trodes with a typical electroencephalographic record shown below. For implantation the snakes were anesthetized with ~ 30 mg per kilogram of sodium pentobarbital administered intraperitoneally. With a dental hand drill small holes were made in the cranium. Small stainless steel jewelers' screws with electrical leads were self-tapped through the skull to the surface of the telencephalon. In all experiments reported here each snake was allowed sufficient time to recover fully from the electrode implantation before any responses were measured. The evoked potentials (EP) were measured with a signal averager (Princeton Applied Research waveform eductor). The eductor was set to average the signal-to-noise ratio 250 msec after the initiation of the stimulus. Signal amplification was recorded in the conventional manner. Evoked potentials to visible stimuli were obtained by the use of a photoflash with an electronic trigger to the eductor. Figure 2A is a typical visual EP with a latency of 30 msec. This latency is a little longer than the 21- to 24-msec latency reported for garter snakes (*10*). Figure 2B is a typical EP record from an 8-msec infrared laser pulse. The total energy given was between 1.5×10^{-5} and 2.7×10^{-5} calorie per square centimeter. The EP latency averaged from three different snakes was between 25 and 30 msec. The snakes were tested on consecutive days with essentially no variance in their record. Electroencephalographic records were obtained routinely during every experiment.

The stimuli we were presenting appeared to be well above the threshold values since a decrease in the shutter speed from 8 to 18 or 33 msec with no change in the laser output gave a record similar in every respect to the 8-msec stimulus. Before each stimulus the position of the snake's head was carefully noted, but here again, at the stimulating intensities used, no correlation with head position was noted, thus suggesting that we were operating in the range far above the threshold stimulus. Figure 2C is a click control; the recording situation is the same as that used in Fig. 2B except that the laser beam was blocked from the snake by means of a firebrick.

Our data strongly suggest that the receptor operates on thermal principles. This conclusion is based on the following observations: (i) there is a high sensitivity to stimuli in the far-infrared region; and (ii) the EP latency after an infrared stimulus is comparable to that of the visual EP. Since the eye is a multisynaptic structure in contrast to the single afferent nerve endings of the infrared receptor, we would expect a very much shorter latency if the infrared receptor operated on a photochemical basis. Bullock and Barrett (*5*) reported a threshold of 1.3×10^{-3} calorie cm^{-2} sec^{-1}, which is very close to our finding. The fact that the energy we used was entirely in the far-infrared region and that our responses were measured by means of evoked potentials from chronic preparations is strong evidence that the mode of operation is thermal.

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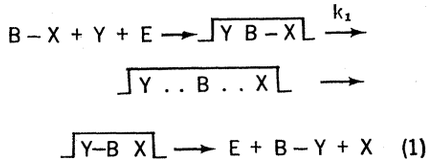
Covalent Enzyme-Substrate Intermediates

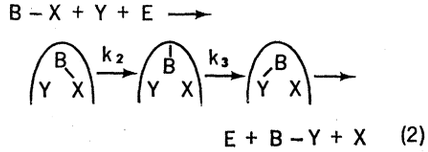
Abstract. *A literature search reveals 60 cases in which there is strong evidence for covalent enzyme-substrate intermediates.*

The manner in which enzymes catalyze reactions is a fascinating problem, relevant both to an understanding of organic chemistry and to the biological role of these catalysts. In all cases, the enzyme must act by polarizing the electrons and the bonds undergoing reaction, but in some cases a covalent intermediate is formed during the course of the enzyme action. The question then arises as to the role of these covalent intermediates. [For general reviews of covalent intermediates, see (*1–7*).]

One possibility is that the covalent intermediate provides a catalytic function. This can be illustrated in Eqs. 1 and 2, where the substrate, BX,



(1)



(2)

is converted to the product BY, either by direct attack of Y on BX (Eq. 1) or indirectly through an enzyme-substrate intermediate (Eq. 2). If the intermediate is to perform a catalytic role, the rate constants k_2 and k_3 must both be greater than k_1, that is, the group on the enzyme surface acts both as a better attacking group than Y and a better leaving group than X. These criteria have been satisfied in certain model systems. Thus the serine of many serine proteinases is both a better leaving group than the amide of peptides and a better nucleophile than water (*8, 9*). Similar properties of pyridoxal intermediates have been demonstrated (*10, 11*). Nevertheless, the data from model systems comparing nonenzymatic analogs for k_2, k_3, and k_1 does not indicate any very large catalytic advantage for the formation of a covalent intermediate.

There may be, of course, reasons other than catalytic ones for such intermediates. They might preserve the high energy character of a bond in a multistage reaction. They might serve the function of preserving the stereochemistry of an asymmetric atom or might provide a sterically more favored course for complex intermediates.

Although advantages can be listed, it is clear that covalent intermediates are not essential for enzyme action. A negative result such as the failure to isolate an intermediate is not conclusive, but the stereochemistry in a single dis-

placement reaction and negative exchange data in certain cases provide strong evidence against their presence (*12*). Thus, covalent intermediates are not essential for enzyme action, and the question arises, therefore, whether they are an occasional aberration or a significant route in enzyme mechanisms. To help clarify this question, a search of the literature was made to ascertain the number of enzymes in which there is strong evidence for a true covalent intermediate. The list is appended here because of its potential usefulness for both teaching and research.

The intermediates have been listed according to six general types: miscellaneous, pyrophosphoryl, phosphoryl, acyl (nonproteolytic enzymes), acyl (proteolytic enzymes), and Schiff base. The evidence available for each intermediate is summarized in the Table 1, together with the amino acid residue

Table 1. Enzyme-substrate covalent intermediates.

Enzyme*	Covalent intermediate		Residue that participates in covalent bond formation		Reference
	Type	Evidence†	Residue	Evidence‡	
A. Miscellaneous enzyme-substrate covalent intermediates					
Glycine amidinotransferase E.C. 2.1.4.1	Amidine-enzyme	I-N, I-T, ES			(*13*)
Sucrose glucosyltransferase (sucrose phosphorylase) E.C. 2.4.1.7	Glucosyl-enzyme	I-D, I-C, I-P, ES, K-PP	Carboxyl	R-P	(*14*)
N-1-(5′-phosphoribosyl)-ATP synthetase	Phosphoribosyl-enzyme	I-D, I-N, I-P, K-B, ES			(*15*)
Succinyl-CoA: 3-oxoacid CoA-transferase E.C. 2.8.3.5	Coenzyme A-enzyme	I-N, I-T, K-PP, E	Glutamic acid	R-C	(*16*)
Polynucleotide ligase	Adenyl-enzyme	I-N, I-T, E			(*17*)
B. Pyrophosphoryl-(phosphoryl) enzyme intermediates					
ATP : D-ribose-5-phosphate pyrophosphotransferase (PRPP synthetase)§ E.C. 2.7.6.1	Pyrophosphoryl-enzyme	I-N, I-T, ES			(*18*)
Phosphoenolpyruvate synthetase	Pyrophosphoryl-enzyme Phosphoryl-enzyme	I-N, I-T, E			(*19*)
Pyruvate phosphate dikinase	Pyrophosphoryl-enzyme Phosphoryl-enzyme	I-N, E			(*20*)
C. Phosphoryl-enzyme intermediates					
ATP : acetate phosphotransferase E.C. 2.7.2.1	Phosphoryl-enzyme	I-N, I-T, E, K-PP			(*21*)
Phosphoglucomutase E.C. 2.7.5.1	Phosphoryl-enzyme	I-N, I-P, E	Serine	R-I	(*22*)
Phosphoglyceromutase E.C. 2.7.5.3	Phosphoryl-enzyme	I-N	Histidine	R-I	(*23*)
Alkaline phosphatase E.C. 3.1.3.1	Phosphoryl-enzyme	I-N, I-P	Serine	R-I	(*24*)
Acid phosphatase E.C. 3.1.3.2	Phosphoryl-enzyme	I-N	Histidine	R-C	(*25*)
Glucose-6-phosphatase E.C. 3.1.3.9	Phosphoryl-enzyme	I-D, I-T, I-P, E	Histidine	R-I	(*26*)
Na^+, K^+ adenosine triphosphatase	Phosphoryl-enzyme	I-D	Glutamic acid	R-C	(*27*)
Phosphoramide-adenosine diphosphate phosphotransferase	Phosphoryl-enzyme	I-N, I-T			(*28*)
Phosphoenolpyruvate hexose transferase	Phosphoryl-enzyme	I-N, I-T, I-P	Histidine	R-I	(*29*)
Phosphoramidate hexose transferase	Phosphoryl-enzyme	I-D, I-N, I-T, E			(*30*)
ATP citrate lyase (citrate cleavage enzyme) E.C. 4.1.3.8	Phosphoryl-enzyme	I-N, I-P	Glutamic acid	R-C	(*31*)
Succinyl-CoA synthetase E.C. 6.2.1.5	Phosphoryl-enzyme	I-D, I-N, I-T, I-P, E	Histidine	R-I	(*32*)
Nucleoside diphosphate kinase	Phosphoryl-enzyme	I-N, I-T, E, K-PP			(*33*)

* The names of the enzymes which have been assigned E.C. numbers are the systematic or recommended trivial names approved by the Enzyme Commission on Enzyme Nomenclature (*74*). In a few special cases a name not recommended by the Enzyme Commission is found in parentheses because of its general use. For those enzymes that have not been assigned E.C. numbers, the names used in the original references were generally used. The Enzyme Commission's report or the original references should be consulted for the reaction catalyzed by each enzyme. † Evidence for enzyme-substrate covalent intermediates: isolation (I); isolated-denatured (I-D) indicates that the substrate was found with the enzyme after denaturation; isolated-native (I-N) means that the substrate was found with the enzyme without denaturation, but appropriate controls ruled out noncovalent binding; isolated and turns over (I-T) indicates that the portion of substrate that is covalently bound to the enzyme turns over upon addition of acceptor; isolated-chemically (I-C) indicates that the enzyme substrate intermediate was trapped by chemical reaction such as sodium borohydride reduction; isolated-peptides (I-P) means that peptides with covalently bound substrate were demonstrated; I′ followed by a letter indicates that a quasi-substrate was used. Kinetic evidence (K); kinetics-ping pong (K-PP) indicates that ping pong kinetics were observed; kinetics-V_{max} (K-V) indicates that a constant V_{max} was observed independent of acceptor; and kinetics burst (K-B) indicates that an initial burst phenomenon upon mixing of substrate and enzyme was observed; exchange data (E); exchange (E) indicates demonstration of appropriate exchange reactions; and exchange-specificity (ES) indicates that the exchange-specificity criterion for enzyme-substrate covalent intermediates has been met (*15*). ‡ Evidence for residue at active site includes the following: residue isolated R(-I) indicates that the residue was identified by isolation and characterization of a peptide or amino acid containing covalently bound substrate; residue protected (R-P) indicates that the residue was identified because the covalently bound substrate protected a particular residue against modification and that subsequent release of the substrate allowed modification of a specific residue; residue-chemically (R-C) indicates that the residue was identified by a characteristic chemical reaction. § Abbreviations: ATP, adenosine triphosphate; PRPP, 5-phosphoribosyl 1-pyrophosphate; ACP, acyl carrier protein; CoA, coenzyme A. || Several other enzymes have been shown to contain essential carbonyl groups, and their mechanism of action probably proceeds through Schiff base intermediates. These include (prosthetic group in parentheses): histidine deaminase (E.C. 4.3.1.3) (dehydroalanine [I. L. Givot, T. A. Smith, R. H. Abeles, *J. Biol. Chem.* **244**, 6341 (1969)]; L-phenylalanine ammonia-lyase (E.C. 4.3.1.5) [E. A. Havir and K. R. Hanson, *Biochemistry* **7**, 1904 (1968); D. S. Hodgins, *Biochem. Biophys. Res. Commun.* **32**, 246 (1968)]; amine oxidase [E. F. Yamasaki, R. Swindell, D. J. Reed, *Biochemistry* **9**, 1206 (1970)].

Enzyme*	Covalent intermediate		Residue that participates in covalent bond formation		Reference
	Type	Evidence†	Residue	Evidence‡	
D. Acyl-enzyme intermediate (nonproteolytic enzymes)					
Glyceraldehyde phosphate dehydrogenase E.C. 1.2.1.12	Acyl-enzyme	I-D, I-N, I-P, E	Cysteine	R-I	(*34*)
Acetoacetyl-CoA thiolase E.C. 2.3.1.9	Acyl-enzyme	I-N, I-P	Cysteine	R-I	(*35*)
β-Hydroxymethylglutamyl-CoA condensing enzyme	Acetyl-enzyme	I-D			(*36*)
Pigeon liver fatty acid synthetase	Acyl-enzyme	I-D, I-N, I-T, I-P	Cysteine and nonthiol	R-P, R-C	(*37*)
Yeast fatty acid synthetase	Acyl-enzyme	I-D, I-N, I-T, I-P	Cysteine and nonthiol	R-P, R-C	(*38*)
β-Ketoacyl acyl carrier protein synthetase	Acetyl-enzyme	I-N, I-T	Cysteine	R-C	(*39*)
Acetyl-CoA : ACP acetyl-transacylase	Acetyl-enzyme	I-D, I-N, I-T			(*40*)
Transglutaminase	Acyl-enzyme	I′-N, I′-P, K-B	Cysteine	R-I	(*41*)
ATP citrate lyase (citrate cleavage enzyme) E.C. 4.1.3.8	Citryl-enzyme	I-N			(*42*)
Formylglycinamide ribonucleotide amidotransferase E.C. 6.3.5.3	γ-Glutamyl-enzyme	I-N, I-D, I-T	Cysteine	R-P, R-C	(*43*)
Gramicidin S synthetase	Peptidyl-enzyme	I-D	Thioester	R-C	(*44*)
Tyrocidine synthetase	Peptidyl-enzyme	I-D	Thioester	R-C	(*45*)
E. Acyl-enzyme intermediates (proteolytic, esteratic enzymes)					
Acetylcholinesterase E.C. 3.1.1.7	Acyl-enzyme	I′-D, I′-P	Serine	R-I	(*46*)
Butyrylcholine esterase	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*47*)
Papain E.C. 3.4.4.10	Acyl-enzyme	I-D, I-N, K-V, K-B	Cysteine	R-I, R-P	(*48*)
Ficin E.C. 3.4.4.12	Acyl-enzyme	I-D, I-N, K-B, K-V	Cysteine	R-C	(*49*)
Thrombin E.C. 3.4.4.13	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*50*)
Plasmin E.C. 3.4.4.14	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*51*)
Subtilopeptidase (subtilisin) E.C. 3.4.4.16	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*52*)
Trypsin E.C. 3.4.4.4	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*53*)
Chymotrypsin E.C. 3.4.4.5	Acyl-enzyme	K-V, K-B, I-D, I-N	Serine	R-I	(*54*)
Elastase E.C. 3.4.4.7	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*55*)
Alkaline proteinase	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*56*)
Beef liver esterase	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*57*)
Horse liver esterase	Acyl-enzyme	I′-D, I′-N, I′-P	Serine	R-I	(*58*)
F. Schiff base intermediates					
D-Amino acid oxidase E.C. 1.4.3.3	Schiff base	I-C	Lysine	R-I	(*59*)
Transaldolase E.C. 2.2.1.2	Schiff base	I-C	Lysine	R-I	(*60*)
Fructose diphosphate aldolase E.C. 4.1.2.13	Schiff base	I-C	Lysine	R-I	(*61*)
4-Hydroxy-2-ketoglutarate aldolase	Schiff base	I-C	Lysine	R-I	(*62*)
Deoxyriboaldolase E.C. 4.1.2.4	Schiff base	I-C	Lysine	R-I	(*63*)
Phospho-2-keto-3-deoxygluconate aldolase E.C. 4.1.2.14	Schiff base	I-C	Lysine	R-I	(*64*)
Acetoacetate decarboxylase E.C. 4.1.1.4	Schiff base	I-C	Lysine	R-I	(*65*)
Histidine decarboxylase ‖ E.C. 4.1.1.22	Schiff base	I-C	Pyruvic acid	R-I	(*66*)
2-Keto-3-deoxy-L-arabonate dehydratase	Schiff base	I-C			(*67*)
δ-Aminolevulinate dehydratase E.C. 4.2.1.24	Schiff base	I-C			(*68*)
Pyruvate-aspartic semialdehyde condensing enzyme	Schiff base	I-C	Lysine	R-I	(*69*)
S-Adenosylmethionine decarboxylase	Schiff base	I-C	Pyruvic acid	R-I	(*70*)
4-Deoxy-5-oxyglucarate hydrolase (decarboxylating)	Schiff base	I-C	Lysine	R-I	(*71*)
Urocanase	Schiff base	I-C	α-Ketobutyrate	R-I	(*72*)
D-Proline reductase E.C. 1.4.1.6	Schiff base	I-C	Pyruvic acid	R-I	(*73*)

which forms the covalent bond when such information is available. Attempts were made to examine the data critically and to include only those cases for which extremely strong evidence was available. Thus, indirect arguments and kinetic evidence in some cases provide strong presumptive evidence, particularly when the reaction parallels that of other similar reactions. For present purposes, however, such cases were excluded. A somewhat arbitrary decision was made to exclude cases involving a covalent intermediate to a coenzyme which in itself is covalently linked to the protein at some stage of the reaction, for example, the pyridoxal reactions. Similarly, proton transfer to the enzyme was excluded, although such bonds are literally covalent. The table would have been too extensive and the theoretical significance would be diminished.

References are provided for accessibility to the literature and usually include the most recent and conclusive evidence. Because, in many cases, many laboratories participated in the delineation of the intermediate, the references should be considered as entries to the literature rather than as the single source for the evidence listed.

Table 1 indicates that covalent intermediates are not a rare and unusual event. Rather, there is widespread occurrence of such intermediates in many types of enzymes. Since establishing the existence of intermediates is difficult experimentally, and since many enzymes have not been isolated to a state of purity which would allow such tests, it can be concluded that this list is only a fraction of the ultimate total. The covalent intermediate must provide important, yet nonessential, functions which may be those listed above. Whatever they are, they have biological survival value.

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