Steric Inhibition of Amide Resonance and its Possible Significance in Enzyme Action[1]

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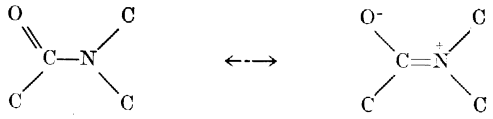
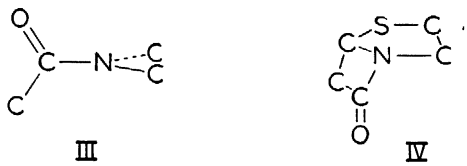
AS A CONSEQUENCE OF AMIDE RESONANCE, I ⟷ II, simple amides are planar (*1–6*). If for any reason the atoms attached to the nitrogen are forced out of



I II

the plane of the remainder of the amide group, as in III, steric inhibition of amide resonance should result



III IV

(*7, 8*). A nonplanar amide, such as III, should have enhanced reactivity (*7, 9*). This is the explanation that has been given for the reactivity of the penicillins (*7*). As shown in IV, fusion of the four-membered β-lactam ring with the five-membered thiazolidine ring forces one of the carbons attached to the nitrogen out of the plane of the amide group. Although the exact magnitude of the effect is uncertain because of limited data and because of the complex structure of the penicillins, it is probable that steric inhibition of amide resonance increases the rate of alkaline hydrolysis of the amide linkage in benzylpenicillin (*10*) by a factor[2] between 10^3 and 10^6.

Since the extent of the inhibition of amide resonance depends on the angle at which the attached atoms are distorted from the plane (*8*), inhibition would be at its maximum in a structure, such as III, in which the plane of the amide group is perpendicular to the plane containing the nitrogen and attached carbons. In the penicillins only one of the carbons attached to the nitrogen is out of the plane of the amide group, and this is out at an angle of approximately 55°.[3] Therefore, the increased reactivity observed in the penicillins is presumably less than the maximum possible increase in reactivity.[4]

Steric inhibition of amide resonance thus offers a means by which a steric effect imposed on an amide group can greatly increase the reactivity of the amide. The purpose of this note is to suggest that steric inhibition of amide resonance may play a role in the enzymatic hydrolysis of amides.

According to this hypothesis, an enzyme may facilitate hydrolysis of a substrate amide group by distorting the susceptible amide group into a nonplanar and, therefore, reactive configuration. Distortion of this nature would be opposed by the resonance energy of the amide group. To overcome this opposition, the forces of attraction between the enzyme and the substrate would have to be greater with the substrate amide group nonplanar rather than planar. It is possible that the hydrogen bonds and other interactions between the substrate and enzyme might be greater in number or of greater energy with the amide group in a nonplanar configuration than in a planar configuration, and as a result a nonplanar configuration should be favored. The nonplanar amide group might then react directly, with water (a hydroxyl ion and a hydrogen ion), or indirectly, by way of some group present on the surface of the enzyme.

It is possible to draw a rough picture of the sort of interactions between enzyme and substrate which might be effective in bringing about steric inhibition of amide resonance. Figs. 1 and 2 indicate conceivable enzyme-substrate interactions that might account for

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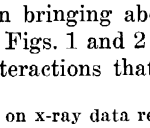
[2] The studies described in (*10*) indicate that the effects of the substituents on the reactivity of the β-lactam in benzylpenicillin either cancel each other or are small in magnitude. The increased reactivity of the β-lactam in benzylpenicillin can therefore be attributed to steric inhibition of amide resonance. Since benzylpenicillin is hydrolyzed by alkali approximately 4×10^3 times as fast as 1-methyl-2-azetidinone (N-methyl-β-propiolactam), a factor of 10^3 would appear to be the minimum for steric inhibition of resonance. According to a suggestion by Woodward (*7*), the fact that β-lactams are more reactive than ordinary amides may also be due to steric inhibition of amide resonance. If this is true, the reactivity of benzylpenicillin should be compared with that of ordinary amides instead of β-lactams, and this comparison gives a factor of 10^5 or 10^6 for the effect of steric inhibition of amide resonance.

[3] This angle is based on x-ray data reported by D. Crowfoot *et al* in (*7*, p. 310).

[4] Maximum reactivity would be observed in a compound such as 2-quinuclidinone:



Unfortunately, all attempts to prepare compounds of this type have been unsuccessful. The synthesis of such a compound and a study of its reactivity would be of great interest.

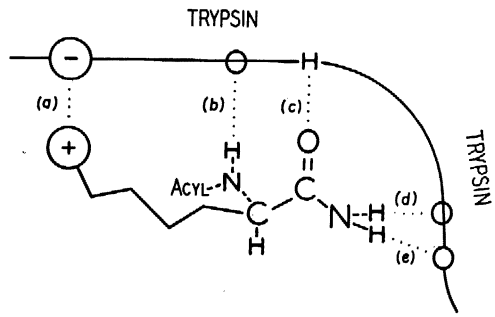
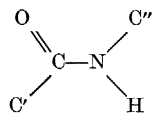


FIG. 1. Hypothetical trypsin surface showing interactions with an acyl-L-arginine amide or acyl-L-lysine amide. The two hydrogens on the nitrogen are distorted out of the plane of the amide group by hydrogen-bonding, (*d*) and (*e*).

the specificity of trypsin. Fig. 3 indicates interactions that might account for the absolute specificity of urease.

It is known that trypsin catalyzes the hydrolysis of amides, peptides, and esters of various acyl derivatives of L-arginine and L-lysine (*11*). Fig. 1 is drawn to indicate conceivable interactions between trypsin and an acyl-L-arginine amide or acyl-L-lysine amide. The interaction (*a*) between a negatively charged group in the trypsin molecule and the positively charged arginine or lysine side chain of the substrate, in conjunction with a hydrogen bond (*b*), determines the position of the substrate on the enzyme surface.[5] Hydrogen-bonding (*c*) of the oxygen of the amide to the trypsin surface, possibly in combination with other steric factors, then determines the plane of the amide group. With these points fixed, it is possible that two oxygen atoms in the trypsin molecule may be so situated that both hydrogens on the nitrogen can be hydrogen-bonded, (*d*) and (*e*), if the hydrogens are distorted from the plane of the amide group. The amide would then be in a nonplanar and, consequently, reactive configuration.

If in place of one of the hydrogens on the nitrogen of the amide there is a substituent, as there would be in an acyl-L-arginylpeptide or acyl-L-lysylpeptide, only one hydrogen is present on the nitrogen and is available for hydrogen-bonding. It might be expected that this would reduce the probability of the amide being distorted into a nonplanar configuration. However, the α-carbon atoms, C′ and C″ (V), of adjacent



V

amino acids in the peptides are ordinarily arranged *trans* with respect to the carbon-nitrogen bond of the amide (*4, 5, 12*). If this *trans*-peptide structure, V, replaces the simple amide of Fig. 1, it is reasonable to expect that, as interactions between trypsin and the substrate bring an acyl-L-arginylpeptide or acyl-L-lysylpeptide close to the surface of the enzyme, steric hindrance would tend to force the amide out of the planar configuration. This tendency, in combination with the formation of one hydrogen bond, may be sufficient to result in a nonplanar configuration of the amide group, as shown in Fig. 2.

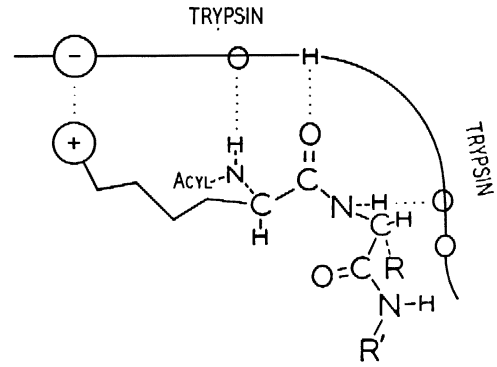
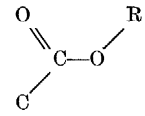


FIG. 2. Hypothetical interactions between trypsin and an acyl-L-arginylpeptide or acyl-L-lysylpeptide with the amide group nonplanar.

The hydrolysis of acyl-L-arginine esters and acyl-L-lysine esters might take place in an analogous fashion. The ester group, like the amide group, is planar because of resonance (*1, 2, 13*). Since esters also exist normally (*13*) in a *trans*-configuration, VI, the alkyl



VI

group of the ester would be forced out of the plane of the ester group when the substrate interacts with the enzyme. Steric inhibition of ester resonance would result, and hydrolysis would be facilitated. Thus, an enzyme surface such as that pictured in Figs. 1 and 2 may account satisfactorily for the specificity of trypsin.

It is known that urease is absolutely specific for the hydrolysis of urea (*14*). In urea, resonance is possible with either or both of the amide groups. Therefore, in order to increase greatly the reactivity of urea, the hydrogens attached to both nitrogens would have to be forced out of the plane. This might be accomplished, as shown in Fig. 3, by the formation of five hydrogen bonds. Since all four hydrogens and the oxygen of urea would take part in interactions with the enzyme, the absolute specificity of urease might be explained.

This hypothesis is, of course, a specialized form of the general theory of enzyme action proposed by Michaelis and Menten (*15*), and it should not be inferred that the author discounts the possible signifi-

[5] The hypothesis does not require that the interactions (*a*) and (*b*) be of a specific type. However, at least two interactions, illustrated by (*a*) and (*b*), are required to determine the position of the amide group, and to account for optical specificity.

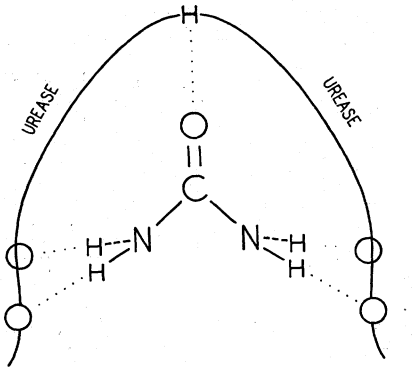


FIG. 3. Hypothetical urease surface showing interactions with urea. Both amides are distorted into nonplanar configurations.

cance of other effects that might make the amide linkage more reactive, or of entirely different effects (*16, 17*). Steric inhibition of amide resonance is of special interest because it has been found in naturally occurring compounds, the penicillins, and it seems likely that it can be studied in other models. A consideration of the possible significance of a specific effect such as this may be of value in the development of experimental approaches to the important problem of enzyme action.

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News and Notes

Scientists in the News

F. K. Bannister has been appointed to a new chair of thermodynamics in the Department of Mechanical Engineering, University of Birmingham. The chair has been created in connection with the organization of the new graduate school in thermodynamics, and Dr. Bannister will be in charge of this school under **G. F. Mucklow,** head of the department. Professor Bannister was appointed lecturer in mechanical engineering in the University of Birmingham in 1941 and reader in thermodynamics in 1951. He has been closely associated with the Air Squadron at the university since its formation in 1941.

Christopher E. Barthel, Jr., has been elected chairman of the board of directors of the National Electronics Conference. Dr. Barthel succeeds **Kipling Adams,** of General Radio Company. The National Electronics Conference is a forum on electronics research, development, and application.

Eric Bradshaw has succeeded **John Hollingworth** in the chair of electrical engineering in the Faculty of Technology in the University of Manchester. He has been special lecturer in high-voltage engineering at the College of Technology since 1944.

E. Lucy Braun, botanist and forestlands expert, has been awarded the Mary Soper Pope Medal granted periodically by Cranbrook Institute of Science, for outstanding achievement in fields related to the study of plant life. Dr. Braun is professor emeritus of botany at the University of Cincinnati.

Colin G. Clark, recently under-secretary of state for labor and industry and financial adviser to the Treasury, Queensland, has been appointed director of the Institute for Research in Agricultural Economics at Oxford, to succeed **A. W. Ashby,** who is retiring under the age-limit provisions.

Eduard Farber, chief chemist of Timber Engineering Company, affiliate of National Lumber Manufacturers Association, has been elected a fellow of the Royal Society of Arts, and an honorary member of the Mark Twain Society.

Continental Can Company has announced the appointment of **Raymond G. Fisher** as director of economic research. His responsibilities will include supervision of the commercial research and sales analysis activities of the company.

Among the recent recipients of medals and awards given by sections of the American Chemical Society are **Reynold Clayton Fuson,** University of Illinois, the 1953 William H. Nichols Medal, from the New York Section, "for studies on nucleophilic substitutions and displacements of an unorthodox character, elucidation of abnormal organic reactions, discovery of stable ene-