that 18 μM cycloheximide reduced protein synthesis by 93% in perfused rat liver but this concentration of drug also reduced protein degradation by 60%; Wildenthal and Griffen[9] observed decreased cathepsin-D-activity due to cycloheximide in cultured fetal mouse hearts. It can be seen from table 2 that in the absence of cycloheximide there was still no difference between control and dystrophic samples.

In addition to diaphragm, we have also studied abdominal muscle because, like diaphragm, it is a flat, thin sheet and lends itself to diffusion of small molecules into and out of the tissue. It also had the advantage of being readily recognizable and removable in these small animals. Tables 1 and 2 show that in this muscle also, the rates of tyrosine release were the same in dystrophic and control hamsters. The present data are tentatively interpreted to mean that increased proteolysis may not be essential to the development of the muscle lesion but rather could be a result. This interpretation is based on our observations that muscles from dystrophic hamsters did not show increased tyrosine release at ages younger than those at which the morphological lesion appeared[6]. Furthermore Goldspink and Goldspink[7] demonstrated increased tyrosine release at 100 and 230 days when the disease is well advanced. In making an interpretation it is well to remember that this method only allows for estimates of overall protein degradation and does not allow one to determine the fate of a particular protein which could be instrumental in the development of a lesion. We also recognize that it is not possible to relate directly tyrosine release to assays of cathepsin activity because cathepsin assays are conducted using synthetic or artificial substrates.

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Studies on substrate specificity of X-prolyl dipeptidyl-aminopeptidase using new chromogenic substrates, X-Y-p-nitroanilides

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Summary. Substrate specificity of X-prolyl dipeptidyl-aminopeptidase (dipeptidyl aminopeptidase IV) was examined by using newly synthesized 8 chromogenic substrates, X-Y-p-nitroanilides. Homogeneous enzyme from human submaxillary gland hydrolyzed glycylproline p-nitroanilide almost specifically, except alanylalanine p-nitroanilide which had 11% activity.

X-Prolyl dipeptidyl-aminopeptidase[1] is an enzyme which cleaves N-terminal X-proline from peptides. The enzyme was purified from either porcine kidney[2-4] or human submaxillary gland[5]. We synthesized several new chromogenic substrates, p-nitroanilides of the dipeptides, glycylproline, alanylproline, lysylproline, arginylproline, glutamylproline, and aspartylproline for X-prolyl dipeptidyl-aminopeptidase purified from human submaxil-

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Table 1. Analytical data for X-Y-p-nitroanilide · tosylates

Compound	Melting point (decomp) °C	Optical rotation $[\alpha]_D$	Temperature (°C)	Molecular formula	Found / calculated (%) C	H	N
Gly-Pro-pNA · TosOH	223 ~ 225	−81.0 (C1 MeOH)	30	$C_{20}H_{24}O_7N_4S$	51.82 51.72	5.19 5.21	12.24 12.06
Gly-Leu-pNA · TosOH	124 ~ 140	−12.3 (C1 DMF)	25	$C_{21}H_{28}O_7N_4S \cdot H_2O$	50.73 50.59	5.90 6.06	11.01 11.24
Gly-Sar-pNA · TosOH	183 ~ 185			$C_{18}H_{22}O_7N_4S$	48.92 49.29	4.98 5.07	13.00 12.78
Gly-Gly-pNA · TosOH	220 ~ 222			$C_{17}H_{20}O_7N_4S \cdot H_2O$	45.92 46.15	4.95 5.01	13.01 12.66
Gly-Hyp-pNA · TosOH	142 ~ 146	−36.3 (C1 DMF)	25	$C_{20}H_{24}O_8N_4S \cdot H_2O$	48.35 48.19	5.24 5.26	10.98 11.24
Gly-Ala-pNA · TosOH	239 ~ 241	−31.1 (C1 DMF)	25	$C_{18}H_{22}O_7N_4S \cdot 1/4H_2O$	48.88 48.81	4.93 5.12	12.82 12.65
Ala-Gly-pNA · TosOH	244 ~ 247	−16.3 (C1 DMF)	25	$C_{18}H_{22}O_7N_4S \cdot 1/4H_2O$	48.88 48.81	4.98 5.12	12.48 12.65
Ala-Ala-pNA · TosOH	122 ~ 141	−11.1 (C1 DMF)	25	$C_{19}H_{24}O_7N_4S \cdot 1/2H_2O$	49.07 49.45	5.21 5.46	11.95 12.14

Table 2. Rate of hydrolysis of X-Y-p-nitroanilides (pNA) with X-prolyl dipeptidyl-aminopeptidase

Substrate	Enzyme activity μmole/min mg protein (37 °C)	%
Gly-Pro-pNA	11.02	100
Gly-Leu-pNA	0.000	0.000
Gly-Sarcosine-pNA	0.009	0.080
Gly-Gly-pNA	0.002	0.016
Gly-Hyp-pNA	0.201	1.82
Gly-Ala-pNA	0.176	1.60
Ala-Gly-pNA	0.011	0.10
Ala-Ala-pNA	1.194	10.83

Homogeneous enzyme from human submaxillary gland was used as enzyme. Activities were measured at pH 8.7 in 71 mM glycine-NaOH buffer and at a substrate concentration of 1.4 mM. The values are the mean of duplicate experiments.

lary glands in order to examine the specificity of the N-terminal amino acid, and found that glycylproline p-nitroanilide had the highest activity among the substrates at the optimum pH (8.7), followed by p-nitroanilides of alanine, lysine, arginine, glutamic acid, and aspartic acid in a decreasing order of activity[6]. Since the homogeneous enzyme from human submaxillary gland did not hydrolyze glycylphenylalanine β-naphthylamide at all[5], the enzyme was supposed to be specific for the 2nd amino acid proline. However, the purified enzyme from porcine kidney was found to hydrolyze not only X-Pro-Y, but also X-Ala-Y[3,4]. Therefore, in order to examine the specificity of the 2nd amino acid, we have synthesized new chromogenic substrates, p-nitroanilides of the dipeptides, Gly-Pro, Gly-Leu, Gly-Sarcosine, Gly-Gly, Gly-Hyp, Gly-Ala, Ala-Gly and Ala-Ala.

These compounds were synthesized in a tosylate form by coupling Boc-Gly or Boc-Ala N-hydroxysuccinimide ester with several amino acid p-nitroanilides used in this study in N,N-dimethylformamide followed by the treatment with p-toluenesulfonic acid in acetic acid at room temperature. Amino acid p-nitroanilides used as the starting material were synthesized by coupling corresponding carbobenzoxy amino acids with p-nitroaniline applying phosphorus oxychloride method[7]; the carbobenzoxy group was removed by the treatment with 25% HBr-acetic acid at room temperature.

Analytical data for the final products were summarized in table 1. X-Prolyl dipeptidyl-aminopeptidase in human submaxillary gland was purified to a nearly homogeneous form from the materials obtained at autopsy by a procedure described previously[5]. The enzyme activity was assayed by directly measuring the liberated p-nitroaniline by the method described previously[6]. Incubation mixture contained 75 μmoles glycyne-NaOH buffer (pH 8.7), 1.5 μmoles of each X-Y-p-nitroanilide, and enzyme plus water to 1.05 ml. Incubation was carried out at 37 °C for 30 min.

The results are shown in table 2. Among various substrates having the sequence of Gly-Y-p-nitroanilide, Gly-Pro-p-nitroanilide was hydrolyzed almost specifically. Only Gly-Hyp-p-nitroanilide and Gly-Ala-p-nitroanilide had slight but significant (2%) activity. However, Ala-Ala-p-nitroanilide had about 11% of the activity. The results suggest that the dipeptidyl-aminopeptidase is highly specific for the 2nd amino acid proline, but hydroxyproline and alanine can also be the 2nd amino acid.

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Energy transfer from the second excited singlet state of spirobifluorene

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Summary. Energy transfer from the second excited state of spirobifluorene is examined by polarization spectroscopy. Evidence against spiroconjugation and evidence in favor of a coulombic mechanism is reviewed and discussed. This coulombic interaction is categorized as an intramolecular energy transfer caused by the 'extrinsic factors' of the Weber nomenclature.

Bis-(2,2'-biphenylene)-methane (common name spirobifluorene, SBF) with its spirolinked planes has been used as a model compound in studying intrachromophoric energy transfer (ET)[2]. The 1 excited singlet state of fluorene (F) is reported to be long axis polarized while the 2 excited singlet is short axis polarized[3]. When the flurenic moieties are joined via the spirocarbon linkage to form SBF, the 1 excited states of the dimeric components posess dipoles that are perpendicularly oriented, and thus no dipole-dipole interaction would be expected. But, if the chromophores should be excited to the 2 excited singlet state, where the dipoles are oriented in a parallel fashion, the possibility of ET occurring through a dipolar interaction exists.

Energy transfer between the favorably oriented 2 excited singlet dipoles would be expected to produce an excitonic splitting of the degenerate levels. This excitonic splitting is difficult if not impossible to measure because it is a radiationsless decay from a perturbed 2 singlet and hydrocarbons usually emit from the lowest vibronic level of the lowest excited single state (Kasha's Rule). However, an indirect indication of whether ET is occurring might be found by measuring the polarization of the resulting singlet state emission. Depolarization of the fluorescence of SBF as compared to F would offer strong evidence for the occurrence of intramolecular ET.

If ET is to occur through a coulombic mechanism, the dipoles must be oriented in such a way that the projection