effect is localized on the glycolytic pathway and mainly on the reaction catalized by phosphofructokinase[2]. This fact, however, does not eliminate the possibility that some metabolic differences observed in mice submaxillary glands[1] may be related to the oxidations of Krebs cycle and to the oxidative phosphorylation carried out by isolated mitochondria.

C3H, Balb/c and DBA/2 mice from AR/IRE colony, in continuation since 1956, were used. The animals were sacrificed by cervical dislocation and the submaxillary glands immediately removed and placed in either ice-cold 0.25 *M* sucrose or distilled water. The glands, blotted, weighed and minced, were homogenized using a precooled glass homogenizer with a teflon pestle[4].

The mitochondria were isolated according to JOHNSON and LARDY[5]. Oxygen consumption of homogenates and ADP: O ratio was measured polarographically with an Oxygraph (Mod. KM, G.M.E.) equipped with an oxygen electrode (Yellow Spring Instruments) polarized at −0.8 volts. The reaction mixture for the homogenates was composed of 20 µmoles KH_2PO_4 (pH 7.3 with NaOH), 8.0 µmoles of $MgCl_2$, 325 µmoles of sucrose and 220 µl of 10% homogenate in a final volume of 2.0 ml. Substrates were added in order to achieve a final concentration of 5×10^{-4} *M*. An aliquot of the homogenate was dried for 3 h at 120 °C for dry weight determination.

For the mitochondria, the reaction mixture was made up as follows: 0.25 *M* sucrose, 10 m*M* *Tris*-HCl pH 7.4, 10 m*M* KH_2PO_4, 5 m*M* $MgCl_2$, 25 m*M* KCl, 1×10^{-3}*M* EDTA, 1% BSA and 200 µl of mitochondrial suspension (~ 2.0 mg of protein) to a final volume of 2.0 ml. In both cases the experiments were performed at 30 °C and the concentration of dissolved oxygen was 240 umoles[6]. The determination of succinic-oxidase activity was carried out according to POTTER[7]. The protein concentration was

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