On the mechanism of amylase synthesis

The increase of amylase activity in a soluble system from acetone-dried pigeon pancreas was reported previously[1]. The detailed account of this work[2] and further results[3,4] are in press. Owing to external difficulties in the publication of these papers, we wish to summarize our recent findings.

In the soluble system referred to above, amylase activity increases if ATP, the salts of a Krebs saline solution and a mixture of amino acids are added. The amino acid mixture can be replaced by 0.5 mg/ml L-arginine + 0.5 mg/ml DL-threonine. Addition of other amino acids did not further influence the increase of amylase activity (Table I, Expt. 1). It is remarkable that the increase in amylase activity is inhibited by the addition of minimal amounts of D(−)-*threo*-chloramphenicol, *p*-fluorophenylalanine, or ribonuclease (Table I, Expt. 2–4). In our opinion, amylase is synthesized in this system from a precursor protein. The two amino acids are used for this synthetic reaction and it will proceed only in presence of a ribonucleic acid. The precursor seems to be adsorbed on this surface, as is indicated by the *p*-fluorophenylalanine inhibition.

TABLE I

Reaction mixture: 0.4 ml water extract of acetone-dried pigeon pancreas + 0.6 ml incubation mixture. The latter contained 1 ml 10% neutral ATP solution, 3.2 ml of doubly-concentrated Krebs-saline solution and 0.3 ml amino acid mixture. The mixture was incubated at 37° C. Amylase activity is expressed in Smith and Roe units.

Expt. No.	*Inhibitor*	*Amylase units/ml mixture*	
		At zero time	*Change in 45 min*
1 a. Casein hydrolysate	—	4950	+ 560
b. Threonine + arginine	—	3930	+ 600
2 a. Threonine + arginine	—	4150	+ 950
b. Threonine + arginine	Chloramphenicol (1 μg/ml)	4360	− 290
3 a. Threonine + arginine	—	3590	+ 590
b. Threonine + arginine	*p*-Fluorophenylalanine (100 μg/ml)	3810	− 120
4 a. Threonine + arginine	—	4540	+ 1060
b Threonine + arginine	Ribonuclease (16 μg/ml)	4700	− 80

We found that the observed synthesis of amylase in a granular fraction ("mitochondria") of pigeon pancreas[5] requires the same constituents: ATP, salts, threonine and arginine. HOKIN[6] has already found the synthesis of amylase in pancreas slices to suffer more by the absence of threonine and arginine, than by the absence of the other amino acids.

It seems likely that this secretory enzyme is formed *in vivo* in at least two steps, the second step being a partial synthesis from precursor protein and arginine + threonine.

F. B. STRAUB
Á. ULLMANN

Institute of Medical Chemistry, University of Budapest (Hungary)

[1] F. B. STRAUB, Á. ULLMANN AND G. ÁCS, *Biochim. Biophys. Acta*, 18 (1955) 439.
[2] Á. ULLMANN AND F. B. STRAUB, *Acta Physiol. Hung.*, (in the press).
[3] T. GARZÓ, K. PERL, M. T. SZABÓ, Á. ULLMANN AND F. B. STRAUB, *Acta Physiol. Hung.* (in the press).
[4] Á. ULLMANN AND F. B. STRAUB, *Acta Physiol. Hung.* (in the press).
[5] Á. ULLMANN AND F. B. STRAUB, *Acta Physiol. Hung.*, 10 (1956) 137.
[6] L. E. HOKIN, *Biochem. J.*, 50 (1951) 216.

Received January 3rd, 1957

Molecular interaction between purines and steroids

A discussion of the results of WEIL-MALHERBE[1], and of WEBER[2] concerning the ability of purines to form molecular complexes with polycyclic aromatic hydrocarbons and riboflavin led to the suggestion that purines might also form complexes with steroid hormones. During the past few years exploratory experiments have been conducted, and we wish to describe here the results of these experiments. A more detailed study of one of the observed interactions has been recently completed in collaboration with Dr. ALLAN U. MUNCK and will be reported separately.

The method of investigation has been to measure the solubility of steroids in buffered aqueous solutions of purine compounds. Finely divided solid steroid was added to purine solutions and

after prolonged mixing at a fixed temperature the excess solid remaining was removed by filtration through ultrafine sintered glass filters. In other experiments the solid was always confined within a sac of dialysis membrane during saturation of the solution. The total steroid present in solution was extracted with a suitable immiscible solvent ($CHCl_3$, CH_2Cl_2, or cyclohexane) in which the purine is insoluble. The concentrations of steroid and of purine were then determined by ultraviolet absorption of the separate solutions. The assumption is made that the dissolved steroid in excess of that present in aqueous buffer alone represents steroid held in solution by virtue of complex formation with the purine.

In early studies it was found that the solubilities of progesterone, deoxycorticosterone, and testosterone were definitely increased in the presence of adenine, adenosine, and the isomers of adenosine monophosphate.

The interaction of testosterone with adenosine was chosen for a more careful study of the relation of steroid solubility to purine concentration. The results of this study are presented in Fig. 1. It is evident from the linear relation between concentrations of steroid and adenosine that only one mole of adenosine is involved per mole of complex. Since the concentration of steroid in this system cannot be varied independently, the mole ratio of adenosine to steroid could not be established. Assuming, however, that the mole ratio is 1:1, one can write

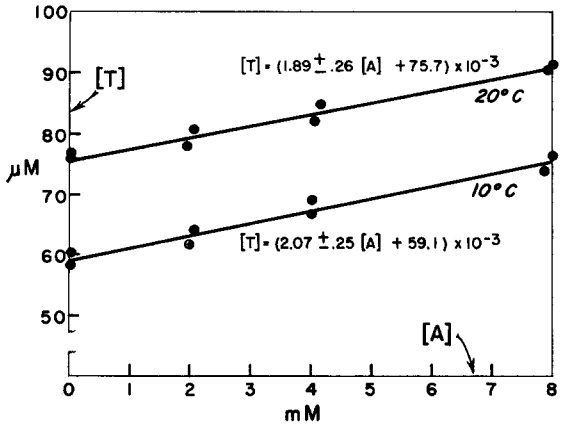

Fig. 1. Solubility of testosterone in 0.01 *M* phosphate buffer, pH 7.0 as a function of adenosine concentration. Lines drawn from the regression equations given in the figure, which were in turn calculated from the correlation coefficient. [T] is total testosterone in solution (T_o + T of TA); [A] is adenosine in solution.

$$T_s \rightleftharpoons T_o$$
$$T_o + A \rightleftharpoons TA$$

where T_s is solid testosterone, T_o unbound testosterone in solution, A is unbound adenosine in solution, and TA is the complex. In the presence of an excess solid, T_o will be the saturation concentration of testosterone.

Let the standard state be solid steroid at 20° and assume that the activity of the solid changes inappreciably over the range 10° to 20°. The activities of T_s and T_o are therefore unity at these temperatures. It is reasonable to assume that in the dilute solutions involved here the activity coefficients of adenosine and of complex are essentially unity. Moreover, since the amount of adenosine bound as complex is small compared with the free adenosine we may then write for the equilibrium constant of the interaction

$$K = [TA]/[A].$$

With these assumptions and approximations the over-all ΔH of both equilibria given above may be calculated from the data in Fig. 1. This was found to be -1.5 kcal/mole. The heat of solution for testosterone can also be calculated from these data and is found to be $+4.2$ kcal/mole, which is in good agreement with the value found by BISCHOFF AND STAUFFER[3]. The difference of these two ΔH's, the heat of the complex formation, is -6 ± 4 kcal/mole. The standard error was calculated from the standard error of the regression coefficients (equilibrium constants). The solubility values for testosterone ([A] = 0) were not included in the calculation of the correlation coefficient, in order to test the extrapolation of the regression line to the intercept.

The solubility characteristics of testosterone are such[4] that it is quite easy to obtain supersaturated solutions under some conditions of equilibration. Further evidence for complex formation was obtained by study of the distribution coefficients of testosterone between cyclohexane and buffered aqueous solutions of adenosine. The results are given in Table I. In these experiments the concentration of testosterone was far below saturation.

This study was initiated with the idea that should the complex-forming properties of purines extend to the steroids it was conceivable that the physiological effects of the steroids could be

TABLE I

EFFECT OF ADENOSINE ON DISTRIBUTION COEFFICIENT OF TESTOSTERONE BETWEEN AQUEOUS BUFFER AND CYCLOHEXANE

Adenosine concentration mM	*k*
0	10.3
9.50	8.05
17.5	6.44
35.0	4.49

Temperature 25° C. Phosphate buffer 0.01 *M*, pH 7.0.

$$k = \frac{\text{concn. testosterone in cyclohexane}}{\text{concn. testosterone in aqueous phase}}$$

mediated by formation of complexes with purine nucleotides. The behavior of coenzymes containing such nucleotides, the nucleic acids, or both might thereby be altered. The results described in this paper afford evidence of complex formation between some steroids and compounds containing purines. Attempts to demonstrate complex formation between deoxynucleic acid and testosterone were inconclusive because of the difficulty of separating finely divided solid testosterone from the high polymer.

Recent work by MARCUS AND TALALAY[5,6], LANGER AND ENGEL[7], and RYAN AND ENGEL[8] on several enzyme systems which utilize steroids as substrates, and by VILLEE[9], and VILLEE AND GORDON[10,11] on the rate-accelerating effect of estrone and estradiol on an *iso*citric dehydrogenase system derived from placenta and uterus are of considerable interest. All of these reactions require pyridine nucleotides and it is not clear whether the substrate specificity or the effect on the reaction rate is dependent on the enzyme, the coenzyme, or a combination thereof.

A subsequent paper will present the effect of small changes in either the steroid or the purine structure on the equilibrium constant of complex formation. This information forms the rational basis for an experimental approach to the biochemical significance of such complex formation.

Acknowledgements. We should like to acknowledge the contribution of Mr. ARTHUR GRAYZEL, Mr. DAVID FEINGOLD, and Mr. PHILIP LEDER to various phases of this project. We also gratefully acknowledge the support of the American Cancer Society (Grants BCH-17 and H-15) upon recommendation of the Committee on Growth, National Research Council, a grant No. C-1393(C4) from the National Cancer Institute of the National Institutes of Health, and a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

JESSE F. SCOTT*
LEWIS L. ENGEL

The John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University; the Department of Biological Chemistry and Department of Anatomy, Harvard Medical School, Boston, Mass. and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. (U.S.A.)

1 H. WEIL-MALHERBE, *Biochem. J.*, 40 (1946) 351, 363.
2 G. WEBER, *Biochem. J.*, 47 (1950) 114.
3 F. BISCHOFF AND H. R. PILHORN, *J. Biol. Chem.*, 174 (1948) 663.
4 F. BISCHOFF AND R. D. STAUFFER, *J. Am. Chem. Soc.*, 76 (1954) 1962.
5 P. I. MARCUS AND P. TALALAY, *Proc. Roy. Soc. (London)*, 144*B* (1955) 116.
6 P. I. MARCUS AND P. TALALAY, *J. Biol. Chem.*, 218 (1956) 661, 675.
7 L. LANGER AND L. L. ENGEL, *Federation Proc.*, 15 (1956) 296.
8 K. RYAN AND L. L. ENGEL, *J. Am. Chem. Soc.*, 78 (1956) 2654.
9 C. A. VILLEE, *J. Biol. Chem.*, 215 (1955) 171.
10 C. A. VILLEE AND E. E. GORDON, *ibid.*, 216 (1955) 215.
11 E. E. GORDON AND C. A. VILLEE, *Endocrinology*, 58 (1956) 1950.

Received January 7th, 1957

* Scholar in Cancer Research of the American Cancer Society.