the replication involved a newly manufactured wing, a different hot-wire sensor operating on a separate calibration curve, and a drive system rebuilt to offset wear. While the replication differed sufficiently from the original to suggest that any consideration of minor trends in the data is unrealistic, the major trend was preserved.

The influence on lift of the rate of incidence change is given (Fig. 2B) at various forward velocities. In all cases the large increasing incidence rate was 10,600 deg/sec, the decreasing rate −16,000 deg/sec, the instantaneous downstroke velocity 654 cm/sec, and the flapping mode one of simple harmonic motion. The large positive incidence rate effected a greater lift, as compared to the large negative rate, at all forward velocities tested. The decreasing incidence rate was associated with reduced lift, severe stalling, or both.

Two things may explain the effect of rapid changes of incidence. First, is the Magnus effect, wherein a rotating surface produces a circulation which, in combination with a velocity of translation, yields lift. The dynamic incidence change may be viewed as a form of rotation within this analogy; a positive rate of change corresponds to an increase of circulation and a negative rate corresponds to a decrease. An analysis of this matter (*6*) in the case of an oscillating wing subjected to large perturbations indicates qualitative agreement between the experimental results (rate of change of incidence versus lift) and predicted performance. A second rationale is based on viscous effects, in particular, destalling attendant upon certain unsteady motions. An impulsive change of incidence given to an airfoil on the verge of stalling will, if positive, delay stall until a larger angle of incidence and more lift is developed than are manifest in a steady state (*7*). Conversely, a negative incidence impulse delivered near stall will create an exaggerated stall effect, compared to steady-state values. As many of the experimental results were gathered at large angles of incidence, suggestive of the stall domain, it is possible that the influence of impulsive incidence on stall is significant with respect to the lift generated. The relative significance of these two factors in causing high lift is uncertain. Both are probably active; however, the violent stalls produced by a negative incidence rate and the low lift at small angles of incidence, regardless of incidence rate, suggest that viscous effects are dominant.

In view of the large rate of change of incidence values employed in these experiments, it is useful to examine flight values. Of the insects whose flight characteristics have been evaluated to the necessary degree of accuracy, the desert locust *Schistocera gregaria* (*8*) displays a small rate of incidence change in downstroke. The diptera studied by Nachtigall (*9*) experienced a linearized rate of incidence change at mid-downstroke of roughly 20,000 deg/sec. The latter value is compatible with my largest values from simulated tests. The paucity of available measurements in vivo precludes any general statement concerning augmentation of lift by insects through rapid changes of incidence. However, it may be that at least some insects do this.

Insect flight has been treated as reducible to a quasi-steady approximation, wherein the wing takes on instantaneous lift values corresponding to steady-state values at equivalent velocities and attitudes. Such an assumption is unwarranted where the angle of incidence changes rapidly. Through use of large positive rates of change of incidence at mid-downstroke, insects may develop lift values several times those apparent in quasi-steady models. I suggest that the apparent gap between requisite and available force coefficients for certain insects may reflect the quasi-steady analysis by investigators and the employment of an incidence change strategy by the insects.

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10. I thank graduate students R. Newman, M. Cytter, and W. Lorell for experimental and theoretical efforts and the NSF for support (GK-1637).

23 June 1969; revised 29 September 1969 ■

Absorption of Proteins and Peptides in the Far Ultraviolet

Abstract. *An absorption peak for the peptide bond at 187 nanometers has been confirmed; a protein assay at this wavelength allows quantitation of proteins in aqueous solution at concentrations between 0.1 and 25 micrograms per milliliter. Assays are conducted at 6°C to take advantage of the reduction of end-absorption of water with temperature.*

Methods for protein assay are complicated by the contribution of aromatic side chains to the final colorimetric or spectrophotometric determination (*1*). Since the content of such side chains varies considerably from protein to protein, it is necessary to construct a separate standard curve for each species of protein; furthermore, when mixtures of proteins are to be assayed the contribution of each may be difficult to judge. This situation holds true for both colorimetric assays such as the Lowry method (*2*) and spectrophotometric assay at 280 nm (*1*). Because of this there have been several attempts to develop an assay based on the ultraviolet absorption of the peptide bond which would be relatively free of side chain contribution and would yield a true measure of the protein content.

Although an absorption peak for the peptide bond has been described at about 186 nm (*3*, *4*), there have been severe limitations on the capacity of conventional spectrophotometers to operate in this range. Apart from optical limitations of the ultraviolet range, the absorption of oxygen below 200 nm has required nitrogen purging of the light path. In addition, the end absorption of water and salt solutions in the far ultraviolet is so large that stray light becomes a limiting factor. The contribution of stray light leads to spurious absorption peaks (*5*) and is further complicated by the need to stabilize most protein solutions by the

addition of salts that add heavily to the end absorption. The result of these difficulties has been to force the peptide bond assay away from its peak to longer wavelengths where instrument capabilities are better, stray light is less, and end absorption is lower. Measurements at 205 (*3*) and 210 nm (*6*) have been proposed, and such assays are reasonably successful despite the fact that lower absorbancies are obtained and interference from aromatic side chains is apparent (*6*).

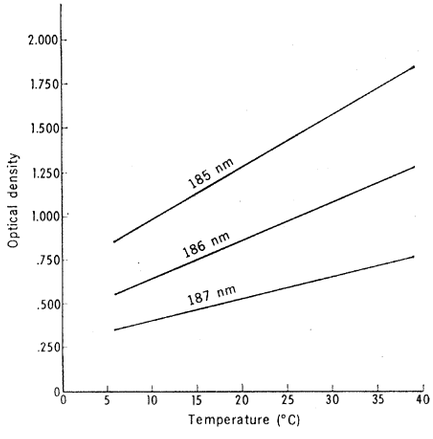
One method of reducing solvent absorption is to shorten the light path by using narrow cells (*7*); another, reported here, is related to the decrease in end absorption of water and salt solutions with a decrease in temperature. The magnitude of this decrease is apparent in Fig. 1. The relation between absorption and temperature is roughly linear and amounts to a doubling of optical density with every increase of 25°C from 0° to at least 50°C (*8*). The spectrophotometer is equipped to maintain a cell compartment temperature of 6°C. It consists of a Beckman DU monochromator equipped with DK optics capable of transmission down to 185 nm and adapted for nitrogen purging. The monochromator is coupled with a Gilford 2000 photometer. It was necessary to maintain nitrogen flows of 10 liter/min and to allow sufficient time for temperature equilibration before reading. Determinations of stray light were made for each wavelength (*5*), and corrections for stray light were made by a program written for the Programma 101 computer. Buffers used were 0.05*M* borate or 0.005*M* phosphate at *p*H 7.4.

Absorption spectra of representative proteins and peptides showed a peak at 187 nm. This peak is fairly sharp for glycylglycine and glycylglycylglycine (*3*). The peak for bovine albumin (fraction V) is broader, extending from 186 to 192 nm. Absorption data for a series of peptides and proteins at 187 nm are given in Table 1. The calculated absorption of each peptide bond is very nearly 10,000 for all molecules except those containing aromatic residues. It is apparent that considerable side chain contribution is still present at 187 nm in these small molecules (*9*); however, the close agreement in values between the glycine peptides and albumin indicate that this is unlikely to cause significant interference in large molecules except where large amounts of tyrosine or tryptophan are present, as in insulin or gramicidin. It is also possible that the large protein molecules are demonstrating a hypochromic effect due to parallel arrangement of peptide bonds in the α-helical configuration, and that this effect offsets the influence of aromatic side chain absorption. Should protein denaturation occur, an increase in absorbance of up to 30 percent may be encountered (*10*).

The increase in absorbance at 187 nm is threefold over that at 205 nm and fourfold over that at 210 nm, which illustrates the considerable loss of sensitivity incurred by operating on the shoulder of the absorption peak. In addition, the considerable difference in peptide bond absorption between glycylglycine and glycylglycylglycine at 205 nm (*3*) is not found at 187 nm; this suggests there may be less sensitivity to either the zwitterion (*3*) or carboxyl effects.

Adherence to Beer's Law is demonstrated in Fig. 2, where absorbancies for bovine albumin at 187 nm and 6°C for a 1-cm light path are plotted, both uncorrected and corrected for 2.5 percent stray light. The corrected data show linearity through a concentration of 25 μg/ml, above which there is progressive departure. The uncorrected data are linear only through 10 μg/ml. Along the left ordinate of Fig. 2 is plotted the contribution of stray light for each absorbancy. From this it can be seen that uncorrected data begin to deviate from linearity when the stray light contribution exceeds about 20 percent of total transmitted light. Correction for stray light preserves linearity through about 80 percent contribution. Above this the effect of stray light becomes too great for correction. Therefore, in terms of protein concentration, the assay at 187 nm is reliable only up to concentrations of about 25 μg/ml. More concentrated solutions may be handled conveniently if cells with a reduced light path are used.

The greatest virtue of this assay is



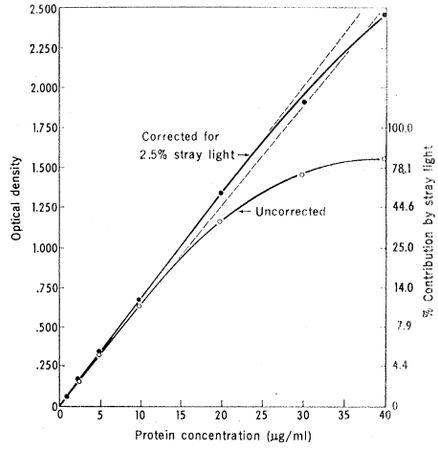


Fig. 1 (left). Absorption of water in the far ultraviolet as a function of temperature. Values are for a 1-cm light path. Fig. 2 (right). Adherence to Beer's Law at 187 nm, 6°C, and 1-cm light path for bovine albumin (fraction V). Solid lines show experimental data. Dashed lines represent theoretical linearity. The left vertical axis shows the percent of total transmitted light contributed by stray light.

Table 1. Absorption data for peptides and proteins at 187 nm. The extinction per peptide bond was calculated by dividing the molar extinction coefficient (E_M) by $n-1$, where n is the number of amino acid residues per molecule.

Compound	Extinction coefficient		
	E 0.1%	E_M	E peptide bond
Glycine	4	30	
Glycylglycine	81.6	1.08×10^4	1.08×10^4
Glycylglycylglycine	94.0	1.85×10^4	0.92×10^4
Glycylglycylglycylglycine	108.6	2.92×10^4	0.97×10^4
Glycyltyrosine	132.0	3.14×10^4	3.14×10^4
Glycyltryptophan	161.0	4.20×10^4	4.20×10^4
Bovine fraction V	82.0	5.81×10^6	1.00×10^4
Human fraction V	86.0	5.67×10^6	0.98×10^4

its adaptability to monitoring column effluents. The required instrumentation includes special prisms for the monochromator, modification of the monochromator and cell housings for nitrogen purging, and a set of thermospacers for the cell compartment connected to a source of refrigerant, all of which are relatively inexpensive. The increase in sensitivity at 187 nm over that at either 205 or 210 nm would seem to justify this modest outlay.

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25 June 1969; revised 17 October 1969

L-Asparaginase-Deficient Mutants of Yeast

Abstract. *Yeast* L-*asparaginase is a multimeric enzyme for which only a single structural gene has been found. Fourteen mutants deficient in* L-*asparaginase have been isolated, and they have been located at one site on the genetic map of* Saccharomyces cerevisiae. *The* L-*asparaginase gene* (asp1) *is located about 18 centimorgans from a gene governing tryptophan synthesis* (trp4) *on fragment 2 of the map.*

The amino acid L-aspartate is a key intermediate in the synthesis of pyrimidines and of several other amino acids, and it functions as a link between the tricarboxylic acid cycle and amino acid metabolism. Many of the metabolic pathways involving L-asparate have been extensively studied in yeast. One exception is the interconversion between L-asparate and its amide, L-asparagine. In *Escherichia coli,* L-asparaginase [L-asparagine amidohydrolase (E.C. 3.5.1.-1)] catalyzes the conversion of L-asparagine to L-aspartate and ammonia (*1*). The existence of yeast L-asparaginase has been known for many years (*2*). We present here the results of part of our studies of the genes involved in the synthesis of L-asparaginase in yeast.

We wished to isolate L-asparaginase mutants by selecting cells that would not grow when L-asparagine was the only available source of L-aspartate. In yeast, however, two enzymes that generate endogenous L-aspartate can be present, L-aspartate aminotransferase [L-aspartate: 2-oxoglutarate aminotransferase (E.C. 2.6.1.1)], which synthesizes L-aspartate from L-glutamate (*3*), and L-asparaginase. Since both these enzymes can generate L-aspartate, active aminotransferase would be expected to interfere with the isolation of L-asparaginase mutants. Mutants have been isolated, however, in which the activity of the aminotransferase is reduced or absent (*3, 4*). In these mutants (denoted *asp5*) (*5*), growth occurs if L-aspartate is supplied exogenously. We found that *asp5* mutants can use exogenous L-asparagine in place of L-aspartate for growth. Therefore, by selecting mutants of *asp5* strains that are unable to utilize exogenous L-asparagine for growth, we have been able to isolate L-asparaginase-deficient cells. We used these mutants (denoted *asp1*) to determine how many genes are involved in the synthesis of L-asparaginase and to find where these genes are located on the yeast genetic map.

We also tried to isolate mutants that have an obligate requirement for L-asparagine. Such mutants, if deficient in L-asparagine synthetase, would not be able to grow on exogenous L-aspartate alone. We have found no stable mutants that exhibit this characteristic.

L-Asparaginase mutants were induced in heterothallic strains of *Saccharomyces cerevisiae* by treating haploid cells of strains X2902-21B (*α trp4 asp5*) and X2902-67B (*a trp4 asp5*) with 3 percent ethyl methanesulfonate (*6*). Treated cells were plated on enriched agar plates and allowed to grow at 30°C. After colonies had formed, they were replica plated onto minimal medium supplemented with 100 μg of L-asparagine monohydrate per milliliter and then onto minimal medium supplemented with the same amount of L-aspartic acid. The plates with minimal medium were incubated at 30°C for 1 day and then at room temperature (about 24°C) for several more days. The pairs of replicas were compared periodically, and those clones that grew on minimal medium plus L-aspartic acid but not on minimal medium plus L-asparagine were isolated, purified by selecting single colonies, and tested further. Fourteen stable mutants were found among 30,000 clones grown from treated cells.

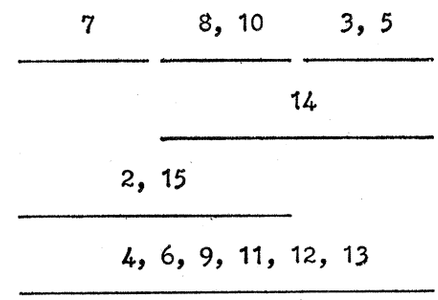

Fig. 1. Complementation map derived from pairwise crosses of 14 L-asparginase mutants of *S. cerevisiae.* Allele numbers of noncomplementing mutants appear on overlapping lines. Mutants that complement are represented by numbers on nonoverlapping lines.

The 14 mutants were crossed to *asp5* strains, the diploid cells were sporulated, and tetrad analysis was performed on the haploid spores formed during sporulation (*7*). In these crosses, the *asp5* mutation was homozygous so that the synthesis of L-aspartate from L-glutamate could not occur. We followed segregation of the inability to utilize L-asparagine by replica plating the haploid segregants onto selective media. All 14 mutations segregate in the 2:2 fashion expected of chromosomal genes

Table 1. Segregation of the *asp1* gene relative to *trp4.* The PD refers to parental ditype asci, NPD to nonparental ditype asci, and T to tetratype asci (*7*). From the totals, the distance between the *asp1* and *trp4* genes, *x*, can be calculated (*9*) from the equation $x = 50(T + 6NPD)/(PD + NPD + T)$.

Allele	Number of asci		
	PD	NPD	T
2	35	0	11
3	4	0	4
4	9	1	6
5	8	0	7
6	7	0	2
7	8	0	2
8	9	0	1
9	18	0	8
10	5	0	1
11	4	0	4
12	11	0	5
13	5	0	3
14	8	0	7
15	5	0	7
Totals	136	1	68