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Some Comments on Allosteric Transitions

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In a recent paper on allosteric transitions [1] it has been proposed that activating and inhibiting molecules can be bonded at a considerable distance from the substrate and still drastically change the activity of an enzyme. The effect should be achieved by stabilization or destabilization of the most active enzyme conformation. It is important to be able to distinguish such an effect from a direct interaction, and a quantitative model has been developed [1] which permits experimental tests of the existence of allosteric effects.

The theory deals particularly with the fraction of sites bound by the substrate or an activating or inhibiting ligand. This fraction can be determined by kinetic or equilibrium studies. (Direct measurements of the free ligand concentration give the same results.)

In the following comments some aspects of that theory will be emphasized or briefly developed.

1. For a *monomeric enzyme* it is not possible to distinguish with such experimental methods between direct and allosteric effects. This is obvious from the formula given for a binary complex [1] but not from the formula for a ternary complex.

By simple algebraic calculations it can be shown, however, that the fraction of sites bound is exactly the same function of the concentrations of the ligands whatever mechanism is valid. The results can be explained *either* as due to an *allosteric transition* where the enzyme conformations *preserve their affinities* for the ligand *or* as a direct effect *changing* the *affinity* for the ligand. The preference for one of the theories must be given by other experimental methods.

2. For an *oligomeric enzyme* the concentration dependence will differ for the two alternatives, but for *very low* or *very high concentrations* of the ligand an *allosteric enzyme will behave as if only one conformation were significant.* We have made a large number of calculations, varying the relative affinities for the ligand and the relative stabilities of the enzyme conformations and have found under which conditions an apparent dissociation constant for only one conformation will fit the data. At low ligand concentrations a dissociation constant will be obtained which is equal to or smaller than the assumed value for the weakest enzyme-ligand interaction. At high ligand concentrations a dissociation constant will be obtained which is equal to or higher than the assumed value for the strongest enzyme-ligand interaction. As a general rule it can be stated that an allosteric effect will be discovered by equilibrium studies if the determination of the constant is made in a concentration range including a *ligand concentration of one tenth to ten times the apparent constant determined.*

3. The sigmoid curve derived for an oligomeric allosteric enzyme does not require the very limited symmetry conditions of the model proposed. [1] The model is thus based on the assumption that for example a dimeric enzyme must have the same symmetry in both enzyme conformations. This means that both subunits should be changed cooperatively. It is, however, easy to show by practical calculations that the sigmoidal curve is obtained even if a mixed conformation exists with the same stability as the one in which the symmetry is preserved. The curvature of the sigmoidal curve is reduced but the effect is still there.

4. If both "on" and "off" velocity constants have been determined for the binary and ternary complex formation with the ligands, there is a further possibility to prove or at least find indication of

a conformational change. If the "on" velocity constants are equal for the addition of a ligand to form a binary and a ternary complex it is reasonable to assume that the "on" reactions take place with similar conformations. If the "off" velocity constants differ in the same system it is a strong indication that the ternary complex has another enzyme conformation than the binary complex. This is the case with horse-liver alcohol dehydrogenase, for which Theorell and McKinley-McKee [2] observed this regularity for the velocity constants and for which we have subsequently shown that the ternary complex has another crystal structure than the binary complex.[3,4] The results have been corroborated by optical rotation studies.[5,6]

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Über die Biosynthese von Cycloalliin

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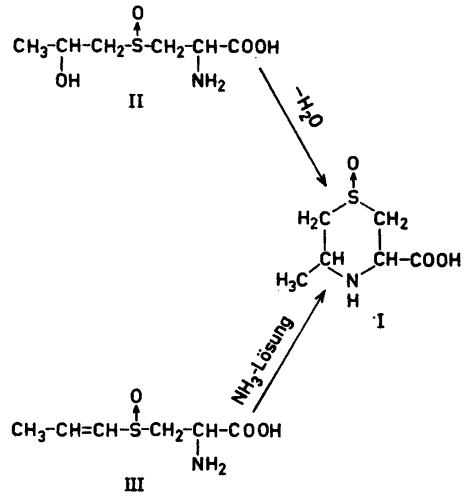
Cycloalliin (I) in reduzierter Form wurde erstmals in diesem Laboratorium von Virtanen und Matikkala [1] aus der Küchenzwiebel, *Allium cepa*, isoliert. Dass die Verbindung als Sulfoxid in der Zwiebel vorkommt wurde später bewiesen.[2] Nach der Aufklärung der Struktur des Cycloalliins und der Synthese desselben wurde es möglich gehalten, dass die Biosynthese von I über die bisher unbekannte Aminosäure II verläuft.[3]



Etwas später wurde in diesem Laboratorium die Vorstufe der tränentreibenden Substanz der Zwiebel isoliert und als S-(1-Propenyl)-L-cystein-S-oxid (III) charakterisiert. [4,5] Diese Verbindung (LP) cyclisiert in schwach ammoniakalischer Lösung zu I. Da beide Substanzen in der Zwiebel in grösseren Mengen nachgewiesen werden konnten, lag der Schluss nahe, dass auch die Biosynthese des Cycloalliins in der Zwiebel über die Stufe des LP verläuft.

Um diese Vermutung zu beweisen, wurde ^{35}S-(1-Propenyl)-L-cystein-S-oxid (LP-^{35}S) synthetisiert und Zwiebeln, welche eine Woche in feuchtem Sand gewesen waren, in Form einer wässrigen Lösung eingespritzt. Einen bzw. sieben Tage nach der Injektion wurden die Zwiebeln aufgearbeitet und die Aminosäuren auf einem 2-dimensionalen Chromatogramm getrennt (vgl. Ettala und Virtanen [6]). Die Lage und Grösse der radioaktiven Flecken wurde durch Autoradiogramme festgestellt, die Intensität mit einem β-Zählrohr gemessen. Aus diesen Daten wurde der Anteil der ^{35}S-Aktivität im Cycloalliin an der gesamten auf dem Chromatogramm vorhandenen ^{35}S-Aktivität bestimmt. Da sich LP während des Aufarbeitens der Zwiebeln teilweise in I umlagert, wurden Kontroll-