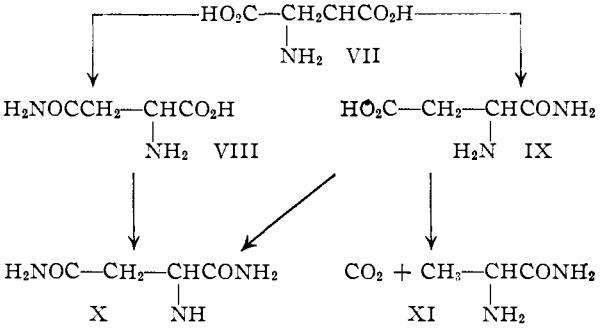

other hand, the β-amide (VIII, asparagine) formed first, then decarboxylation did not occur and VIII was further amidated to IX.

When crystalline beef insulin was subjected to ammonolysis, the only C-terminal acid which could be detected, with certainty, was alanine, although traces of asparagine and glycine were also found (see Table I). The failure to find more than a trace of asparagine was attributed to the conversion of asparagine to aspartic diamide.

Conclusions.—Table II summarizes the applicability of the ammonolysis procedure to the determination of the C-terminal amino acids in peptides. Of the 16 different C-terminal amino acids studied, only 3, asparagine, cystine and glutamic acid, could not be detected in peptides by this procedure.

The main disadvantage of the present ammonolysis procedure is that it cannot be applied routinely to proteins. However, modification of the separation scheme may obviate this difficulty. It should be noted that the ammonolysis procedure has been investigated more extensively with known compounds than any other chemical method of determining C-terminal amino acids. The irregularities encountered in the experiments reported here demonstrate the dangers inherent in drawing conclusions from any C-terminal method until it has been tested on a wide variety of known compounds in which most of the possible C-terminal amino acids are represented. This is particularly significant in view of the conflicting reports in the literature concerning the identity of the C-terminal amino acids in certain proteins, such as insulin. It should also be noted that some of the side reactions encountered in the ammonolysis reaction might be expected to occur in the hydrazinolysis procedure of Akabori.[5] In fact Locker has just recently reported[21] that arginine, cysteine, cystine, aspartic acid, asparagine, glutamic acid and glutamine are destroyed on heating in anhydrous hydrazine at 108° for 10 hours. These are the same amino acids which we judged to be non-detectable in the ammonolysis procedure described here.

TABLE II

DETECTABILITY OF THE C-TERMINAL AMINO ACIDS IN PEPTIDES BY THE AMMONOLYSIS PROCEDURE

Investigated		Not investigated		
Detectable		Not detectable	Probably detectable	Probably not detectable
Alanine	Norvaline	Asparagine	Isoleucine	Cysteine
Aspartic acid	Serine	Cystine	Lysine	Glutamine
Glycine	Methionine	Glutamic acid	Threonine	Isoasparagine
Histidine	Tryptophan			Isoglutamine
Leucine	Tyrosine			Arginine
Phenylalanine	Valine			
Proline				

(21) R. H. Locker, *Biochim. Biophys. Acta*, **14**, 533 (1954).

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENTS OF PHARMACOLOGY, UNIVERSITY OF TENNESSEE, AND BIOCHEMISTRY, UNIVERSITY COLORADO MEDICAL SCHOOL AND OAK RIDGE NATIONAL LABORATORY]

Enzyme Inhibition by Complexing of Substrates: Inhibition of Tyrosinase by Titanium Compounds

BY D. J. CAVANAUGH, JOSEPH HARRIS[1] AND J. Z. HEARON

RECEIVED OCTOBER 13, 1954

The oxidation of O-dihydric phenolic substrates by tyrosinase was found to be inhibited by Ti^{IV} and triethanolamine titanate. The kinetics of this inhibition were in accord with the hypothesis that it was due to competition between enzyme and inhibitor for the substrate rather than between substrate and inhibitor for the enzyme. The details of the kinetics with regard to 3,4-dihydroxyphenylalanine as the substrate have been presented here. This type of inhibition clearly demonstrated one of the possible modes of action in enzyme systems of metals capable of forming complexes with the substrates.

The effects of metal–substrate reactions[2,3] and of complex ions[4] on enzymatic reactions have been examined to some extent. The case of a type of competitive inhibition arising from competition between enzyme and metal or metal–complex for the substrate has not received detailed consideration. This paper is concerned with such inhibition as it occurs between tyrosinase and the complex, triethanolamine titanate (TAT).[5]

The formation and analytical employment of Ti complexes, *e.g.*, with 1,2-dihydroxybenzene-3,5-disulfonate, have been studied.[6–9] The structural

(1) A portion of the preliminary work was done by J. H. at Baxter Laboratories, Inc., Morton Grove, Illinois.

(2) R. J. P. Williams, *Biol. Rev. (Cambridge)*, **28**, 381 (1953).

(3) E. L. Smith, *Adv. Enzymol.*, **12**, 191 (1951).

(4) F. P. Dwyer, E. C. Gyarfas, W. P. Rogers and J. H. Koch, *Nature*, **170**, 190 (1952).

(5) TAT-21 and titanium lactate were obtained from E. I. du Pont de Nemours & Co., Pigments Dept., Wilmington, Del. TAT-21 contains Ti in the form of the diisopropyl ester chelated with 2 molecules of triethanolamine. The authors are grateful to the du Pont Co. for generous supplies of these compounds.

(6) J. H. Yoe and A. R. Armstrong, *Anal. Chem.*, **19**, 100 (1947).

(7) A. E. Harvey and D. L. Manning, THIS JOURNAL, **74**, 4744 (1952).

(8) E. Hines and D. F. Boltz, *Anal. Chem.*, **24**, 947 (1952).

(9) J. Piccard, *Ber.*, **42**, 4343 (1909).

similarity between such complex-forming compounds and the O-dihydric substrates of tyrosinase suggested the possibility of Ti inhibition of tyrosinase through complex formation with its substrates.

Several catechols, including 3,4-dihydroxyphenylalanine (dopa) and pyrocatechol, gave the antic-i pated reactions with Ti in the forms of both the lactate and TAT. The reaction products were intensely colored (orange-yellow). Monohydroxy compounds such as tyrosine did not form colored reaction products. Preliminary experiments showed that both the lactate and TAT inhibited the oxidations catalyzed by tyrosinase and that TAT was a much more potent inhibitor than the Ti ion. This property was used to advantage in making the tyrosinase preparations described below and formed the subject of the present investigation.

Experimental

Enzyme Activity.—Rates of oxygen consumption were measured manometrically, using the standard Warburg apparatus. The gas phase was air and in most cases the temperature was 25°. The reaction mixtures were buffered to *p*H 6.5 with 0.067 *M* phosphate buffer. In all cases the reagents were added to the main compartment and the enzyme was contained in the side arm. Control experiments showed that there was no gas evolved by the enzyme and that any decarboxylation of the substrate was not sufficient during the first 15 min. to affect the initial rate measurably. Accordingly, base was omitted from the center well in most cases. All reagents were dissolved in buffer immediately before using. The enzyme was dissolved in deionized water. In all cases the data given here refer to rates determined from readings made at 2–3 minute intervals after adding the enzyme.

Reagents.—Standard reagents were AR grade. TAT solutions were prepared using the assumed formula weight 462.44 in calculating the molality. The extent of hydrolysis and polymerization of TAT could not be determined.

Triethanolamine[10] was not purified and contained small amounts of mono- and diethanolamines.

Spectrophotometry.—Data for the continuous variation experiments were obtained using a Beckman model DU spectrophotometer.

N Determination.—Protein content of the enzyme solution was computed from N determinations using a direct nesslerization method.[11]

Enzyme Preparation.—Mushrooms were obtained from commercial sources and stored frozen at −20°. The frozen mushrooms were cut and blendorized without thawing, using 1500 ml. of water to which 7.5 ml. of TAT had been added per 700 g. of mushrooms. The mince was filtered through muslin and the filtrate was passed through a medium paper such as Whatman #1. The temperature was kept at about 10°. The filtrate was chilled in an ice-bath and, with constant stirring, was brought to *p*H 4.8 with HCl. To facilitate flocculation of the precipitate 30 ml. of saturated KCl solution/liter of filtrate was added. The precipitate was collected by centrifugation and extracted with 100 ml. of water to which enough dilute NaOH was added to bring the *p*H to 7.5. The mixture was centrifuged and the sediment was discarded. The supernatant was chilled and mixed with cold acetone (−20°) to make an 80% (v./v.) acetone concentration. The mixture was chilled at −20° for 30 min. and centrifuged. The precipitate was taken up in about 30 ml. of water and centrifuged. The supernatant was brought to 0.8 saturation with solid $(NH_4)_2SO_4$. After several hours in the cold the precipitate was spun down and taken up in a few ml. of water. The mixture was centrifuged and the supernatant was again brought to 0.8 saturation with $(NH_4)_2SO_4$. The previous step was repeated and the enzyme, after one further precipitation, was dialyzed against water for 48 hours. The dialyzed solution was stored frozen. Repeated freezing and thawing did not affect the activity. The final solutions were clear and light brown in color. There was no blackening upon standing in air and the light brown color did not bleach upon adding ascorbic acid to such solutions. It was concluded that melanin was probably absent and that the TAT had effectively removed substrates which often give rise to melanin in such enzyme preparations. Some of the properties of a typical preparation are given in Table I.

TABLE I

Q_{O_2} values refer to the uptake during the first ten minutes per mg. protein (calculated from N determinations). The concentrations given refer to those used in the runs from which these data were calculated.

Substrate	Q_{O_2}, *M*	*Ks*	*Vm*
Catechol	685 (2×10^{-3})		
Dopa	717 (2.5×10^{-3})	2.3×10^{-3}	13.17
Tyrosine	228 (10^{-3})		
Tyrosinol	111 (10^{-3})		

Although TAT was used primarily to avoid substrate-removing procedures such as washing with cold acetone,[12] it apparently contributed to the protection of the enzyme during acidification, which inactivates it.[13] In preliminary experiments lowering the *p*H to 4.5–5.0 in the absence of TAT always resulted in extensive inactivation.

TAT was strongly bound by the initial precipitate. This was shown by its liberation from such precipitates by water extraction. The complex was easily detected by simply adding a little dopa to the solution in question. The intense color formed was easily distinguished from that due to dopa oxidation by adding a little ascorbic acid.

Color due to TAT was intensified by the ascorbic acid, while that due to substrate oxidation was bleached by the reducing agent. TAT in concentrations of 10^{-5} *M* could be detected in this manner. In no case did the final enzyme solution contain detectable TAT.

The use of cold acetone to precipitate the enzyme has been described.[13,14] In the present case a good deal of inert material precipitated with the enzyme and the subsequent salt precipitations were essential to remove the bulk of the inactive material.

Results and Discussion

I. Inhibition of Dopa Oxidation.—Inhibition of the oxidation of catechol, dopa, tyrosine and tyrosinol[15] was observed. In the two latter cases this was presumably due to inhibition of the oxidation following hydroxylation to dopa or its analog. Only the case of dopa will be considered here.

Plotting $1/v$ *vs.* $1/S$, v and S, respectively, being the initial rate of O_2 uptake and substrate concentration, resulted in curves typified by those in Fig. 1. This result is generally taken to indicate the usual type of competitive inhibition.[16] However, in the light of the observed reaction between the inhibitor and substrate in this case it appeared that an alternative explanation would account for the inhibition. The alternative was to consider that the competition was between enzyme and inhibitor for the substrate.

In addition to the rate dependency upon the substrate concentration at fixed inhibitor levels it was desirable to examine the effects of inhibitor concentration. Figure 2 shows that there is an apparent linearity in the $1/v$ *vs.* [TAT] curve until low TAT concentrations are reached. It will be shown (eq. 12) that according to the hypothesis mentioned

(10) Mono-, di- and triethanolamines were generously supplied by Jefferson Chemical Co., Inc., New York.

(11) M. J. Johnson, *J. Biol. Chem.*, **137**, 575 (1941).

(12) M. F. Malette, *et al.*, *Arch. Biochem.*, **16**, 283 (1948).

(13) F. Kubowitz, *Biochem. Z.*, **292**, 221 (1937).

(14) J. D. Ponting and M. A. Joslyn, *Arch. Biochem.*, **19**, 47 (1948).

(15) Obtained from the Baxter Laboratories, Inc., through the courtesy of Dr. L. Ginger.

(16) P. Wilson in H. A. Lardy, ed., "Respiratory Enzymes," Burgess Pub. Co., Minneapolis, Minn., 1948, pp. 16–57.

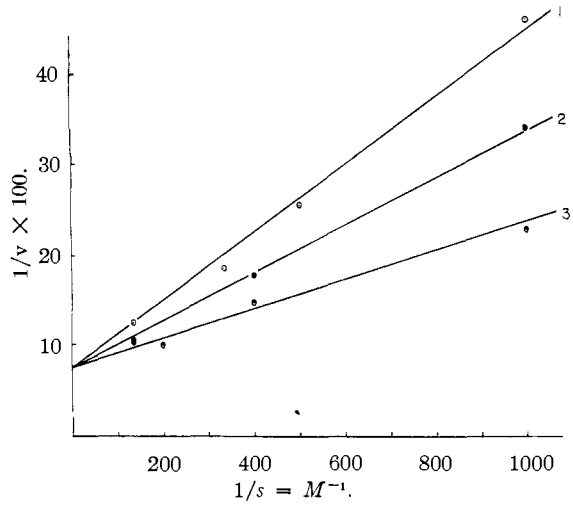

Fig. 1.—Curve 1, 2.7 × 10^{-3} M TAT; curve 2, 1.35 × 10^{-3} M TAT; curve 3, control; substrate *dl*-dopa.

above such a linear relationship would obtain. The cause and nature of deviations from linearity at low [TAT] will be discussed.

If it is assumed that no appreciable enzyme–TAT interaction occurs but that TAT and substrate react according to M + S $\rightleftarrows$ MS, then in terms of the association constant, K, for this reaction and the total substrate concentration, S_0 = [S] + [MS], (and with the assumption [ES] << [S] + [MS]) the rate equation takes the form

$$\frac{1}{v} = \frac{K_s(1 + K[M])}{V_m S_0} + \frac{1}{V_m} \qquad (1)$$

where the routine Michaelis–Menten enzyme–substrate interaction has been assumed. The formal relation between (1) and the equation usually discussed in connection with competitive inhibition[16] is evident. However, [M], the concentration of free TAT is not constant except when it is nearly equal to the *total* TAT concentration, M_0. In the following it is shown that (1) with [M] = M_0 may be considered as a limiting case of a more general situation. Consider the TAT–substrate interaction to be described by the reactions

$$MS_{i-1} + S \rightleftarrows MS_i \quad i = 1, 2, \ldots, n \qquad (2)$$

with association constants K_i, where it is understood that MS_i with $i = 0$ represents free TAT or M. Denoting the concentrations [MS_i] by X_i, the mass action expressions take the form

$$X_i = X_0 P_i [S]^i \qquad (3)$$

where $P_k = K_1 K_2 \ldots K_k$, $1 \leqslant k \leqslant n$, and $P_0 = 1$. The conservation equations for total TAT and substrate are

$$M_0 = \sum_{j=0}^{n} X_j = X_0 \sum_{j=0}^{n} P_j [S]^j \qquad (4)$$

$$S_0 = [S] + \bar{n}_e E_0 + \bar{n} M_0 \qquad (5)$$

where E_0 is the total enzyme concentration and $\bar{n}_e$ and $\bar{n}$ are the mean number of S-molecules bound per mole of enzyme and TAT, respectively. Now $\bar{n}$ is given by the familiar[17]

(17) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase, Copenhagen, 1941, pp. 24-29.

$$\bar{n} = \sum_{j=0}^{n} iX_j \Big/ \sum_{j=0}^{n} X_j = \sum_{j=0}^{n} iP_j [S]^j \Big/ \sum_{j=0}^{n} P_j [S]^j \qquad (6)$$

immediately obtained from (3), (4) and the definition of $\bar{n}$. If the enzyme–substrate interaction is described by

$$ES_{i-1} + S \rightleftarrows ES_i \longrightarrow ES_{i-1} + \text{product}, \; i = 1, 2, \ldots, m \qquad (7)$$

the steady-state concentrations [ES_i] are given by known equations[18] and $\bar{n}_e$ can be written at once in terms of [S]. The problem is completed by solving

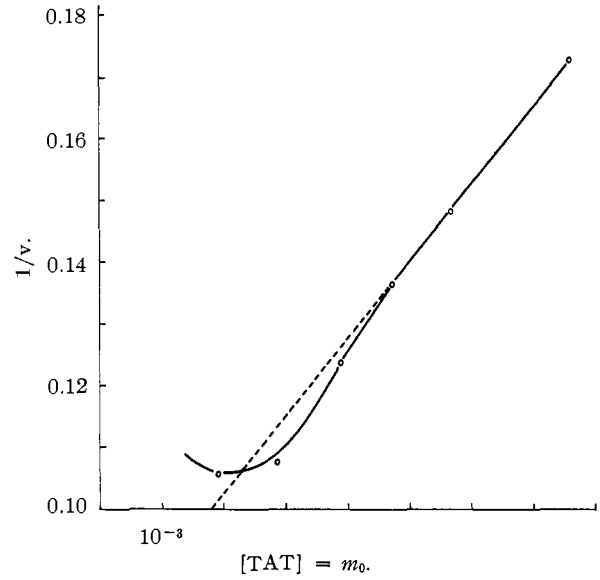

Fig. 2.—Experimental curve showing rate dependency on TAT (M_0) concentration; substrate 5 × 10^{-3} M *dl*-dopa.

(5) for [S] and substituting the result in the appropriate rate expression to obtain the rate as a function of S_0, M_0 the P_j and the parameters of reactions (7). In particular, if $m = 1$, it is evident that

$$\bar{n}_e E_0 = [ES] = E_0[S]/(K_s + [S]) \qquad (8)$$

The solution of (5), with (6) and (8), for [S] and substitution of the result in the rate equation

$$v = V_m[S]/(K_s + [S]) \qquad (9)$$

where V_m and K_s (which is not necessarily an equilibrium constant) have their usual meaning, gives the effect of reactions (2) upon a "zone B" enzyme system.[19] But even in this case (5), with (6) and (8), is of degree $n + 2$ in [S] and the requisite operations can be performed in principle only. An approximate but tractable result can be obtained as follows. From (8), $\bar{n}_e E_0$ cannot exceed E_0 and if $S_0 > > E_0$, as we shall assume here,[20] (5) can be written as

$$[S] = S_0/(1 + \bar{n}M_0/[S]) \qquad (10)$$

From (6), if [S] is small it is approximately true that

$$\bar{n}/[S] = P_1 \qquad (11)$$

(18) J. Botts and M. Morales, *Trans. Faraday Soc.*, **49**, 696 (1953).

(19) O. H. Strauss and A. Goldstein, *J. Gen. Physiol.*, **26**, 559 (1943).

(20) This assumption is convenient, usually justified and here unnecessary. For if it is assumed that [S]<<θ, where θ is the smaller of K_s and $1/P_1$, we have from (5), [S] = $S_0/(1 + E_0/K_s + P_1M_0)$ in place of (10) and (11). The arguments to follow go through with trivial modification.

and in the limit (11) is exact. With (10) and (11) a conventional rearrangement of (9) gives

$$1/v = \frac{K_s(1 + P_1M_0)}{V_m}\frac{1}{S_0} + \frac{1}{V_m} = \left(\frac{K_sP_1}{V_mS_0}\right)M_0 + \frac{1}{V_m}(K_s/S_0 + 1) \quad (12)$$

Equation 12 justifies the remarks made relative to (1) and predicts a linear relation between $1/v$ and M_0 provided the assumption (11) is justified. It is expected that obvious departure from linearity will occur when the error entailed in (11) exceeds the error of measurement. That, for certain S_0, departure from linearity may be expected as M_0 becomes small may be seen as follows. If P_1 [S] $< < 1$, (11) is justified provided[21] $P_1^k \geqslant P_k$, for under these conditions $1 > > P_1[S] > > (P_1[S])^k \geqslant P_k[S]^k$, for all $k \geqslant 2$. Clearly [S] $< S_0$ and if $P_1S_0 < < 1$, the conditions for (11) are satisfied. But [S] depends upon M_0 and in fact can be shown, from (10) to be a monotone decreasing function of M_0 in consequence of the fact that $\partial\bar{n}/\partial[S] > 0$ for all (finite) [S]. Therefore, while the condition $P_1S_0 < < 1$ assures the validity of (11) for *any* M_0, the goodness of the approximation (11) for an S_0 such that this condition is not well satisfied improves as M_0 increases and the error entailed in (11) approaches its greatest possible value as M_0 approaches zero.

These facts are easily seen under the conditions $E_0 < < S_0$, $n = 1$ and there is evidence (*cf.* next section) that this is the actual case for the dopa–TAT–tyrosinase system. Under these conditions [S] is obtained from (5) as

$$2[S] = (S_0 - K - M_0) + [(K + M_0 - S_0)^2 + 4S_0K]^{1/2} \quad (13)$$

where $K = 1/P_1$. If $S_0 < < K$, which is just the condition discussed above, then the quantity

$$4S_0K/(K + M_0 - S_0)^2 \cong 4S_0K/(K + M_0)^2 \quad (14)$$

is small relative to unity since its order of magnitude is at *most* S_0/K. Then the radical in (13) can be expanded and linear terms retained with the result, equivalent to (10) and (11)

$$[S] = S_0/(1 + M_0/K) \quad (15)$$

But the quantity in (14) is also small if $S_0 < < M_0$ and (15) likewise results from this condition. In general, given any value of the ratio S_0/K, (15) can be made to hold with any desired degree of approximation by making M_0 sufficiently large.

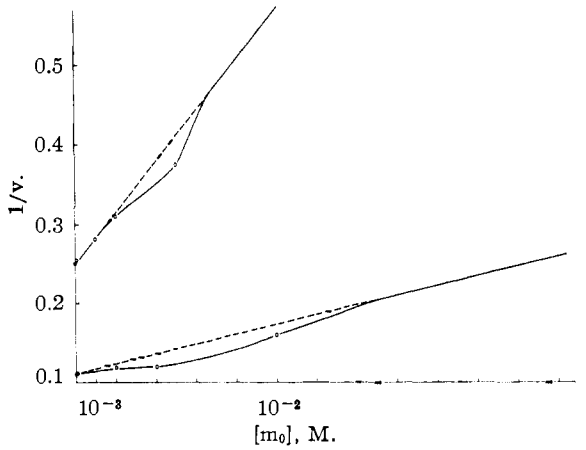

Fig. 3.—Theoretical curves of rate dependency on M_0: upper curve, $S_0 = 10^{-3}$ M; lower curve, $S_0 = 5 \times 10^{-3}$ M (see Table II).

(21) Note that $P_1^k \geqslant P_k$ can hardly be considered a *physical* restriction since it requires only that $K_1 \geqslant K_2 \geqslant \ldots \geqslant K_n$ and this is true both in the absence of interaction of bound S-molecules and in the presence of the usual (repulsive potential) types of interactions.

TABLE II

S_t and S_a are true S and approximate S, respectively, where S_t was calculated from eq. 13 and S_a was computed from the approximation of (14). $K = 5 \times 10^{-3}$; $n = 1$. The curves of Fig. 3 were obtained by computing $1/v$ from the S_t of this table.

M_0	S_0 5×10^{-3} $S_t \times 10^{-3}$	$S_a \times 10^{-3}$	S_0 10^{-3} $S_t \times 10^{-3}$	$S_a \times 10^{-3}$
0	5.00	5.00	1.000	1.000
10^{-4}	4.95	4.90	0.984	0.980
2×10^{-4}	4.90	4.80	.968	.962
4×10^{-4}	4.80	4.63	.937	.926
10^{-3}	4.53	4.17	.854	.833
2×10^{-3}	4.10	3.13	.742	.714
4×10^{-3}	3.39	2.78	.583	.555
10^{-2}	2.07	1.67	.348	.333
5×10^{-2}	0.50	0.455	.0925	.0909
10^{-1}	0.25	0.238	.0481	.0476

As has been remarked, $\partial[S]/\partial M_0 < 0$ and from this it follows that $\partial(1/v)/\partial M_0 > 0$ so that $1/v$ is a continuously increasing function of M_0. Further since the approximations involved in obtaining (12) underestimate [S], the *actual* curve will be everywhere under the linear approximation al-

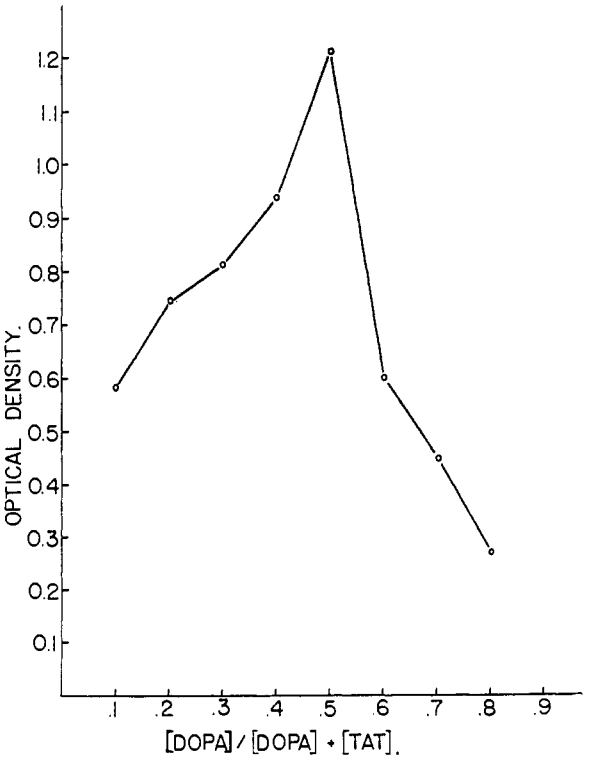

Fig. 4.—Continuous variation curve: run at 385 mμ, light path 1 cm., slit width 0.085 mm., room temp. (approx. 27°); curves identical in water and in 0.067 M phosphate buffer at pH 6.5.

though in practice the two may be indistinguishable over a considerable M_0 range (see Fig. 3).

In Fig. 3 a value of K was assumed but $1/v$ was computed from the S_t (see Table II) obtained using this K value and the actual V_m and K_s of the enzyme used experimentally. It can be seen that the experimental curve (Fig. 2) probably contains only one or two points on the truly linear part of the curve. The apparent linearity over most of the concentration range is due to the very slight concavity of the deviation region (compare with Fig. 3). At very low M_0 the inhibited rate was not sufficiently different from the uninhibited rate to allow a precise determination of the curve form in this region and relatively small errors in v would markedly alter the curve. It is likely that such errors were responsible for the up-curving seen in Fig. 2. Table II gives a comparison of approximate and true values of S for two values of S_0. It can be seen from the table that the error in S_a decreases markedly as S_0 becomes less than K and a corresponding reduction in the amount of deviation in the $1/v$ *vs.* M_0 curves is apparent from Fig. 3.

II. The Dopa–TAT Reaction.—The theory of inhibition discussed in the last section has been based upon the assumption that a dissociable complex was formed between dopa and TAT. It was not possible to obtain detailed information regarding the reaction product, but an estimate of the ratio in which the components occurred was made by means of the method of continuous variation.[22] These experiments (see Fig. 4) showed that the dominant complex species was a 1:1 complex. If hydrolysis was not appreciable and polymerization did not occur to any great extent, it would be reasonable to assume that this complex formation came about through the replacement of a molecule of triethanolamine by one of dopa. Triethanolamine had a negligible effect and Ti^{IV} from the lactate was a weak inhibitor, but an approximately equimolar mixture of these reagents was synergistic, giving considerably greater inhibition than either alone. This strongly suggested that in some manner prior complexing with triethanolamine facilitated formation of a dopa complex (Table III) or contributed to its stability.

(22) For a general discussion see A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York, N. Y., 1952, pp. 28-34.

TABLE III

Substrate in all cases 5×10^{-3} M dopa. Concentrations are final concn. in reaction vessels.

Inhibitor	% Inhibition (total uptake)
2.5×10^{-3} M Triethanolamine	2.04
2.0×10^{-3} M Titanium lactate	29.20
Triethanolamine 2.5×10^{-3} M plus Ti-lactate, 2.0×10^{-3} M	53.50

While no proof of the nature of the dopa–TAT product can be offered, the nature of the inhibition kinetics and the experiments above lead to a tentative conclusion that a 1:1 complex was formed.

Acknowledgments.—We wish to thank Mrs. Rooney Weaver for the computation of theoretical S values. This work was supported by the University of Tennessee reserve for research.

MEMPHIS, TENN.

[CONTRIBUTION FROM THE DEPARTMENTS OF MEDICINE (SECTIONS OF DERMATOLOGY AND CARDIOLOGY) AND BIOCHEMISTRY, UNIVERSITY OF CHICAGO]

Studies of the *in vitro* Lipid Metabolism of the Human Skin. I. Biosyntheses in Scalp Skin[1]

BY N. NICOLAIDES, OSCAR K. REISS AND ROBERT G. LANGDON

RECEIVED OCTOBER 5, 1954

The incorporation of isotopic carbon from 1-C^{14} acetate into the lipids by human scalp skin slices was studied. Acetate was incorporated into the fatty acids, squalene, sterols and probably the wax alcohols. Of the total activity, 40% was incorporated into squalene while only 2.6% was found in the sterols. Squalene has been recently implicated as a sterol precursor. The high squalene content of human scalp skin made direct isolation of this hydrocarbon possible. Its specific activity was found to be 10 times higher than that of the sterols, an observation which supports the above implication.

Introduction

The composition of the lipids of human surface fat has been receiving increasing attention recently.[2] Important constituents shown to be present are free fatty acids (25–35%), esterified acids (28–40%), squalene (8–13%), wax alcohols (9–12%) and cholesterol (2–4%).[3] In order to investigate the biosynthesis of these lipids, a study of the incorporation of radioactivity from labeled acetate into the lipids of skin slices was undertaken.

In rat skin, the *in vitro* synthesis of cholesterol from acetate has been demonstrated.[4] The experiments to be reported here show that human skin can also synthesize lipids from 1-C^{14} acetate. These findings are in accord with the established fact that acetate is a precursor of fatty acids, cholesterol and squalene.[5,6] The amount of isotope incorporated into the squalene of skin lipids, however, is considerably greater than that found for other tissues.

While this work was in progress two publications have appeared in which was reported the specific

(1) This investigation was supported in part by the Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-411.

(2) S. Rothman, "Physiology and Biochemistry of the Skin," University of Chicago Press, Chicago, 1954, Chapter 13.

(3) R. E. Bloom, S. Woods and N. Nicolaides, *J. Invest. Dermatol.*, in press.

(4) P. A. Srere, J. L. Chaikoff, S. S. Treitman and L. S. Burstein, *J. Biol. Chem.*, **182**, 629 (1950).

(5) R. G. Langdon and K. Bloch, *ibid.*, **200**, 129 (1953).

(6) R. G. Langdon and K. Bloch, *ibid.*, **200**, 135 (1953).