ken wir der Firma Sandoz AG. Basel und der Stiftung für Staatsstipendien in Griechenland für die gewährte Unterstützung.

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Summary

In a series of model reactions, it is shown that residues of α-aminoacids may be inserted by a particular rearrangement into certain carboxyl or carbonylamido groups. Repeated insertion results in the formation of a peptide derivative. It is concluded that natural peptides or proteins must not necessarily be formed by head to tail combination of aminoacids. Other implications of the new principle are discussed.

The Effect of Ferrimyoglobin on the Oxidation of Succinic Acid by Horse Heart Muscle Preparations[1]

Not much is yet known about the physiology of myoglobin. Millikan[2] has discussed the possible functions that myoglobin may have *in vivo* and has excluded the possibility that it can act as an oxygen carrier within muscle fibre; he has considered the possibility, which is not yet demonstrated, of a catalytic action of myoglobin, which has moreover intermediate properties between those of haemoglobin and those of the oxidases; he has concluded that the myoglobin principal property should be of oxygen storage for muscle activity.

Therefore, from Millikan's considerations, even accepting the prominent storage function of myoglobin, we cannot exclude other properties. Regarding haemoglobin, it has been universally demonstrated and accepted that a small part of it (1–3 %) is transformed in the red cells by autoxidation into (HbOH), and afterwards reduced by glycolysis. It has also been observed by Gibson[3] that a lack of this normal mode of reduction of HbOH causes the appearance of methaemoglobinaemia. If we consider that the velocity of autoxidation of myoglobin is much higher than that of haemoglobin, we can also assume that a normal enzymatic pathway should be present in the muscle for the reduction of ferrimyoglobin which could be formed in the muscle.

In these researches, the action of MbOH on two enzymatic systems of horse heart muscle has been studied. It has been observed that MbOH has no influence on the cytochrome-oxidase-system; on the contrary, the MbOH strongly inhibits the succinic oxidase system.

This inhibitory effect seems to be specific on the succinic dehydrogenase; in fact the inhibition is not removed by adding methylen blue but is completely reversed by adding KCN. This may be explained by suggesting that MbOH might oxidize SH groups of the succinic dehydrogenase; KCN may reverse the inhibitory effect by opening the S–S bridge formed after the oxidation; the succinic dehydrogenase activity is thus immediately restored. These results obtained with Warburg apparatus are quite clear from Figure 1.

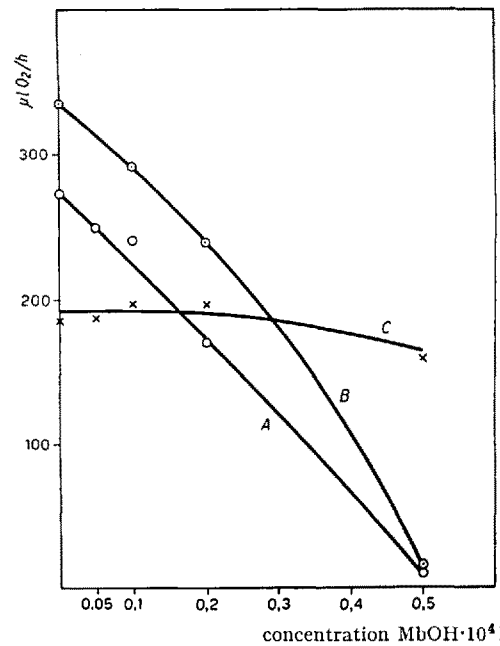

Fig. 1.–The inhibitory effect of the MbOH on the succinic oxidase and on the succinic dehydrogenase. In the center well 0·2 ml NaOH 30%; in the side arm 0·2 ml enzyme preparation and increasing amounts of MbOH; in the flask 0·2 ml Na succinate 0·4 M and phosphate buffer 0·18 M pH 7·25 up to 3 ml. Temperature 38°. Curve *A* enzyme + MbOH; curve *B* enzyme + MbOH + 0·3 ml methylene blue 0·01 M; curve *C* enzyme + MbOH + methylene blue + 0·2 ml KCN 0·1 M (neutralized with acetic acid).

The inhibitory effect of MbOH is not modified by adding variable and increasing amounts of cytochrome C. It is also conserved after denaturation of MbOH with NaOH. A very slight inhibition has also been observed after the addition of increasing amounts of HbOH in the same concentration of MbOH. In this case our results do not agree with those of Keilin and Hartree[1] who have observed a higher oxygen uptake by adding HbOH 2 % to succinic oxidase system.

This inhibitor effect is immediate and gradually increases. After one hour of incubation of the enzyme in contact with MbOH, the inhibition is more than doubled. This increasing inhibition after prolonged incubation is only slightly reversed by KCN. In these experiments we have used small amounts of MbOH in order to follow the gradual increase; these amounts are calculated in 0·75 milliequivalents for ml of enzyme. The results are clearly explained in Figure 2.

In addition the inhibitory effect of MbOH does not remain long in the solutions of MbOH, and even the crystals of MbOH conserved for some months at 0° have, after dialysis, a notable decrease of inhibitory effect. The highest inhibition has been observed with MbOH solutions of recent crystallization.

The experimental conditions were as follows: horse MbOH crystallized according to Rossi-Fanelli[2] has been dialyzed and used in concentration 0·3 mM; the concentration of the solution has been measured according to the De Duve's method; horse haemoglobin, crys-

[1] Aided by a grant of the Rockefeller Foundation and the Consiglio Nazionale delle Ricerche.

[2] G. A. Millikan, Physiol. Rev. *19*, 503 (1939).

[3] Q. H. Gibson, Biochem. J. *42*, 14 (1948).

[1] D. Keilin and E. F. Hartree, Biochem. J. *41*, 500, 503 (1947).

[2] A. Rossi-Fanelli, *Haemoglobin* Butterworths Sci. Publ., 115 (1949).

tallized according to DRABKIN[1], has been oxidized by adding $K_3Fe(CN)_6$ in molar ratio 8:1; the solution has been dialyzed to remove the $K_3Fe(CN)_6$ and used in concentration equal to that of MbOH, after spectrophotometric measurements; the enzyme has been prepared according to KEILIN and HARTREE[2]; succinate,

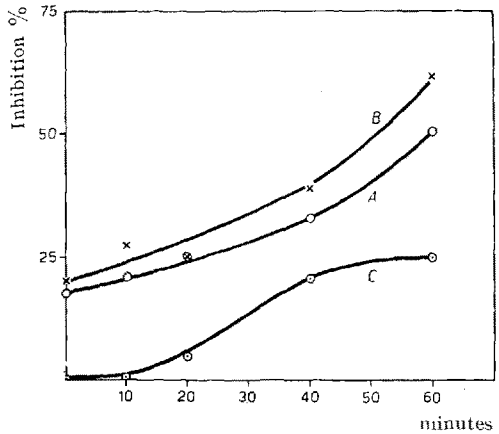

Fig. 2.–The gradual inhibitory effect of MbOH on the succinic oxidase and on the succinic dehydrogenase. In the center well 0·2 ml NaOH 30%; in the side arm 0·2 ml enzyme and 0·1 ml MbOH 3×10^{-4} M; in the flask 0·2 ml Na succinate 0·4 M and phosphate buffer 0·18 M pH 7·25 up to 3 ml. After 20 min of equilibration the side arm content is tipped into the first flask and this is considered time 0 min. Then the other side arms content is tipped at the time marked on the figure an the O_2 uptake for 60 min is measured. Curve *A* enzyme + MbOH; curve *B* enzyme + MbOH + 0·3 ml methylene blue 0·01 M; curve *C* enzyme + MbOH + methylene blue + 0·2 ml KCN 0·1 M neutralized with acetic acid.

KCN, methylene blue have been used in concentrations indicated above; cytochrome C with 0·456 Fe^3 has been used in concentration 0·75 mM; to measure the cytochrome oxidase activity p-phenylendiamine (chlorhydrate) 0·5 M neutralized with sodium carbonate, has been used.

In our experiments we exclude the possibility that the inhibitory effect may be due to the presence of haematin which has also been observed from KEILIN and HARTREE to inhibit succinic dehydrogenase.

As we have suggested above, the inhibitory effect may be explained by the oxidation of succinic dehydrogenase SH groups by MbOH, and the reversing action of the KCN by the opening of S–S bridge.

More detailed accounts of the present results will be published in forthcoming papers.

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Institute of Biological Chemistry, University of Rome, June 15, 1955.

Riassunto

L'autore ha studiato l'azione della metamioglobina sul sistema succinossidasico. È stata riscontrata una netta inibizione che sembra essere specifica per la succinodeidrogenasi. Tale inibizione non è influenzata dall'aggiunta di bleu di metilene ma invece completamente rimossa dal KCN in presenza di bleu di metilene. L'inibizione provocata dalla metamioglobina sulla succinossidasi aumenta nel tempo.

[1] D. L. DRABKIN, J. Biol. Chem. *185*, 231 (1950).
[2] D. KEILIN and E. F. HARTREE, Biochem. J. *41*, 500, 503 (1947).
[3] Kindly sent by Sierotherapic Institute of Milan.

Interaction of Pepsin with Lysozyme

Interaction of proteins with other large molecules has been found by many research workers in studies with nucleic acids, with polysaccharides, and with numerous polymeric materials. It has also been shown that similar interactions can be realized between proteins; and thus, precipitation of proteins by protamines, first reported by KOSSEL[1], has been ascribed to the presence of opposite net charges on the reactans.

From the biological point of view the most important interactions are such as occur among protein and protein in some natural systems, such as milk (NITSCHMANN and ZÜRCHER[2]), as blood serum (GREEN[3]; ONCLEY, ELLENBOGEN, GITLIN and GURD[4]), or gastric juice (CAPUTO[5]).

It is the purpose of this note to report some information on the pepsin-lysozyme complex, which may play an important role in studies of gastric juice.

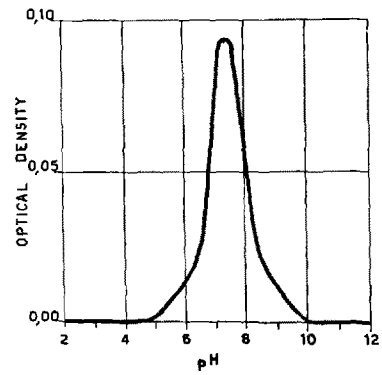

Fig. 1.–Turbidimetric behaviour of mixture of $1^0/_{00}$ pepsin and $1^0/_{00}$ lysozyme in acetic acid/sodium acetate buffer 0·02 M.

Figure 1 shows the reaction of crystalline pepsin with crystalline lysozyme at different pH values; from the turbidimetric behaviour of mixtures of pepsin and lysozyme, it appears that the maximum of turbidity is confined to regions of pH in which the proteins carry charges of opposite sign, and therefore the anionic protein precipitates the cationic protein only on the acid side of the isoelectric point of the latter. As shown in Table the solubility of protein-protein complex is very sensitive to ionic strength, and sodium chloride tends to dissociate the complex.

Table

NaCl molarity	Δ% of complex dissociation
0·05–0·10	20
0·10–0·15	17
0·15–0·20	15
0·20–0·25	14
0·25–0·30	13

Electrophoretic analysis has revealed that the complex exhibited a single sharp boundary when the mixture is made in about equal proportions of pepsin and lyso-

[1] A. KOSSEL, Dtsch. Med. Wschr. *20*, 147 (1894).
[2] H. NITSCHMANN and H. ZÜRCHER, Helv. Chim. Acta *33*, 1698 (1950).
[3] A. A. GREEN, J. Amer. Chem. Soc. *60*, 1108 (1938).
[4] J. L. ONCLEY, P. ELLENBOGEN, D. GITLIN, and F. R. N. GURD, J. Phys. Chem. *56*, 85 (1952).
[5] A. CAPUTO, G. Biochim. *1955* (in press).