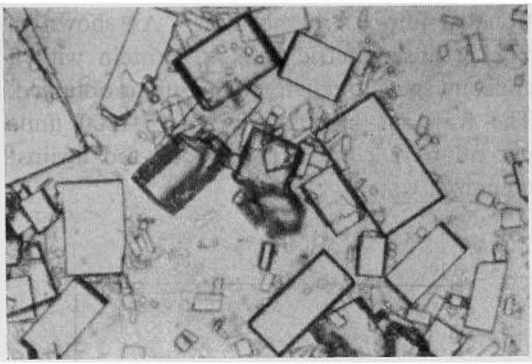

FIG. 4. Crystals of Lipase from *Geotrichum candidum* Link.

TABLE I. PURIFICATION PROCEDURE OF THE LIPASE FROM *Geotrichum candidum* LINK

Culture filtrate

- fractionated with ammonium sulfate (0.25 ~ 0.75 saturation)

Precipitate

- dissolved in deionized water and desalted with a column of Sephadex G–25

Desalted solution

- chromatographed on DEAE-Sephadex column equilibrated with 0.01 M acetate buffer, pH 5.0, and eluted by NaCl

Eluted solution

- chromatographed on Sephadex G–100 column equilibrated with 0.01 M acetate buffer, pH 5.0, and lyophilized

Lyophylized powder

- dissolved in 0.01 M acetate buffer, pH 5.0, and chromatographed on Sephadex G–200 column equilibrated with 0.01 M acetate buffer, pH 5.0.

Eluted solution

- dialyzed against deionized water and concentrated by means of a collodion bag under reduced pressure

First crystals

- recrystallized

Second crystals

crystallization. The microphotograph of crystals of lipase is shown in Fig. 4. The crystals formed were washed three times with cold deionized water and lyophilized.

The overall procedures of the purification and results are summarized in Tables I and II. The purification results in a 40 fold increase in specific acitivity with a recovery of 20% of the original activity.

2. *Physical properties of the crystalline lipase*

The homogeneity of the crystallized preparation of lipase from *G. candidum* Link was examined by analytical electrophoresis, disc electrophoresis on polyacryl amide gels and sedimentation. From the results shown in Figs. 5, 6 and 7, it is revealed that the preparation is homogeneous.

The sedimentation constant, $s_{20,\mathrm{w}}$ was determined to be 4.0, when the experiment was made from 0.856% to 0.428% in protein concentration. Figure 8 shows a plot of sedimentation constants in various protein concentrations.

The molecular weight of the lipase was estimated to be 53000–55000 by the method of gel filtration using Sephadex G–200 column (1.6×93 cm). Chymotrypsinogen A (M.W. 25000), ovalbumin (M.W. 45000), bovine

TABLE II. LIPASE ACTIVITIES IN THE COURSE OF PURIFICATION

Fraction and step	Total volume (ml)	Total activity (U)	Specific activity (U/mg protein)	Recovery of activity (%)
Culture filtrate	23300	1.53×10^6	11	100
Solution of precipitate	1060	1.29×10^6	37	84
Desalted solution	2780	1.16×10^6	50	76
DEAE-Sephadex Chromatography	252	1.00×10^6	259	65
Sephadex G–100 Chromatography	120	7.42×10^5	385	49
Solution of lyophilized powder	60	5.73×10^5	377	37
Sephadex G–200 Chromatography	258	4.57×10^5	447	30
Solution of first crystals	100	3.83×10^5	447	25
Recrystals	—	3.07×10^5	447	20

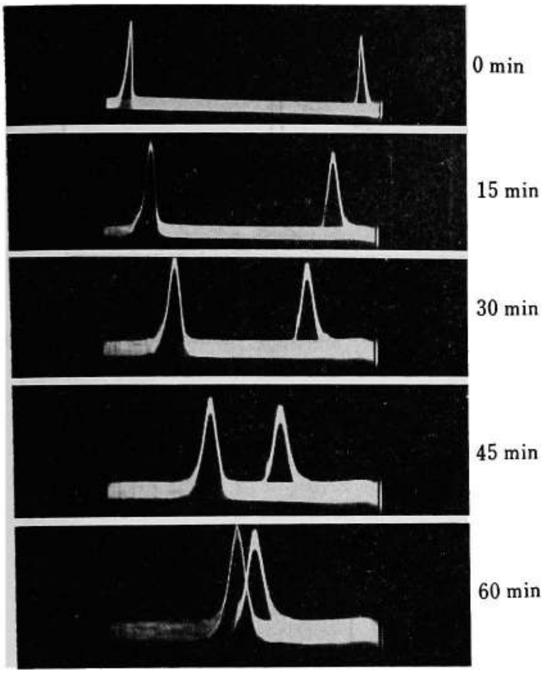

FIG. 5. Electrophoretic Patterns of Lipase from *G. candidum* Link.

The experiment was carried out at a concentration of 1% lipase in 0.1 ionic strength phosphate buffer at pH 7.9 under the condition of temperature 15°C, Voltage 100 volt, Ampere 15 mA. The photographs were taken at 0, 15, 30, 45 and 60 min.

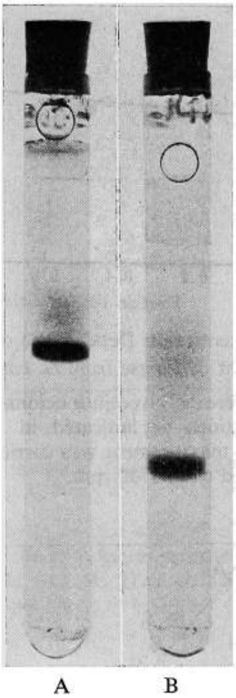

FIG. 6. Disc Electrophoretic Pattern of Lipase from *G. candidum* Link.

The enzyme, 200 μg was applied to the stacking gel and was subjected to electrophoresis in 0.1 M glycine-NaOH buffer, pH 9.4, at a current of 5 mA for 35 min (A) and 70 min (B).

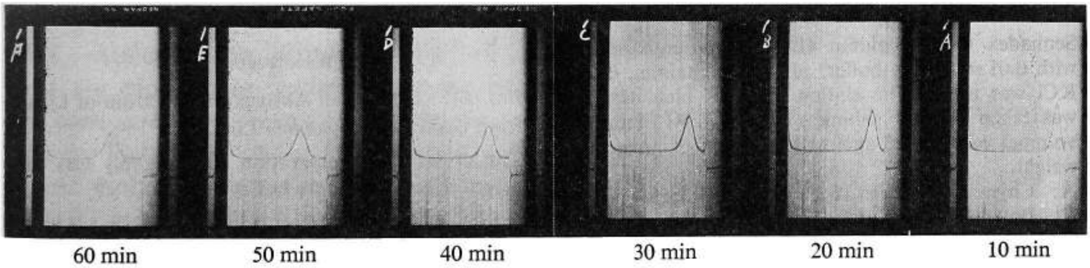

FIG. 7. Sedimentation Patterns of Recrystallized Lipase.

The experiment was carried out at a concentration of 0.856% lipase in deionized water at 21°C. The photographs were taken every 10 min after reaching the final speed (55700 rpm).

serum albumin (M.W. 67000) and γ-globulin (M.W. 160000) were used as standard proteins for molecular weight determination (Fig. 9). From the result of amino acid analysis as described later, it is considered that the value of M.W. 53000 is reasonable.

The isoelectric point of the lipase was determined by isoelectric-focusing method. It was estimated to be pH 4.33 from the effluent pattern shown in Fig. 10.

The ultraviolet absorption spectrum is shown in Fig. 11, from which $E^{1\%}_{280\,\mathrm{nm},1\,\mathrm{cm}}$ was calculated to be 10.8.