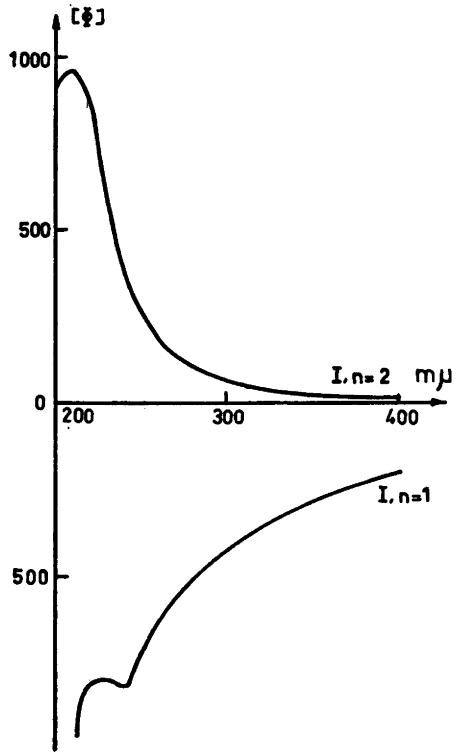

Fig. 2. Rotatory dispersion curves of L-albizziine and L-norcitrulline in water.

first extrema located at about 215 mμ or 223 mμ, respectively.

Experimental. Rotatory dispersion curves were measured with the Bellingham and Stanley/Bendix-Ericsson "Polarmatic 62" automatic recording spectropolarimeter, modified as previously described.[2] The measurements were performed at 18–25° in a 0.1 dm cell; the concentrations were about 1 mg/ml in water and 1.1 N HCl and about 2 mg/ml in 0.5 N KOH. The wave-length range studied was 400–200 mμ and the results are expressed as molecular rotations (Φ).

L-Citrulline, and the hydrochlorides of L-lysine, L-ornithine, and L-arginine were commercial preparations. L-Albizziine and L-2,3-diaminopropionic acid hydrochloride were prepared in the laboratories of one of the authors (A.K.).

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The Purification of an Alkaline Phosphatase from Baker's Yeast

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The occurrence in baker's yeast extract of several phosphatases with different enzymatic properties has been reported.[1-3] However, no alkaline phosphatase has previously been purified and investigated extensively. This paper describes the purification of an alkaline phosphatase from baker's yeast. The enzyme has a rather high activity towards O-monophosphate esters (*p*-nitrophenyl phosphate, phenyl phosphate, sodium β-glycerophosphate, threonine phosphate, serine phosphate, and different O-phosphorylated serine peptides).

The alkaline phosphatase activity was measured by incubating the enzyme with the substrate *p*-nitrophenyl phosphate

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(Sigma-104), 0.15 mg/ml in 0.5 M Tris—HCl buffer (pH 8.10) at 37°, in the presence of 5 mM $MgCl_2$. The increase in absorbancy at 400 mμ was determined at 30 sec intervals in Zeiss PMQII spectrophotometer in a 1 cm cuvette. The liberation of inorganic phosphate from other substrate was determined according to Beerenblum and Chain.[4] Units of the enzyme activity is defined as μg of *p*-nitrophenyl phosphate hydrolysed per minute under the conditions described above. Specific enzyme activity is expressed as units per milligram of total nitrogen. Nitrogen was determined by micro-Kjeldahl analysis.

Purification procedure. The fractionation involved the following main steps: precipitation, gel filtration and zone electrophoresis.

Step 1. *Precipitation of the crude enzyme.* Lyophilized baker's yeast (1.3 kg) was extracted two times with 3.0 l 0.01 M Tris—HCl buffer, pH 8.4, during 3 h with slow mechanical stirring at 40°. The solid part was removed by centrifugation (2600 *g*, 30 min) and the solution precipitated at 4° with protamine sulphate (1 mg/l supernatant solution). The precipitation was discarded after centrifugation (9000 *g*, 50 min).

The supernatant fluid was dialysed against running distilled water for 16 h at 4°. The inactive precipitate formed during the dialysis was removed by centrifugation (9000 *g*, 20 min).

Step 2. *Fractionation with acetone.* (a) The pH of the clear solution was adjusted to 7.2 and $MgCl_2$ was added to 1 mM concentration. The solution was cooled down to 0° and 0.12 volume acetone at −17° was added with stirring. The precipitate was discarded after centrifugation (16 000 *g*, 10 min).

(b) The supernatant solution was cooled to 0° and precipitated with 0.39 volume acetone at −17°. The active precipitate was collected by means of centrifugation (9000 *g* 30 min) and the precipitate dissolved in 200 ml of 0.025 M Tris—HCl buffer, pH 8.0, during 1 day with slow magnetic stirring at 4°.

(c) The pH was readjusted to 7.2 and the enzyme solution precipitated with 0.065 volume acetone as in step (a) with exception of $MgCl_2$ addition. The precipitate was discarded.

(d) Cold acetone (1.9 volume) was added with stirring to the clear solution and the active protein precipitated as in the step (b). It was collected and dissolved in 200 ml of water during 24 h and 4° with slow magnetic stirring. Finally the solution was freeze-dried.

Step 3. *Gel filtration.* A part of the freeze-dried substance (350 mg) was dissolved in 0.02 M Tris—HCl buffer, pH 8.0, and filtered through a column (2.5 × 90 cm) with Sephadex G-100 (water regain 10 ± 1 g/g) in equilibrium with 0.02 M Tris—HCl buffer, pH 8.0, with 0.2 M NaCl. Fig. 1 shows the results of one such experiment.

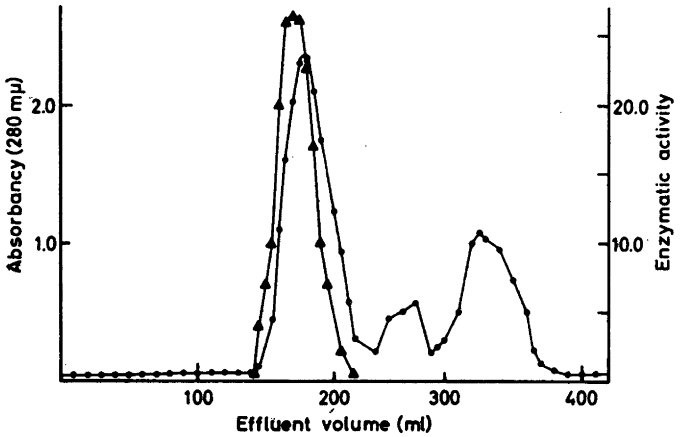

Fig. 1. Gel filtration on Sephadex G-100. Column dimensions: 2.5 × 90 cm; buffer: 0.02 M Tris—HCl, pH 8.0, with 0.2 M NaCl; elution: 0.02 M Tris—HCl buffer, pH 8.0, with 0.2 M NaCl; elution rate: 20 ml/h; fraction volume: 5 ml. Fraction IV is not included in the figure. (●) absorbancy at 280 mμ; (▲) enzyme activity.

The phosphatase-containing fractions from the gel filtration were pooled, cooled to 0° and 2.3 volume acetone were added at −17°. The white precipitate formed was collected by centrifugation (7000 *g*., 20 min).

Step 4. *Zone electrophoresis.* Zone electrophoresis experiments were carried out according to Porath [5] on a column with Pevicon (Superfosfatbolaget, Stockholm) as supporting medium. The white precipitate after gel filtration was dissolved in 8 ml Barbital Na-buffer, pH 8.5, and applied to the column (3.5 × 50 cm) in equilibrium with 0.05 M Barbital Na-buffer, pH 8.5. Dinitrophenyl-aspartic acid was used as a marking substance. The column was cooled with tap water (6°). A voltage of 340 V was applied, giving a current of 20 mA; after 19 h the current was discontinued. Elution was performed at a rate of 10 ml/h with a fraction volume of 7.0−7.2. Fig. 2 shows the results of an electrophoresis experiment. The distribution of material in the gel filtration and zone electrophoresis experiments were estimated from absorbancy measurements at 280 mμ in Zeiss PMQII spectrophotometer. Starch gel electrophoresis [6] of the purified enzyme in the 0.3 M H_3BO_3−NaOH buffer at pH 8.0 and pH 8.5 revealed only one protein component with coinciding phosphatase activity. The electrophoresis time was 6 h at 18 mA; and 210 V. The position of the protein zone was detected after staining with Amido Black and the enzyme activity with the α-naphthyl phosphate as substrate; see Fig. 2, upper part. The electrophoretically purified enzyme in ultracentrifugation experiments gave only one, slightly asymmetric, sedimenting boundary at pH 7.0. The sedimentation experiments were performed with a Spinco Model E ultracentrifuge at 59 780 rev./min at 20° in Na-phosphate buffer, pH 7.0, $\mu = 0.2$.

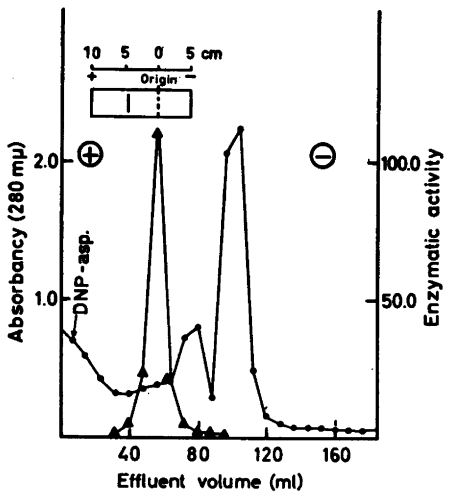

Fig. 2. Pevicon column electrophoresis of the Sephadex G-100 filtrated enzyme. Column dimensions: 3.5 × 50 cm; buffer: 0.05 M Barbital-Na, pH 8.5; current: 20 mA, voltage: 340 V; time: 19 h; elution rate: 10 ml/h; fraction volume: 7.0−7.2 ml. Concerning starch electrophoresis at pH 8.0, see the text. (●) absorbancy at 280 mμ; (▲) enzyme activity.

Expressed as specific activity with *p*-nitrophenyl phosphate as substrate, an approximately 3000-fold purification of the alkaline phosphatase has been achieved by the method described in this communication.

Properties. The enzyme activity is increased by addition of Mg^{2+}, Na^+, or K^+ tested with *p*-nitrophenyl phosphate as substrate at pH 8.10 in 0.5 M Tris−HCl buffer. The phosphatase has a sharp pH optimum at 8.10 at optimal *p*-nitrophenyl phosphate concentration. At this pH the enzyme has activity towards other monophosphate esters (phenyl phosphate, sodium β-glycerophosphate, threonine phosphate, serine phosphate and O-phosphoryl serine peptides). The baker's yeast phosphoserine phosphatase purified by Schramm [7] had a pH optimum at pH 6.5, was inhibited by Mn^{2+} and did not hydrolyse threonine phosphate. The alkaline phosphatase described in this paper is active not only towards many monophosphate esters but also shows activity towards inorganic pyrophosphate as in the case of the alkaline phosphatase of *Escherichia coli*.[8] It is possible that the hydrolysis of monophosphates and the pyrophosphate compounds by the baker's yeast alkaline phosphatase is also due to a single enzyme, but this problem still remains to be examined. Kunitz [9-10] succeeded in isolating a specific inorganic pyrophosphatase from baker's yeast in crystalline form. It was recently shown in this laboratory that this pyrophosphatase hydrolyses O-pyrophosphorylated serine peptides.[11] However, the yeast alkaline phosphatase has not activity towards such substrates. The enzyme has no phosphodiesterase activity, when O-monophenylphosphoryl-DL-serylglycine[12] is used as substrate.

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Alkyl Cyanates

II. A New Route to Alkyl Cyanates

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Alkyl cyanates have recently been prepared by decomposition of 5-alkoxy-1,2,3,4-thiatriazoles.[1-3] Since the properties of these rather unstable compounds are now known it seemed worthwhile to reinvestigate some of the methods which have earlier been tried without success. One of these methods aimed at the removal of hydrogen sulfide from *O*-alkyl thiocarbamates, the reverse reaction of the addition of hydrogen sulfide to alkyl cyanates, which according to our experiences proceeds smoothly.[1,2]

The reaction between *O*-ethyl thiocarbamate (xanthogenamide) and various metal compounds, such as HgO, Ag_2O, Ag_2CO_3, PbO, TlOH, $AgNO_3$, and $CuSO_4$ was studied about one hundred years ago by Debus,[4] Conrad and Salomon,[5] and Mulder.[6] Although, according to these authors, metal sulfides were formed and a sharp smelling substance was recognized no cyanate could be isolated. When performing the reaction between *O*-alkyl thiocarbamates and metal oxides, *e.g.* mercury(II) oxide, in ether solution at 0°C we have, however, been able to isolate the expected alkyl cyanate, formed according to the equation:

$$RO-CS-NH_2 + HgO \rightarrow RO-CN + HgS + H_2O$$

The water formed in the reaction was removed by means of $MgSO_4$. The alkyl cyanates were found by gas chromatography, infrared spectroscopy, and refractometry to be identical with the products prepared from 5-alkoxy-1,2,3,4-thiatriazoles.

The reaction was carried out with *O*-ethyl, *O*-propyl, *O*-isopropyl, and *O*-butyl thiocarbamate. The yields varied from 40 % to 57 % when mercury(II) oxide was used. With Ag_2O as the sulfur removing agent the yields could be raised about 5 %. PbO, PbO_2, Cu_2O, and CuO were found to react in a similar way but more slowly than HgO and Ag_2O.

O-Ethyl selenocarbamate, $EtO-CSe-NH_2$ was found to react very fast with HgO, but the yield of ethyl cyanate was very low (7 %).

Attempts to prepare alkyl cyanates from alkyl carbamates (urethans) by means of a dehydrating agent (P_2O_5, dicyclohexylcarbodiimide) were unsuccessful.

The comparatively low yields seem to be due to side reactions or trimerisation since an oily residue is always left when the cyanate is distilled from the reaction mixture; they may, however, also to some extent be due to the circumstance that our preparations were carried out on a rather small scale (about 0.5 g of the cyanates were prepared).

Experimental. Ethyl cyanate: 2 g of *O*-ethyl thiocarbamate were dissolved in 10 ml of dry ether and 4 g of dry magnesium sulfate were added. The solution was cooled at 0°C and 7 g of mercury(II) oxide were added in one portion (1.6 times the calculated amount) with stirring. The stirring was continued for