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Extrinsic Signals for Monitoring the Association Reaction of Proteins as Introduced by Fluorescent and Non-Fluorescent Labels

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Summary: Two known dansyl labels (I, II) and 5-[2-(iodoacetamido)ethylamino]-1-naphthalenesulfonic acid (III) and three new azo-dyes (IV - VI) were covalently attached to α-chymotrypsin and to basic pancreatic trypsin inhibitor by four different reactive groups. In order to protect the contact region of the proteins the complex of the two proteins was labeled. Advantage was taken of the fact that a group which is buried in the complex reacts about $\sqrt{[C]/K}$ times slower than a group which is always exposed (K = dissociation equilibrium constant, $[C]$ = concentration of the complex). The complex was dissociated at pH 3 and the labeled proteins were isolated by column chromatography. They were fully active. The dansyl label was immobilized when introduced by dansyl chloride but highly mobile when attached via the longer imidoester group (II). Changes of absorption and of fluorescence which occur when differently labeled reaction partners recombine were studied. Changes in absorption (up to 18%) were mainly due to interactions of the label of one protein with the other protein. Fluorescence changes of up to 480% could be obtained. They were interpreted in terms of a Förster type energy transfer between donor and acceptor labels and changes of absorption and quantum yield due to interactions of the labels with the proteins. The kinetic constants of complex formation are not seriously altered by the labels (Bösterling, B. & Engel, J. (1976) *this J.* **357**, 1297 - 1307, succeeding). It is concluded that the labeling technique may be of general value for kinetic and equilibrium studies of protein associations.

Meßsignale für Protein-Protein-Assoziationen, eingeführt durch fluoreszierende und nichtfluoreszierende Markierungsgruppen

Zusammenfassung: Um große Absorptions- und Fluoreszenzänderungen bei der Assoziation von Proteinen zu erhalten, wurden an die Reaktionspartner verschiedene Reaktivfarbstoffe als Markierung gebunden. Die Kontaktregionen zwischen den Proteinen waren dabei durch Komplexbildung geschützt. Eine Gruppe, die im Komplex nicht zugänglich ist, reagiert etwa $\sqrt{[C]/K}$ mal langsamer als eine Gruppe, die immer exponiert ist (K = Gleichgewichtskonstante der Dissoziation und [C] = Konzentration des Komplexes). Diese Markierungstechnik wurde an dem System α-Chymotrypsin/Basischer Trypsin Inhibitor aus Pankreas studiert. Neben zwei bekannten Dansyl-Verbindungen (I, II) und 5-[2-(Jodacetamido)-äthylamino]-1-naphthalinsulfonsäure (III) (Fluo-

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Enzyme: Chymotrypsin (bovine α-chymotrypsin) (EC 3.4.21.1).