run with the crystalline material is not due to artefacts arising during chromatography. Furthermore the specific activities of the purified components also agree with those found in the peaks obtained in the first run of rennin crystals. In each of the A- and B-peaks, the specific activity is constant (within experimental error) across the peak, but over the C-peak there is a slight increase in specific activity. Whether this is due to overlapping contamination with B-rennin, or whether the C-rennin is a mixture itself is not yet clear.

The experiments indicate that the crystalline rennin investigated contains only small amounts of inactive material, and further that the crystals contain at least three components with different enzymatic activity.

Examination of the results of Jirgensons *et al.*[4] shows that the main component in their chromatogram has increasing specific activity over the peak, suggesting that this peak in fact represents a mixture of A- and B-rennins. This assumption is supported by the failure of attempts to separate A- and B-rennins by chromatography at pH 6.2 in this laboratory.

Full details of these experiments and further studies on the rennin fractions will appear in the *Comp. rend. trav. lab. Carlsberg.*

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Enzymatic Hydrolysis of Bifunctional S-Substituted L-Cysteine Derivatives

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Previous publications [1,2] have reported that the C—S-lyase of the endosperm of the seeds of *Albizzia lophanta* can act on a variety of S-substituted cysteine and cysteine sulfoxide derivatives. Whereas a typical monofunctional derivative such as S-ethyl-L-cysteine can undergo complete hydrolysis to pyruvic acid [3], results presented in this communication show that the two bifunctional cysteine derivatives investigated, djenkolic acid and L-lanthionine sulfoxide, are not fully hydrolyzed. The extent of hydrolysis of djenkolic acid, as measured by formation of pyruvic acid, amounted to 66.0, 67.2, and 65 % at substrate concentrations of 1, 2, and 2.5 mM, respectively (Fig. 1). Thus the actual maximum amount of pyruvate formed was pro-

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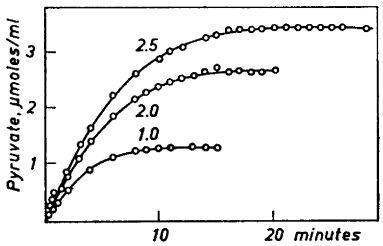

Fig. 1. Course of hydrolysis of djenkolic acid by *Albizzia* C—S-lyase. One ml of reaction mixture at 37° contained 35 μg of enzyme (specific acitivity = 200)[4], 40 μmoles of borate buffer, pH 8.0 and 0.02 μmole of pyridoxal phosphate. Micromoles per ml of djenkolic acid are indicated for each curve.

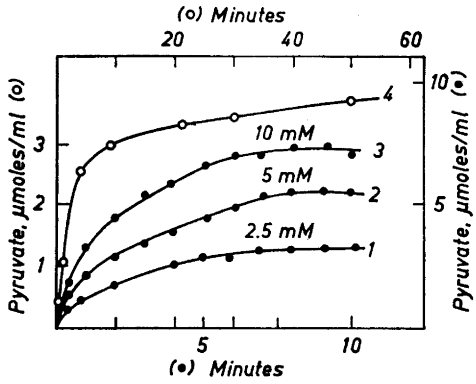

Fig. 2. Curves 1, 2, and 3-course of hydrolysis of L-lanthionine sulfoxide by *Albizzia* C—S-lyases at indicated substrate concentration under the same conditions as in Fig. 1. Curve 4,hydrolysis of mixture of 3,3-bis-(2-aminoethylthio)-*iso*butyric acid and 3-(2-carboxy-2-ethylaminothio)-3-hydroxybutyric acid by 172 μg/ml of enzyme. Concentration of substrate = 3.05 mg/ml, equivalent to 10 μmoles/ml of bisubstituted derivative.

portional to the substrate concentration, or the percentage hydrolysis was independent of the substrate concentration. On the other hand percent maximum hydrolysis of L-lanthionine sulfoxide was found to be dependent on the substrate concentration (Fig. 2). The three values found, 26 %, 22 %, and 14 % for substrate concentration 2.5, 5.0, and 10.0 mM fell on a straight line (in a substrate *vs* hydrolysis plot) which extrapolated to 30 % hydrolysis at zero substrate concentration.

It is suggested that the cessation of enzyme action prior to complete hydrolysis arises as a result of the inactivation of the enzyme by the highly reactive expected end products:

1. $R-S-CH_2-S-R + 2\ H_2O \longrightarrow S-CH_2-S + 2NH_3 + 2CH_3COCOOH$
2. $R-SO-R + 2\ H_2O \longrightarrow HSOH + 2NH_3 + 2CH_3COCOOH$

where $R = CH_2-CHNH_2-COOH$.
Thus djenkolic acid yields methylene dithiol and L-lanthionine sulfoxide yields the hypothetical sulfenic acid. Both of these sulfur-containing products could presumably react with the enzyme and inactivate it.

In addition to the above-mentioned difference between these two substrates, their course-of-hydrolysis curves appear to be quite distinct. Thus the course of hydrolysis of djenkolic acid is characterized by a linearity of rate for the first 20 to 30 % hydrolysis, followed by a progressive decrease in rate until the maximum is reached. The hydrolysis of L-lanthionine sulfoxide does not exhibit a distinct linear rate at the initial stages of the reaction. A linearity in rate at the later stage of hydrolysis prior to achievement of maximum hydrolysis was observed. In this respect, the course of hydrolysis resembles more closely that of a monosubstituted cysteine derivative (S-ethyl-L-cysteine [3]) and suggests that considerable product inhibition of the enzyme occurs during the reaction. The superimposition of product inhibition on product inactivation would then explain why the percent hydrolysis decreases with increasing substrate concentration.

Fig. 2 includes the course of hydrolysis of a preparation consisting mainly of 3,3'-bis-(2-carboxy-2-aminoethylthio)-*iso*butyric acid, which may be considered as an analogue of djenkolic acid [2]. The distinctly biphasic mode of the course of liberation of pyruvic acid probably arises from the presence of the impurity 3-(2-carboxy-2-aminoethylthio)-3-hydroxy*iso*butyric acid.

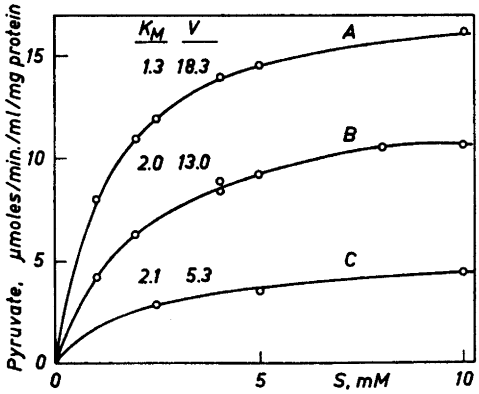

Fig. 3. Effect of substrate concentration on the initial rate of hydrolysis of djenkolic acid (A), S-ethyl-cysteine (B), and L-lanthionine sulfoxide (C) under the conditions of Fig. 1. The smooth curves were calculated from the classical Michaelis equation using values for the constants K_M and V shown in this figure. The points are the experimental values.

The ratio of the initial fast rate to the slow rate at the later stage of hydrolysis is equal to roughly about ten times the estimated ratio of the intensity of the ninhydrin spots on paper chromatograms of this preparation. This suggests that the mono-substituted cysteine derivative is much less susceptible to hydrolysis by the C—S-lyase than is the disubstituted derivative.

Fig. 3 shows the influence of substrate concentration on the rate of hydrolysis of djenkolic acid (A), S-ethyl-L-cysteine (B), and L-lanthionine sulfoxide (C). It will be noted that the Michaelis constants K_M vary considerably less than the values for V, the maximum rate when the enzyme is saturated with substrate. Let us assume as a first approximation, that K_M is a measure of the traditional steady-state constant $(k_{-1} + k_2)/k_1$ according to the usual formation $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E +$ product, and that V is a measure of the magnitude of k_2. If K_M remains constant while k_2 increases, then k_1 (the velocity constant for combination of enzyme with substrate) must also increase. This analysis corroborates the previous conclusion that the susceptibility to hydrolysis as function of the S-substituent depends upon the absence of structures in this substituent which would tend to interfere with hydrogen bond formation between the pyridoxal phosphate moiety of the enzyme and the substrate [2]. Such interference would tend to decrease the rate of formation of enzyme-substrate complex (k_1) and also decrease the rate of decomposition of the complex (k_2). This would tend to maintain K_M at a constant value, as was found experimentally.

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Preparation and Properties of Human Thyroglobulin

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The preparation and properties of thyroglobulin of animal origin has been described by several investigators [1-5]. Some chemical and physico-chemical studies on human thyroglobulin will be described briefly in the following.

The thyroid glands were frozen, sliced and extracted with 0.15 M NaCl for about 20 h at 5°C. The mixture was then centrifuged and the sediment discarded. Neutralized, saturated ammonium sulphate was added to the supernatant at a final saturation of 33 %. The sediment was removed by centrifugation and additional ammonium sulphate was added to the supernatant at a final saturation of 50 %. The sediment was collected by centrifugation and dissolved in distilled water. The solution was dialyzed against distilled water, and the dialysate was treated with ammonium sulphate as described above. The protein fraction precipitated between 33 and 50 % salt saturation was collected and investigated chemically and physico-chemically.

The iodine content of the material isolated from the thyroid glands was determined according to Kendall [6]. Nitrogen was determined by the Kjeldahl method. Ultracentrifugal studies were performed using the Spinco analytical ultracentrifuge working at 59 780 rpm. The protein concentration in the samples used for the ultracentrifugal analyses was 1 % and the total salt concentration in these samples was 0.17 M (0.15 M NaCl and 0.02 M phosphate, pH 7.0).

The main part of the iodine of the thyroid extracts was recovered in the protein fraction precipitated between 33 and 50 % saturation of ammonium sulphate. Table 1 gives the results of the chemical analyses carried out on the proteinaceous material thus obtained. The values for iodine and nitrogen content agree with those reported previously for animal thyroglobulin [2,3].

Table 2 shows the results of the ultracentrifugal analyses. It can be seen that the main high molecular weight component of the analyzed solutions has a sedimentation constant ($S_{20,\,aq}$) of about 17 *S*. Similar