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POSITRONIUM ANNIHILATION
IN AMINO ACIDS AND
PROTEINS

Progress Report

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Abstract

The mean lives of positron annihilation in amino acids and proteins were carefully measured. The positronium pick-off quenching rate in amino acid is found to be related to the carbon number in its molecule. The non-polar part of the molecule tends to have less effect on the pick-off quenching of o-Ps than the polar part. Similar theory can be applied to proteins. This is in agreement with the theoretical treatment by Tao.¹ The effect of the properties of amino acids and proteins on the values of τ_1 and I_2 is also discussed.

I. Introduction

Positron annihilation in common amino acids and several common proteins was investigated by using the lifetime technique. The mean lives of positron annihilation in samples were carefully measured. Then we tried to correlate the measured data and the properties of the samples. This is a brief preliminary report. For details regarding positron or positronium annihilation one is referred to recent review articles.²

II. Experimental

All the samples used in this experiment were obtained from Nutritional Biochemicals Corp. The purest possible forms of the compounds available at reasonable price were used. L-cysteine and L-lysine were obtained as HCl salts. No further purification was made. The samples were degassed under 10^{-4} torr for about 24 hours before the lifetime measurements were made. All the measurements were at room temperature 22°C. All the samples were in solid form.

The resolution of the time-to-amplitude converter used for the lifetime measurement was 400 psec or slightly better. The positron annihilation lifetime spectra obtained were analyzed by our standard method.³ A simple two-exponential component curve fit was used.

III. Results

The annihilation mean life of the first component τ_1 , the annihilation mean life of the second component τ_2 and the intensity of the second component I_2 are the resultant output after the data reduction. The values of τ_1 , τ_2 and I_2 for common amino acids are summarized in Table 1. The values of τ_1 , τ_2 and I_2 for some DL-amino acids and a couple of other compounds are summarized in Table 2. The values of τ_1 , τ_2 , and I_2 for some common proteins and a few other compounds such as RNA and DNA are summarized in Table 3.

IV. Discussion

A. τ_1 , τ_2 and the Properties of Amino Acids

If we disregard the common amino acids for which the intensity I_2 is less than 10%, the annihilation mean life of the second component, in general, is found to increase with the number of carbon atoms in a molecule of the amino acid. This relationship is shown in Figure 1, in which the values of τ_2 are plotted against the number of carbon atoms in a molecule. This indicates that the non-polar part of the amino acid molecule tends to have less effect on the pick-off quenching of the o-Ps than the polar part.

If we extrapolate both the upper bound of the data and the lower bound of the data to zero carbon atoms, the contribution of the

zero carbon annihilation mean life to the whole annihilation mean life in an amino acid will be about 0.3 to 0.4 nsec.

The values of the mean life of the first component τ_1 are found to increase with the value of τ_2 . This relationship can be easily visualized by inspecting Figure 2.

For DL-amino acids the values of τ_1 , τ_2 and I_2 are found to be very close to the values of those for L-amino acids. In general, the values of I_2 for DL-amino acids are found to be slightly higher than those for L-amino acids.

B. I_2 and the Properties of Amino Acids

After a thorough study no systematic relationship can be found between I_2 and many properties of amino acids. However, we have found an interesting anomalous relationship between I_2 and the value of the isoionic point, pI , as shown in Figure 3. For the few acidic amino acids with pI of 5.4 or less, the values of I_2 are found to be less than 10%. For the few basic amino acids with pI of 7 or higher, the values of I_2 are found to be greater than 25%. For most of the amino acids with pI around 6 the values of the intensity I_2 vary from 5% to 57% with most of the values concentrated about 25%.

The phenomenon of lower intensity I_2 for acidic amino acids and higher intensity I_2 for basic amino acids is easy to understand.

The reaction

through which positronium is formed certainly proceeds easier for a basic amino acid than for an acidic amino acid. For a basic amino acid the positive charge at the amino group is more strongly attached to the nitrogen atom than the negative charge at the carboxyl group. It is easier for a basic amino acid to lose an electron to the positron at the carboxyl group than an acidic amino acid. However, it is hard to understand why for neutral amino acids with pI about 6 the intensity of the positronium component can vary from as low as 5% to as high as 57%.

C. Proteins

The positron annihilation mean lives in common proteins vary within limited ranges. The values of τ_1 vary from 0.35 nsec to 0.38 nsec and the values of τ_2 vary from 1.4 nsec to 1.9 nsec. The values of the intensity I_2 range from 21% to 28% except for those of hemoglobin and keratin.

If we consider that proteins are just condensation polymers of amino acids, neglect the water produced during condensation and treat the values of $\lambda_1 = 1/\tau_1$, $\lambda_2 = 1/\tau_2$ and I_2 to be constitutive properties of proteins, we can calculate the values of τ_1 , τ_2 and I_2 for a protein from the values of τ_1 , τ_2 and I_2 for individual amino acids provided the amino acid composition of the protein is known.

We have

$$(1/\tau) = \sum_i (I_i P_i / \tau_i) / \sum_i (I_i P_i), \quad (2)$$

and

$$I = \sum_i (I_i P_i) / \sum_i (P_i), \quad (3)$$

where I_i is the intensity of the component for the i th amino acid; τ_i is the mean life of the component for the i th amino acid and P_i is the fraction of the i th amino acid in the protein.

The calculated values of τ_2 and I_2 are shown with the measured values in Table 3. Most of the calculated values of I_2 are quite close to the measured values of I_2 . But there are three exceptions, i.e. for hemoglobin, keratin and pepsin. All the calculated values for τ_2 are much lower than the measured values. Therefore, the values of τ_2 and I_2 , actually including τ_1 , for a protein are not constitutive properties of individual amino acids in the protein.

Actually, we should treat protein as a polymer or a macromolecular. As mentioned before, the positronium annihilation mean life, or τ_2 , is related to the number of carbon atoms in an amino acid molecule. The carbon atoms form the non-polar part of the molecule of an amino acid. It is concluded that the non-polar part of the molecule of an amino acid tends to have less effect on the pick-off quenching of o-Ps than that of the polar part. The peptide link, $-C-N-$, if it is not non-polar certainly is much less polar than the free amine and carboxyl groups in an amino acid. Therefore, the mean life τ_2 and its intensity I_2 in an ordinary protein should approach those of Nylon. Actually, this is found to be true.⁵ Or we can extend the usefulness of Figure 1 by counting all the number of carbon atoms in a protein. This number can be quite large. However the lines in Figure 1 should level off as the number of carbon atoms per molecule becomes large.

The above theory is further supported by the values of τ_2 for glycineglycine. The data point for glycineglycine (No. 14) lies between the two limiting lines if the number of carbon atoms in glycineglycine is treated as the true number. 4.

V. Conclusions

The positronium annihilation mean life τ_2 for amino acids is related to the number of carbon atoms in the amino acid molecule. The non-polar part of the molecule tends to have less effect on the pick-off quenching of o-Ps than the polar part. The values of τ_1 are found to increase with the values of τ_2 . Obviously the same effect also applies to τ_1 . Protein can be treated as a long chain amino acid. Similar theory can, therefore, also be applied to protein -- the value of τ_2 is related to the polar or non-polar nature of the protein. This is in agreement with the relationship between the pick-off quenching of o-Ps and the polarizability of the medium compound investigated and theoretically proved previously.¹

The intensity I_2 is found to be somewhat related to the isoionic point pI. However, the wide range of I_2 , 5% to 57%, for neutral amino acids with pI around 6 is a puzzle. Further study is required.

It is interesting to find that positron annihilation may occupy a place in the study of some properties of proteins. It is suggested that further research should be undertaken to increase our knowledge in this area. In this way, we may be able to gain a broader understanding of this problem.

Table 1
The Values of τ_1 , τ_2 and I_2 for Common
Amino Acids

Amino Acids	τ_1 (nsec)	τ_2 (nsec)	I_2 (%)
L-Alanine	0.300 ± 0.010	0.68 ± 0.03	30.3 ± 4.5
L-Arginine	0.331 ± 0.006	0.91 ± 0.02	43.2 ± 2.1
L-Aspartic Acid	0.312 ± 0.004	1.12 ± 0.05	8.6 ± 1.0
L-Cysteine (HCl)	0.322 ± 0.004	1.07 ± 0.05	5.7 ± 0.9
L-Cystine	0.314 ± 0.004	1.15 ± 0.07	4.0 ± 0.8
L-Glutamic Acid	0.294 ± 0.004	1.3 ± 0.1	3.3 ± 0.7
Glycine	0.271 ± 0.004	1.07 ± 0.05	5.3 ± 0.7
L-Histidine	0.356 ± 0.010	0.89 ± 0.04	27.6 ± 4.5
L-Hydroproline	0.285 ± 0.021	0.68 ± 0.03	41.6 ± 5.0
L-Isoleucine	0.324 ± 0.007	1.13 ± 0.03	25.3 ± 0.8
L-Leucine	0.300 ± 0.009	1.12 ± 0.02	41.7 ± 1.5
L-Lysine (HCl)	0.315 ± 0.004	0.99 ± 0.04	8.3 ± 1.1
L-Methionine	0.371 ± 0.004	1.42 ± 0.03	29.4 ± 1.2
L-Phenylalanine	0.338 ± 0.005	0.93 ± 0.02	22.0 ± 1.5
L-Proline	0.341 ± 0.006	0.86 ± 0.02	42.0 ± 2.0
L-Serine	0.240 ± 0.015	0.59 ± 0.02	57.0 ± 5.0
L-Threonine	0.322 ± 0.012	0.73 ± 0.03	26.2 ± 5.0
L-Tryptophan	0.363 ± 0.006	1.13 ± 0.02	24.4 ± 1.5
L-Tyrosine	0.367 ± 0.004	1.19 ± 0.02	13.8 ± 1.1
L-Valine	0.316 ± 0.006	0.98 ± 0.02	21.9 ± 1.6

Table 2

The Values of τ_1 , τ_2 and I_2 for Some
DL-Amino Acids and other Compounds

Compound	τ_1 (nsec)	τ_2 (nsec)	I_2 (%)
DL-Alanine	0.317 ± 0.007	0.89 ± 0.03	23.2 ± 2.5
DL-Aspartic Acid	0.324 ± 0.003	0.98 ± 0.03	9.9 ± 0.9
DL-Isoleucine	0.320 ± 0.010	1.18 ± 0.02	43.7 ± 2.0
DL-Leucine	0.347 ± 0.006	1.22 ± 0.01	42.3 ± 1.7
DL-Methionine	0.322 ± 0.003	1.34 ± 0.01	32.7 ± 0.6
DL-Phenylalanine	0.360 ± 0.004	1.05 ± 0.01	36.8 ± 1.2
DL-Serine	0.334 ± 0.005	0.92 ± 0.05	18.1 ± 3.6
DL-Threonine	0.336 ± 0.008	0.86 ± 0.03	27.1 ± 2.9
DL-Tryptophane	0.372 ± 0.005	1.32 ± 0.05	22.7 ± 1.5
DL-Valine	0.324 ± 0.006	0.95 ± 0.01	33.1 ± 1.5
β -Alanine	0.348 ± 0.005	1.00 ± 0.02	18.9 ± 1.5
Glycineglycine	0.340 ± 0.009	0.76 ± 0.02	22.3 ± 3.0
D-Ribose	0.330 ± 0.010	0.81 ± 0.03	34.9 ± 5.1

Table 3
The Values of τ_1 , τ_2 and I_2 for
Some Common Proteins

Protein	τ_1 (nsec)	τ_2 (nsec)		I_2 (%)	
	Measured	Measured	Cal'd	Measured	Cal'd
Casein	0.354 ± 0.005	1.58 ± 0.02	0.96	28.4 ± 0.8	23.4
Collagin	0.354 ± 0.005	1.43 ± 0.02	0.89	25.9 ± 1.0	21.7
Fibrin (Bovine)	0.357 ± 0.005	1.62 ± 0.02	0.87	24.4 ± 0.7	24.1
Gelatin	0.346 ± 0.006	1.33 ± 0.02	0.88	24.6 ± 1.3	22.0
Gluten	0.373 ± 0.004	1.61 ± 0.02	1.19	24.9 ± 0.7	19.8
Hemoglobin (Bovine)	0.369 ± 0.004	1.57 ± 0.03	0.96	13.1 ± 0.8	24.7
Keratin	0.366 ± 0.004	1.66 ± 0.03	0.95	14.8 ± 0.6	21.3
Lactalbumin	0.365 ± 0.008	1.68 ± 0.03	0.98	23.1 ± 1.4	21.3
Pepsin (3 X)	0.361 ± 0.005	1.58 ± 0.02	1.02	23.4 ± 0.9	13.2
Zein	0.379 ± 0.004	1.93 ± 0.02	0.92	25.7 ± 0.8	26.4
Trypsin (1:300)	0.348 ± 0.005	1.35 ± 0.02	----	20.7 ± 1.0	----
Hensin	0.353 ± 0.005	1.4 ± 0.3	----	2.5 ± 1.2	----
RNA	0.353 ± 0.005	1.26 ± 0.04	----	11.8 ± 1.9	----
DNA	0.362 ± 0.004	1.34 ± 0.04	----	14.1 ± 1.3	----

Note: The amino acid compositions of the proteins for the calculation were obtained from the reference 4. The values of τ_2 and I_2 for lysine (HCl) instead of pure lysine were used. The error involved from this substitution should be quite small.

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1. L-Alanine

2. L-Arginine

3. L-Histidine

4. L-Hydroxyproline

5. L-Isoleucine

6. L-Leucine

7. L-Phenylalanine

8. L-Proline

9. L-Serine

10. L-Threonine

11. L-Tryptophan

12. L-Tyrosine

13. L-Valine

14. L-Asparagine

15. L-Glutamine

16. L-Glutamic acid

17. L-Aspartic acid

18. L-Malic acid

19. L-Fumaric acid

20. L-Tartaric acid

21. L-Lactic acid

22. L-Pyruvic acid

23. L-Oxaloacetic acid

Figure 1

Number of Carbon Atoms

1. L-Alanine
2. L-Arginine
3. L-Histidine
4. L-Hydroxyproline
5. L-Isoleucine
6. L-Leucine
7. L-Methionine
8. L-Phenylalanine
9. L-Proline
10. L-Serine
11. L-Threonine
12. L-Tryptophan
13. L-Tyrosine
14. L-Valine
15. β -Alanine

Figure 2

1.0
1.5
 T_2 (msec)

50
I₂ (%)

1. L-Alanine
2. L-Arginine
3. L-Aspartic acid
4. L-Cystine
5. L-Glutamic acid
6. Glycine
7. L-Histidine
8. L-Hydroxyproline
9. L-Isoleucine
10. L-Leucine
11. L-Methionine
12. L-Phenylalanine
13. L-Proline
14. L-Serine
15. L-Threonine
16. L-Tryptophan
17. L-Tyrosine
18. L-Valine

Figure 3