Multienzyme Complexes

BY DR. U. HENNING[1]

INSTITUT FÜR GENETIK, UNIVERSITÄT KÖLN (GERMANY)

In 1948 *D. E. Green*[2] termed the mitochondrial enzymes the *cyclophorase complex*, in order, as he later commented[3] "... to signify that the complex was a unit of enzyme action and not a chance mixture of several hundred enzymes. ... this complex was visualized as an organized mosaic of enzymes in which each of the large number of component enzymes was uniquely located to permit efficient implementation of consecutive reaction sequences".

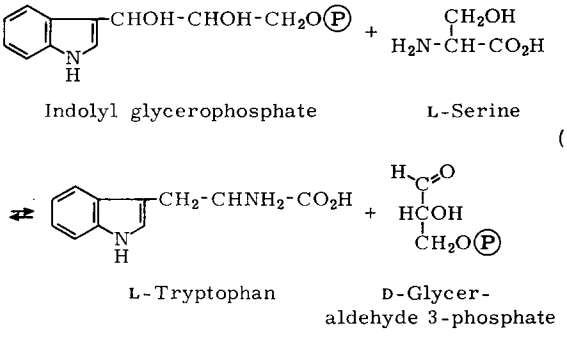
These words contain the concept of the multienzyme complex: ordered association (not involving peptide linkages) of various enzymes that catalyse successive steps in a reaction sequence. The present paper deals with recent findings regarding the properties of such complexes, and the question whether the concept represents a biological principle is discussed.

Predominantly protein-chemical aspects of the association of enzymes were recently described by *Sund* and *Weber*[4], and the "macromolecular organization of enzyme systems" by *Reed* and *Cox*[5].

I. Multienzyme Complexes in a Narrower Sense

1. Tryptophan Synthetase

A clear and relatively simple example is the tryptophan synthetase from *Escherichia coli*. In the presence of pyridoxal phosphate, the enzyme catalyses the last step [Eq. (a)] in the reaction chain leading to the biosynthesis of tryptophan:



Indolyl glycerophosphate L-Serine

(a)

L-Tryptophan D-Glyceraldehyde 3-phosphate

It can also[6,7] bring about the cleavage

indolyl glycerophosphate $\rightleftarrows$ indole + D-glyceraldehyde 3-phosphate (b)

and the condensation

indole + L-serine $\rightleftarrows$ L-tryptophan (c)

The extensive investigations by *Yanofsky* and coworkers, which made this enzyme a component of one of the best-known gene enzyme systems, also showed that the synthetase consists of two sub-units, the proteins A and B. The isolated crystallizable protein A (molecular weight about 30000[8]) consists of only one polypeptide chain α[9], while the protein B (M = 117000) consists of two chains (β_2)[10].

The physiological reaction (a) can take place only when the proteins A and B are in contact with each other; indole does not occur as a free intermediate[6,11].

Isolated protein A is inactive in reactions (a) and (c), but can bring about reaction (b) (not requiring pyridoxal phosphate, as expected) with about 1 % of the activity exhibited in the presence of B[12]. On the other hand, isolated B containing pyridoxal phosphate (2 moles of coenzyme per mole of protein) does not catalyse reactions (a) and (b), but catalyses the condensation (c) with 3 to 10 % of the activity exhibited in the presence of A[12]. It was later found that protein B can also act as an L-serine deaminase[13]

L-serine $\rightleftarrows$ pyruvate + NH_3 (d)

Addition of A causes almost complete inhibition of B in reaction (d).

There is strong evidence that the catalytically active centers of the respective complementary proteins are not involved in the activation of A in reaction (b) and in that of B in reaction (c). Protein B which has lost its enzymatic activity as a result of mutation can activate A in reaction (b) in the same way as unaltered protein B, while the reverse is true of A which has been inactivated by mutation. Moreover, the activity of B in reaction (c) is increased by a factor of about 20 in the presence of a high concentration of NH_4^+ ions[14]. Finally, *Hatanaka*

[1] Present address: Max-Planck-Institut für Biologie, Tübingen (Germany).

[2] *D. E. Green, W. F. Loomis,* and *V. H. Auerbach,* J. biol. Chemistry *172,* 389 (1948).

[3] *D. E. Green,* Harvey Lectures *52,* 177 (1958).

[4] *H. Sund* and *K. Weber,* Angew. Chem. *78,* 217 (1966); Angew. Chem. internat. Edit. *5,* 231 (1966).

[5] *L. J. Reed* and *D. J. Cox,* Annu. Rev. Biochem. *35,* in press.

[6] *I. P. Crawford* and *C. Yanofsky,* Proc. nat. Acad. Sci. USA *44,* 1161 (1958).

[7] *I. P. Crawford,* Biochim. biophysica Acta *45,* 405 (1960).

[8] *U. Henning, D. R. Helinski, F. C. Chao,* and *C. Yanofsky,* J. biol. Chemistry *237,* 1523 (1962).

[9] *B. C. Carlton* and *C. Yanofsky,* J. biol. Chemistry *237,* 1531 (1962).

[10] *D. A. Wilson* and *I. P. Crawford,* Bacteriol. Proc. USA *1964,* 92.

[11] *C. Yanofsky* and *M. Rachmeler,* Biochim. biophysica Acta *28,* 640 (1958).

[12] *C. Yanofsky,* Bacteriol. Rev. *24,* 221 (1960).

[13] *I. P. Crawford* and *J. Ito,* Proc. nat. Acad. Sci. USA *51,* 390 (1964).

[14] *M. Hatanaka, E. A. White, K. Horibata,* and *I. P. Crawford,* Arch. Biochem. Biophysics *97,* 596 (1962).

and *Crawford*[15] have isolated a mutant of *E. coli* producing a defective protein B, which by itself is inactive in reaction (c); in the presence of A, however, the activities of all three tryptophan synthetase reactions are observed.

The two proteins are only loosely associated[16]. L-Serine and pyridoxal phosphate are essential to the complex formation between A and B. Centrifugation of a mixture of A and B in a sucrose density gradient leads to practically complete separation of the components A (2.7 S) and B (5.1 S), while the associated A–B complex (6.4 S) appears after incubation of the mixture with L-serine and coenzyme[16]. The completely associated A–B complex, tryptophan synthetase, contains two moles of A to one mole of B, and its probable polypeptide-chain formula is therefore $\alpha_2\beta_2$[16,17].

The enzymatic activities of the components change, by several orders of magnitude in some cases, on complex formation. The activation by NH_4^+ and the behavior of the various proteins which have become inactive as a result of mutation provide very strong evidence that complex formation *per se* is responsible for the change in enzymatic activity. Association presumably involves changes in the conformation of the proteins, for which the active site of the complementary component is not directly responsible.

It is interesting to compare the tryptophan synthetase of *E. coli* (or other enterobacteria) with that of *Neurospora*. The neurospora enzyme[18,19] (M = 135000) so far has not been dissociated into enzymatically active sub-units. On reduction with mercaptoethanol in 5 M guanidinium chloride, the protein dissociates into four sub-units, possibly made up of two identical pairs[19]. This result led *D. M. Bonner*[20] to assume that the two tryptophan synthetases represent two different stages of evolution. It is conceivable that the tryptophan synthetase reaction initially proceeded in separate steps, with indole as a free intermediate. With the development of the association of the two proteins, indole is no longer liberated; the binding of the two proteins may have become "tighter" during the further evolution.

2. Pyruvate and α-Ketoglutarate Dehydrogenases

In animal tissues, as well as in many fungi and bacteria, the principal route for the degradation of the two α-keto acids is by oxidative decarboxylation:

$$R{-}COCO_2H + CoASH + NAD^+ \rightarrow R{-}COSCoA + CO_2 + NADH + H^+ \quad (e)$$

It is now known[5,21] that this overall reaction (e) proceeds *via* the steps (f) to (k).

$$R\text{-}CO\text{-}CO_2H + TPP\text{-}E1 \rightleftarrows R\text{-}CHOH\text{-}TPP\text{-}E1 + \underline{CO_2} \quad (f)$$

$$R\text{-}CHOH\text{-}TPP\text{-}E1 + \text{S—S (E2)} \rightleftarrows TPP\text{-}E1 + \text{SH S-CO-R (E2)} \quad (g)$$

$$\text{SH S-CO-R (E2)} + CoA\text{-}SH \rightleftarrows \text{SH SH (E2)} + \underline{R\text{-}CO\text{-}SCoA} \quad (h)$$

$$\text{SH SH (E2)} + FAD\text{-}E3_{ox.} \rightleftarrows \text{S—S (E2)} + FAD\text{-}E3_{red.} \quad (i)$$

$$FAD\text{-}E3_{red.} + NAD^+ \rightleftarrows FAD\text{-}E3_{ox.} + \underline{NADH + H^+} \quad (k)$$

The best-known case is that of the pyruvate dehydrogenase of *E. coli*, which has been isolated as a homogeneous protein ($M = 4.8\times10^6$)[22]. Electron micrographs[23] of the complex show (Fig. 1) a polyhedral structure with a diameter of 300 to 350 Å.

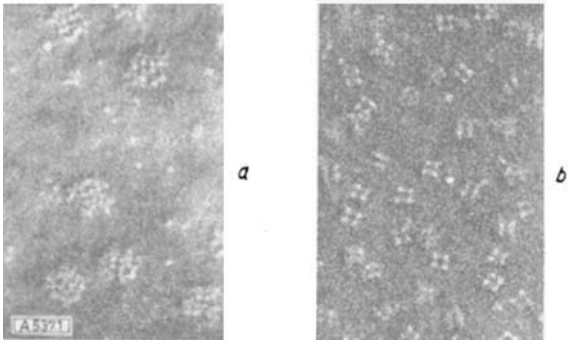


Fig. 1a). Pyruvate dehydrogenase complex from *E. coli*. Magnification factor 260000. b) Lipoate reductase transacetylase component of the enzyme complex, same magnification. The tetrad structure of the component is visible in the center of the complex (1a). In these projections, the diameter of the complex is about 300 Å and that of the components is about 120 Å. Both samples were obtained by negative staining with phosphotungstic acid.

The complex can be dissociated, under mild conditions, into three proteins[24], to which the activities of reactions (f) to (k) could be assigned with the aid of model reactions: the thiamine pyrophosphate(TPP)-dependent pyruvate decarboxylase (E1), the lipoate reductase transacetylase (E2) containing lipoic acid, and the flavoprotein dihydrolipoate dehydrogenase (E3). The proteins re-associate spontaneously *in vitro*[24]; E1 does

[15] *M. Hatanaka* and *I. P. Crawford*, Bacteriol. Proc. USA *1965*, 88.

[16] *T. Greighton* and *C. Yanofsky*, J. biol. Chemistry *241*, 980 (1966).

[17] *D. Wilson* and *I. P. Crawford*, J. biol. Chemistry *240*, 4018 (1965).

[18] *W. C. Mohler* and *S. R. Suskind*, Biochim. biophysica Acta *43*, 288 (1960).

[19] *M. Carsiotis, E. Apella, P. Provost, J. Germershausen*, and *S. R. Suskind*, Biochem. biophysic. Res. Commun. *18*, 877(1965).

[20] *D. M. Bonner, J. A. DeMoss*, and *S. E. Mills* in *V. Bryson* and *H. J. Vogel*: Evolving Genes and Proteins. Academic Press, New York 1965, p. 305.

[21] *I. C. Gunsalus* in *W. D. McElroy* and *B. Glass*: The Mechanism of Enzyme Action. Johns Hopkins Press, Baltimore 1964, p. 545.

[22] *M. Koike, L. J. Reed*, and *W. R. Carroll*, J. biol. Chemistry *235*, 1924 (1960).

[23] *H. Fernandez-Moran, L. J. Reed, M. Koike*, and *C. R. Willms*, Science (Washington) *145*, 930 (1964).

[24] *M. Koike, L. J. Reed*, and *W. R. Carroll*, J. biol. Chemistry *238*, 30 (63).

not associate with E3, but E2 binds both E1 and E3. One molecule of the complex contains about twelve molecules of the decarboxylase (M = 1.83×10⁵), one molecule of E2 (M = 1.6×10⁶), and about six molecules of the flavoprotein (M = 1.12×10⁵)[5]. The lipoate reductase transacetylase is probably centrally situated (cf. Fig. 1), and surrounded by the two other enzymes.

The lipoate reductase transacetylase has also been reversibly dissociated; it dissociates at pH 2.6 into subunits having $M \approx 7\times10^4$[5]. The enzyme contains one mole of lipoic acid per 3.5×10⁴ g of protein, and may therefore consist, not of about 24, but of about 48 polypeptide chains having M = 3.5×10⁴. The composition of the decarboxylase is not yet known; that of the flavoprotein will be discussed below. The lipoic acid is covalently bound to the ε-amino group of a lysine residue[25]. The dithiolan ring of the coenzyme must react with (a) the hydroxyethyl TPP of the decarboxylase, (b) the flavin-adenine dinucleotide (FAD) of the dihydrolipoate dehydrogenase, and (c) the coenzyme A. Since the intermediates of the overall reaction remain bound to the enzyme, there appear to be steric difficulties opposing the interaction of one and the same lipoic acid residue with all the prosthetic groups mentioned. The lipoic acid:FAD ratio in the complex is about 4:1. The lipoic acid linkage forms a "flexible arm" (about 14 Å long), and the coenzyme may be able to rotate about the α-C atom of the lysine residue in the enzyme complex[24,26]. It was shown that all the lipoic acid residues can take part in the oxidation of pyruvate. It was therefore suggested[24] that different lipoic acids could participate in the various reaction steps (Fig. 2), which indeed would be a remarkable type of electron transport in a protein molecule.

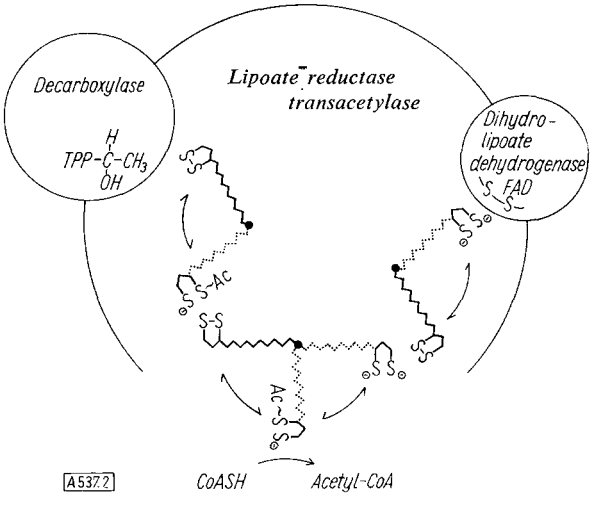
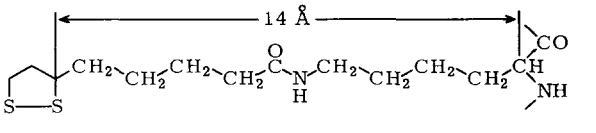


Fig. 2. Scheme of the hypothetical interaction between the coenzymes of the pyruvate dehydrogenase [24]. The lipoic acid residue pivots about the α-C atom of a lysine residue.



[25] *H. Nawa, W. T. Brady, M. Koike,* and *L. J. Reed,* J. Amer. chem. Soc. *82,* 896 (1960).

[26] *R. M. Bock* and *R. S. Criddle,* quoted in *D. E. Green,* Comparat. Biochem. Physiol. *4,* 81 (1962).

A similar situation is found with the α-ketoglutarate dehydrogenase of *E. coli* (M = 2.4×10⁶)[22,24]. This also could be reversibly dissociated into α-ketoglutarate decarboxylase, lipoate reductase transsuccinylase, and dihydrolipoate dehydrogenase. The flavoproteins of the two α-ketoacid dehydrogenases are functionally interchangeable, that of the pyruvate dehydrogenase being bound by the lipoate reductase transsuccinylase and *vice versa*; no "hybrid" binding of the decarboxylases takes place[27].

It is possible that the two flavoproteins are identical[5,28]. If this were the case, it would raise an interesting problem in connection with the regulation of the synthesis of the dihydrolipoate dehydrogenase, since the syntheses of the pyruvate and α-ketoglutarate dehydrogenases are not regulated by the same mechanism[29].

It is not yet known whether the structure of the enzyme complex in the cell is the same as that in which it is obtained after purification. However, since the intermediates of the overall reaction (e) remain bound to the enzyme, some form of association of the enzymes is necessary. It is conceivable that the large complex isolated is an artifact resulting from the aggregation of smaller complexes that were bound to other structures in the cell. There are several arguments against this view; *e.g.*, mutants in which the decarboxylase component has lost its enzymatic activity were found to contain a "partial" complex consisting of the lipoate reductase transacetylase and the flavoprotein[30]. This partial complex appears to be the same as that obtained by the removal of the decarboxylase from the pyruvate dehydrogenase complex *in vitro*.

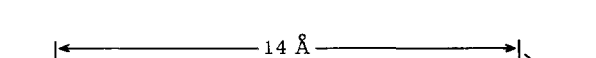
Concerning the α-keto acid dehydrogenases from animal cells in which the enzymes are components of mitochondria, the reader is referred to the review by *Reed* and *Cox*[5].

3. Fatty Acid Synthetase

A similar organization of structure and function is found in the synthesis of unbranched saturated fatty acids. Investigations by *Lynen et al.*[31] have shown that the overall reaction (l)

$$CH_3{-}CO{-}SCoA + n\,HO_2C{-}CH_2{-}CO{-}SCoA + 2n\,NADPH + 2n\,H^+ \rightarrow CH_3(-CH_2-CH_2-)CO{-}SCoA + n\,CO_2 + n\,CoASH + 2n\,NADP^+ + n\,H_2O \quad (n = 7 \text{ for palmityl-CoA}) \quad (l)$$

in yeast is catalysed by a homogeneous protein (M = 2.3×10⁶), the principal products being palmityl and stearyl CoA. The net reaction takes place on the fatty acid synthetase in the following steps. The acetyl transfer [Eq. (m)] is the initiation reaction.



[27] *B. B. Mukherjee, J. Matthews, D. L. Horney,* and *L. J. Reed,* J. biol. Chemistry *240,* PC 2268 (1965).

[28] *U. Henning, G. Dennert, R. Hertel,* and *S. W. Shipp,* Cold Spring Harbor Symp. Quant. Biol. *31,* in press.

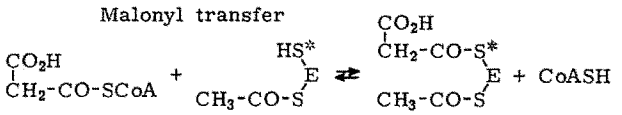
[29] *U. Henning* and *G. Deppe,* unpublished work.

[30] *U. Henning, C. Herz,* and *K. Szolyvay,* Z. Vererbungslehre *95,* 236 (1964).

[31] *F. Lynen,* Angew. Chem. *77,* 929 (1965).

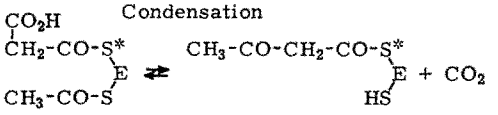
The five subsequent reactions lead to lengthening of the chain; they begin with the malonyl transfer [Eq. (n)].

Malonyl transfer



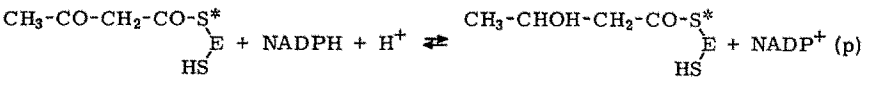
(n)

Condensation



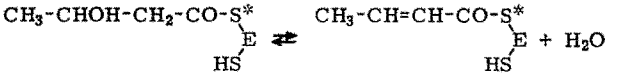
(o)

First reduction



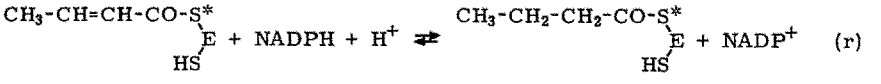
(p)

Dehydration



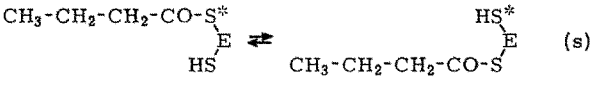
(q)

Second reduction



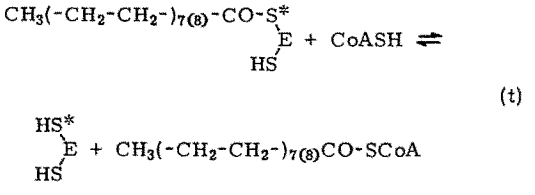
(r)

The SH group which accepts the malonyl residue is then liberated by the butyryl transfer [Eq. (s)].



(s)

Finally, when the chain length becomes C_{16} to C_{18}, the stearyl or palmityl CoA is formed.



(t)

The existence of the functionally different SH groups (HS*-, HS–) has been demonstrated [32]. As with the other enzyme complexes, all intermediates remain enzyme-bound.

The seven reaction steps (m) to (s) apparently correspond to seven sub-units in the synthetase; however, dissociation of the enzyme into enzymatically active sub-units has not been achieved. An electron micrograph [33] of the synthetase (Fig. 3) shows its ordered structure in particles having a diameter of 200 to 250 Å. As in the case of tryptophan synthetase, the enzyme system from *E. coli* which carries out the fatty acid synthesis easily can be separated into enzyme fractions with different functions [34].

Details of bacterial and animal systems cannot be discussed here. The electron micrographs, and the fact that the synthetase from yeast can be dissociated into inactive sub-units only under fairly drastic conditions strongly indicate that the isolated enzyme is not an artifact produced during purification.

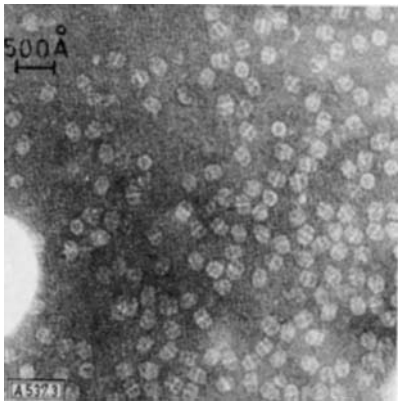


Fig. 3. Fatty acid synthetase from yeast [33]. (Preparation as in Fig. 1, magnification factor 250000).

4. Other Multienzyme Complexes

The examples described undoubtedly are not special cases of little general importance. There is evidence that a number of other reaction sequences are catalysed by a functional complex. This is true of the four enzymes that synthesize valine from pyruvate and isoleucine from pyruvate and α-ketobutyrate in *Neurospora* [35,36], and also of the synthesis of isoleucine in *Salmonella* [37].

[32] *F. Lynen* in *M. Sela*: New Perspectives in Biology. B.B.A Library Vol. 4, Elsevier Publishing Comp., Amsterdam-London-New York 1964, p. 132.

[33] *A. Hagen* and *P. H. Hofschneider* in *M. Titlbach*: Electron Microscopy 1964 (Proc. 3rd European Regional Conference). Publishing House Czechoslovak Acad. Sci., Prague 1964, Vol. 13, p. 69.

[34] *P. R. Vagelos*, Annu. Rev. Biochem. *33*, 139 (1964).

[35] *K. Kiritani, S. Narise, A. Bergquist*, and *R. P. Wagner*, Biochim. biophysica Acta *100*, 432 (1965).

[36] *R. P. Wagner, A. Bergquist*, and *T. Barbee*, Biochim. biophysica Acta *100*, 444 (1965).

[37] *C. S. Cronenweit* and *R. P. Wagner*, Proc. nat. Acad. Sci. USA *54*, 1643 (1965).

Three enzymes involved in the biosynthesis of tryptophan appear to act as a functional unit in *Neurospora* [38], as do three enzymes involved in the biosynthesis of histidine in *Neurospora* [39]. In the last case, since the three enzymes in question correspond, not to consecutive reaction steps, but to the second, third, and tenth steps, it is reasonable to suspect that *all* relevant enzymes *in vivo* are associated in a functional unit.

The features common to all these examples are readily recognized, and have been characterized by *Lynen* [32] as "strict compartmentalization in the smallest possible space". If enzymes that operate in sequence are also physically adjacent, the activity of the overall reaction sequence presumably may be greater than if these enzymes were separated. In the latter case the action would be optimal only when the concentrations of diffusing intermediates reach certain values. Moreover, reaction chains in which the intermediates are bound to the enzyme avoid competition between different enzymes for the same intermediate.

The specific activities of the fatty acid synthetase from yeast and of the pyruvate dehydrogenase are high; one synthetase complex can catalyse the conversion of 2.4×10^3 molecules of malonyl CoA per minute into fatty acid [32], and one pyruvate dehydrogenase complex at 30 °C can produce at least 1.25×10^4 molecules of acetyl CoA per minute from pyruvate.

One might conclude that the concept of the multienzyme complex represents an example of the biological principle of "increasing the efficiency". However, this view probably is still too narrow. In higher organisms at least, the large enzyme complexes discussed are localized in the mitochondria. The same is true of the above mentioned enzymes of the biosyntheses of valine and isoleucine in *Neurospora* [35, 36]; the isoleucine system in *Salmonella* [37] occurs in the membrane fraction. It appears reasonable to combine enzyme systems physically when they are bound to large cell structures, even if they are not also components or "supply" enzymes of further processes (*e.g.* the respiratory chain). Another interesting speculative aspect [39a] is that the structure-bound enzymes could greatly increase their surface if the substrate can be retained by the structure (*e.g.* membrane) and can migrate along the latter to the enzyme.

II. Extension of the Concept: Homofunctional and Heterofunctional Enzyme Complexes

With regard to the enzyme protein, the definition of the multienzyme complex as described in the introduction is really teleological, and therefore is too specialized. This can be seen particularly well with hemoglobin and myoglobin [41–43], which *Frieden* [40] aptly described as "perfect" enzymes, since they permit the study of the enzyme-substrate interaction without the formation of interfering products. The four heme groups, and hence the four active sites, corresponding to the four polypeptide chains of hemoglobin, are situated in "pockets" in each of the two α and β chains on the surface of the molecule, without being in contact with one another. The tertiary structure (and also the position of the heme) of myoglobin, which consists of only one polypeptide chain, is extremely similar to the tertiary structure of each of the hemoglobin chains, and in this sense myoglobin may be regarded as a naturally-occurring sub-unit of hemoglobin. The activities of all the polypeptide chains are the same. In contrast to myoglobin, however, the binding of O_2 by hemoglobin leads to a heme-heme interaction, *i.e.* a co-operation of the binding sites [44–46]. The rate of reaction of a heme with O_2 depends on the state of loading of the other hemes with O_2, and increases with the amount of O_2 already bound. Thus the association of different sub-units leads (in a wider sense) to an increase in efficiency.

The question arises whether the fact that a protein molecule consists of several sub-units, *i.e.* the presence of a quarternary structure, is enougn to constitute a multienzyme complex. It may be assumed that this is not the case, but that the cell also makes use of the association to *produce* an active site. This appears to be the situation with the dihydrolipoate dehydrogenase [cf. reactions (i) and (k)], as *Massey*'s investigations [47,48] have shown in the case of the pig's heart enzyme.

The enzyme consists of two probably identical polypeptide chains, each with $M = 50000$; 2 moles of FAD and 4 moles of cystine units (as well as 12 cysteine units) are present per mole of enzyme. The intermediate is the reduced enzyme [reaction (i)], in which, in contrast to several other dehydrogenases containing FAD, the flavin coenzyme takes up only one electron. The difference spectrum of the resulting reduced enzyme is similar to that of the semiquinoid form of FMN which occurs as a charge-transfer complex in an equilibrium mixture of FMN and $FMMH_2$. This form ("red" enzyme) has been obtained by the action of NADH or dihydrolipoic acid on stoichiometric quantities of the dehydrogenase. However, spectrophotometric titration of this last reaction with either of the substrates shows that the red form of the protein corresponds to the uptake of two electrons per FAD. This discrepancy was resolved when a disulfide was discovered as a second prosthetic group of the enzyme, and it was found by amperometric titration that the addition of NADH leads to the formation of a dithiol. When the NAD formed in a mixture of NADH and enzyme is removed with an NADase (which does not attack NADH), the enzyme is completely reduced and becomes colorless; four electrons are taken up per FAD. The colorless enzyme can react with NAD to give again the red form.

The dihydrolipoic dehydrogenase in the oxidized yellow state is unusually stable towards urea (in 6.5 M urea at 0 °C only

[38] *J. A. De Moss* and *J. Wegman*, Proc. nat. Acad. Sci. USA *54*, 241 (1965).

[39] *A. Ahmed, M. E. Case*, and *N. H. Giles*, Brookhaven Symp. Biol. *17*, 53 (64).

[39a] *M. Delbrück*, personal communication.

[40] *C. Frieden*, Brookhaven Symp. Biol. *17*, 98 (1964).

[41] *J. C. Kendrew*, Angew. Chem. *75*, 595 (1963).

[42] *M. F. Perutz*, Angew. Chem. *75*, 589 (1963).

[43] *G. Braunitzer, K. Hilse, W. Rudloff*, and *U. Hilschmann*, Advances Protein Chem. *19*, 1 (1964).

[44] *R. Benesch* and *R. E. Benesch*, J. molecular Biol. *6*, 489 (1963).

[45] *H. Muirhead* and *M. F. Perutz*, Cold Spring Harbor Symp. Quant. Biol. *28*, 451 (1963).

[46] *R. E. Benesch, R. Benesch*, and *G. McDuff*, Proc. nat. Acad. Sci. USA *54*, 535 (1965).

[47] *V. Massey, T. Hofmann*, and *G. Palmer*, J. biol. Chemistry *237*, 3820 (1962).

[48] *V. Massey* in *P. D. Boyer, H. L. Lardy*, and *K. Myrbäck*: The Enzymes. Academic Press, New York, 1963, Vol. 7, p. 275.

about 30 % of the enzyme activity is lost after one month); the red form of the enzyme also retains its activity in urea. In the presence of excess NADH or dihydrolipoic acid in 6.5 M urea, the spectrum is initially that of the semi-reduced enzyme, but this soon gives way to that of the fully reduced protein. The completely reduced enzyme is unstable in the urea solution. The $FADH_2$ (or FAD after the admission of air) becomes separated from the protein and dialysable; the protein becomes insoluble when the urea concentration is decreased to less than 2 M, and the molecular weight of the resulting reduced dehydrogenase falls to half that of the native protein.

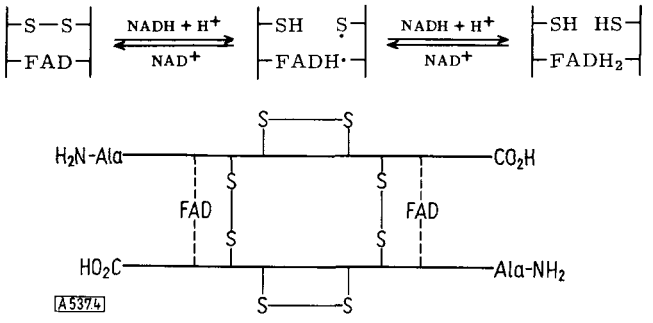


Fig. 4. Scheme of the dihydrolipoate dehydrogenase [47]. Model representation of the steps of the enzyme reduction as described in the text.

Figure 4 shows the model derived by *Massey* from these and other data. Both polypeptide chains appear to be involved, with the disulfide bridges, in the catalysis. The active sites literally are situated *between* the polypeptide chains, since at least cystine residues of both chains act as components of one active site. However, it is not known whether other amino acid residues belonging to one active site originate from both chains.

The example described is not intended to show implicitly that enzymes of this type must necessarily consist of identical polypeptide chains. An association of different polypeptide chains can give almost the same catalytic activity, as is well known from various forms of lactic dehydrogenase [49,50]. These can consist either of four identical chains (the so-called isozymes I and V, consisting of chains H and M) or mixtures of integral molar quantities of chains H and M (isozymes II, III, and IV: 1H2M, 2H2M, 1H3M). *Holbrook* [51] recently reported results that led him to conclude that the active sites in this case also are composed of parts of adjacent sub-units. A similar possibility exists for aldolase, which has only one active site, but consists of at least three (or six) polypeptide chains [52–55].

However, the direct participation of several sub-units of an enzyme molecule in one active site is hypothetical. If this hypothesis is true, it remains to be seen whether it is correct to classify such enzymes separately, since the transition to proteins of the tryptophan synthetase type may be continuous. (Incidentally, it would not be easy to give a good account for the biological evolution of enzymes with "mixed"centers of activity, as dihydrolipoate dehydrogenase).

From the examples described, it appears that a protein may be regarded with certainty as a multienzyme complex if the centers of activity for various steps in an overall reaction chain are situated on different sub-units. As far as the protein is concerned, however, there is no fundamental difference between such complexes and enzymes in which similar active centers are also present in their sub-units and are not formed only by the combination of the sub-units in such a manner that two or more sub-units contribute in forming an active site. If a classification is desired, it appears more reasonable to distinguish between *homofunctional* and *heterofunctional* multienzyme complexes, depending on whether the sub-units and active centers are identical or different.

The so-called allosteric effect [56,57], for which association is evidently a prerequisite, fits in easily with the "heterofunction" on association. An example is the heme-heme interaction in hemoglobin described earlier. Another extreme case was reported in the aspartate transcarbamylase (ATC) of *E. coli* [58].

The enzyme initiates the biosynthesis of cytidine triphosphate (CTP) with the reaction: carbamyl phosphate + aspartate → carbamyl aspartate. It is regulated by feed-back, its activity being inhibited by CTP. The ATC ($M \approx 3\times10^5$) consists of enzymatically inactive regulatory sub-units ($M \approx 3\times10^4$) and enzymatically active sub-units ($M \approx 9\times10^4$); only the first type binds CTP. The enzymatically active sub-unit, when dissociated from the regulating sub-unit, is not inhibited by CTP.

It may be concluded that multienzyme complexes as defined initially do not occupy a special position in enzyme structures. It remains to be seen whether the concept of homofunctional and heterofunctional complexes provides a better insight, since the organization of enzymes is more elaborate than has been described here. We have not considered systems which act at the level of large cell structures, *e.g.* the vectorial enzymes of the transport system on membranes or the mechanochemical systems of contractile or other mobile structures.

The author is grateful to Prof. L. J. Reed and Dr. R. M. Oliver (Fig. 1) and to Prof. F. Lynen (Fig. 2) for the use of illustrations. The author's own investigations were supported by the Deutsche Forschungsgemeinschaft.

Received: June 1st, 1966 [A 537 IE]
German version: Angew. Chem. *78*, 865 (1966)
Translated by Express Translation Service, London

[49] *N. O. Kaplan*, Brookhaven Symp. Biol. *17*, 131 (1964).

[50] *T. Wieland* and *G. Pfleiderer*, Angew. Chem. *74*, 261 (1962); Angew. Chem. internat. Edit. *1*, 169 (1962).

[51] *J. J. Holbrock*, Biochem. Z. *344*, 141 (1966).

[52] *L. F. Hass*, Biochemistry *3*, 535 (1964).

[53] *J. A. Winstead* and *F. Wold*, J. biol. Chemistry *239*, 4212 (1964).

[54] *E. W. Westhead, L. Butler*, and *P. D. Boyer*, Biochemistry *2*, 927 (1963).

[55] *E. Grazi, T. Cheng*, and *B. L. Horecker*, Biochem. biophysic. Res. Commun. 7, 250 (1962).

[56] *J. Monod, J. P. Changeux*, and *F. Jacob*, J. molecular. Biol. *6*, 306 (1963).

[57] *J. Monod, J. Wyman*, and *J. P. Changeux*, J. molecular. Biol. *12*, 88 (1965).

[58] *J. C. Gerhart*, Brookhaven Symp. Quant. Biol. *17*, 222 (1964).