Structural changes in the activation of chymotrypsinogen

Experimental evidence has been recently provided[1,2] to support the view that the conversion of chymotrypsinogen to π-chymotrypsin is accompanied by the hydrolysis of the arginyl-*iso*leucyl bond in the sequence leucyl-seryl-arginyl-*iso*leucyl-valine. The possibility exists that other more or less profound intramolecular changes likewise occur, creating a configuration endowed with enzymic functions[3].

Since the optical rotation of proteins has been shown to be very sensitive to structural changes[4,5,6,7], this method was applied in the work described in this communication.

Optical rotations were measured in a Rudolph Precision polarimeter equipped with a sodium vapor lamp. A 20 cm tube, 2 ml capacity, was maintained at 0° ± 0.5, using a water jacket. Activation mixtures were prepared under conditions leading, predominantly, to the formation of π-chymotrypsin. The composition of the solutions was as follows: chymotrypsinogen 5 × or 9 × crystallized, 40 mg per ml; trypsin 0.4 mg per ml; sodium phosphate buffer 0.05 *M*, pH 7.8, containing 0.1 *M* β-phenylpropionate. Activation was carried out at 0°. Optical rotation was measured continuously for the first 50 minutes and then at appropriate time intervals. Enzymic activity (acetyl-L-tyrosine ethyl ester as substrate[8]) was measured in 0.1 ml aliquots which were removed at predetermined time intervals and diluted to 10 ml with 0.005 *M* HCl to stop the activation. Measurements were extended up to 1000 minutes but no further changes in rotation beyond 400 minutes were found. The ordinate values are the per cent change of specific rotation or enzymic activity (the latter expressed as percentage of maximum activity attained after 200 minutes). The results are the average of duplicate experiments which were indistinguishable from one another over the initial 90 minute time interval.

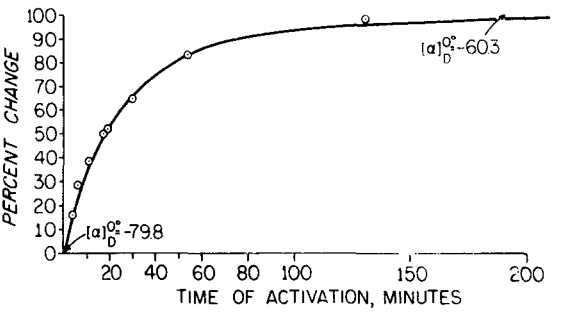

Fig. 1. Curve, optical rotation change during activation; O, esterase activity determinations.

It can be seen that the changes in optical rotation correspond exactly to the appearance of enzymic activity. A δ-activation mixture (without β-phenylpropionate) yielded a similar relationship, the final values of $[\alpha]_D^0$ for π- and δ-chymotrypsins being identical within the limits of the error. Hence no changes in either specific rotation or enzymic activity occur during the π- to δ-conversion.

Since the two enzymes, in contrast to chymotrypsinogen, dimerize[3] reversibly at pH 7.5, it was important to determine whether the changes in optical rotation are to be attributed to dimerization. To this end the rotation of chymotrypsinogen and δ-chymotrypsin was measured at various pH values in water and in the presence of 0.2 *M* NaCl. As can be seen from Fig. 2, the difference of specific rotations between chymotrypsinogen and δ-chymotrypsin varies little within the limits of pH 5–8. Since at pH 5, unlike pH 7.5, δ-chymotrypsin remains as a monomer even at high concentrations, the variation of rotation of δ-chymotrypsin with pH is thus attributable to structural changes other than dimerization.

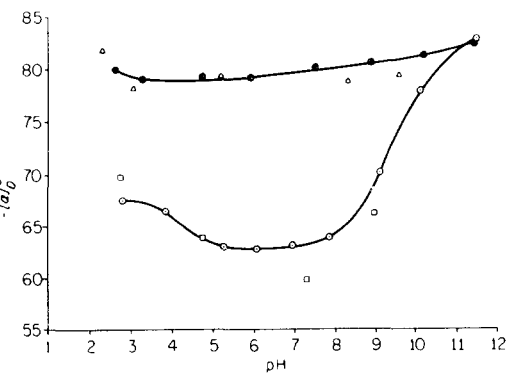

Fig. 2. ●, chymotrypsinogen in 0.2 *M* NaCl; △, chymotrypsinogen in water; O, chymotrypsin in 0.2 *M* NaCl; □, chymotrypsin in water.

It is worthy of note that the change in optical rotation attending activation is in opposite direction to that previously noted for the denaturation of proteins by urea[4] and heat[9] and for the denaturing effect ascribed to the action of trypsin on β-lactoglobulin[6]. In the light of recent interpretations[4,10] the decrease in levorotation observed herein might be attributed to a change toward a more constrained orientation of the peptide chains. In view of the virtual identity of the

extrapolated sedimentation constants of chymotrypsinogen, π- and δ-chymotrypsins[3], the three proteins must possess nearly equally compact structures. However, the greater sensitivity of the optical rotation of δ-chymotrypsin, as compared with chymotrypsinogen, to changes in pH might imply a greater flexibility of structure in the active enzyme. While no explanation can be offered for the discrepancy between the values for the specific optical rotation of chymotrypsinogen reported herein and some of those found in the literature[11,12,13] for the zymogen and α-chymotrypsin, it should be stated that the present data were obtained with two different preparations of chymotrypsinogen both of which were homogeneous by electrophoretic and end group analyses, and essentially free of active enzyme. Details of this work and analogous studies of the activation of trypsinogen will be published alsewhere.

This work has been supported by the United States Public Health Service, Research Grant C-2286 and by funds made available by the people of the State of Washington, Initiative 171.

JOHN A. RUPLEY
WILLIAM J. DREYER
HANS NEURATH

Department of Biochemistry, University of Washington, Seattle, Wash. (U.S.A.)

1 W. J. DREYER AND H. NEURATH, *J. Am. Chem. Soc.*, 77 (1955) 814.
2 W. J. DREYER AND H. NEURATH, *J. Biol. Chem.*, in press.
3 H. NEURATH AND W. J. DREYER, *Discussions Faraday Soc.*, in press.
4 W. KAUZMANN, in *The Mechanism of Enzyme Action*, ed. by W. D. MCELROY AND B. GLASS. The Johns Hopkins Press, Baltimore, 1954.
5 J. T. YANG AND J. F. FOSTER, *J. Am. Chem. Soc.*, 76 (1954) 1588.
6 L. K. CHRISTENSEN, *Compt. rend. trav. lab. Carlsberg, Ser. chim.*, 28 (1952) 37.
7 K. LINDERSTRØM-LANG, *Lane Medical Lectures*, Stanford University Press, Stanford (1952).
8 L. W. CUNNINGHAM, Jr., *J. Biol. Chem.*, 207 (1954) 443.
9 H. A. BARKER, *J. Biol. Chem.*, 103 (1933) 1.
10 C. COHEN, *Nature*, 175 (1955) 129.
11 J. T. YANG AND J. F. FOSTER, *J. Am. Chem. Soc.*, 77 (1955) 2374.
12 M. A. GOLUB AND E. E. PICKETT, *J. Poly. Sci.*, 13 (1954) 427.
13 B. JIRGENSONS, *Arch. Biochem. Biophys.*, 41 (1952) 333.

Received June 18th, 1955

Über die Messung des Energieumsatzes bei der Photosynthese mit dem Grossflächen-Bolometer

Das Grossflächen-Bolometer, von LUMMER UND KURLBAUM[1] in der Physikalisch-Technischen Reichsanstalt um 1900 entwickelt, ist bei den grundlegenden Arbeiten der Photochemie von EMIL WARBURG[2] als Strahlungsmesser benutzt worden. Das gleiche Instrument wurde bei den Messungen des Energieumsatzes der Photosynthese angewendet, 1920 in der Physikalisch-Technischen Reichsanstalt[3] und in den folgenden Jahren im Kaiserwilhelm-Institut für Biologie in Dahlem[4].

Da es keinen, für photosynthetische Experimente geeigneteren absoluten Strahlungsmesser gibt als das LUMMER-KURLBAUM-Bolometer, veranlasste ich 1945 in Berlin die Wiederherstellung dieser Instrumente mit dem Erfolg, dass die Firma Lassen*-Berlin vor kurzem 2 Grossflächen-Bolometer nach den U.S.A. liefern konnte, das eine nach Madison (Wisconsin) in das Laboratorium von FARRINGTON DANIELS, das andere nach Berkeley (Calif.) in das Laboratorium von MELVIN CALVIN.

Der Energieumsatz bei der Photosynthese ist dadurch in den U.S.A. in bemerkenswerter Weise angestiegen. Während RABINOWITSCH[5] aus dem Laboratorium von EMERSON, noch im Jahre 1952 einen mittleren Quantenbedarf von 12 pro Molekül Sauerstoff meldete, nennt 1955 DANIELS[6] als Mittelwert des von ihm gefundenen Quantenbedarfs die Zahl 8.9 und CALVIN[7] sogar die Zahl 4.9, wenn bei niedrigen Intensitäten — unter den Bedingungen unserer Versuche[3] von 1920 — die Dunkelatmung zu dem im Licht entwickelten Sauerstoff addiert wurde.

Trotz dieser Besserung schienen mir die Differenzen zwischen DANIELS und CALVIN noch so erheblich zu sein, dass man Fehler in ihren Arbeiten vermuten musste. Tatsächlich sind in beiden Arbeiten, wie im folgenden gezeigt wird, wesentliche Fehler bei der Messung der Lichtabsorption gemacht worden.

* Jetzt Firma Röhrig, Berlin S.O., Erkelenzdamm 59.