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Resonance Transfer of Excitation Energy between Non-steroidal Anti-inflammatory Drugs and Aromatic Amino Acids[1]

Koji Mizuno, Shun-ichi Hata, and Suiichi Tomioka[2]

Research Laboratories, Chugai Pharmaceutical Co., Ltd.[2]

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The critical distance, R_0, which is the rate parameter for the transfer of excitation energy by resonance between aromatic amino acids and 14 kinds of non-steroidal anti-inflammatory drugs or some of other aromatic compounds was calculated. The rank correlation between the R_0 values and anti-inflammatory activity were found to be significant, and the activity of various nonsteroidal anti-inflammatory drugs were illustrated uniformly at a molecular level. Finally, a possibility of formation of electronic excited state of aromatic amino acid residues in living cell was discussed.

Because of strong untoward effects in clinical use, steroidal anti-inflammatory drugs are not always accepted by clinical physicians. Hence, non-steroidal anti-inflammatory drugs are used frequently in various clinical fields, and also are studied very actively by many research workers.[3]

Main non-steroidal anti-inflammatory drugs which are used presently in the clinical field have various chemical structures, and hence, if a common theory, by which the activity of these non-steroidal anti-inflammatory drugs may be illustrated uniformly at a molecular level, can be presented, it would be very useful for the future development of non-steroidal anti-inflammatory drugs.

In the present work, using the theory of Förster[4] as applied by Karreman *et al.*,[5] critical distance, R_0 (the distance, in Å, between an energy donor and an acceptor molecule over which excitation energy is transferred by resonance with the same probability that it is emitted as fluorescence), was calculated for the pairs between the aromatic amino acids as the donor and various non-steroidal anti-inflammatory drugs as the acceptor. Namely, critical distance in aqueous solution is calculated from the following formula:

$$R_0 = \sqrt[6]{0.95\times10^{-33}\frac{\tau\cdot J_{\tilde{\nu}}}{\tilde{\nu}_0^2}}$$

where τ is the lifetime of the excited states of the donor, $\tilde{\nu}_0$ is the average of the wave numbers of the peak of the fluorescence spectrum of the donor and of the longest wavelength peak of the absorption spectrum of the donor, and $J_{\tilde{\nu}}$ the overlap integral. The latter is defined by

$$J_{\tilde{\nu}} = \int_0^\infty \varepsilon_A(\tilde{\nu})\varepsilon_F(2\tilde{\nu}_0-\tilde{\nu})d\tilde{\nu}$$

1) This work was reported at the Annual Meeting of Pharmaceutical Society of Japan, April, 6 1969.
2) Location: *Takada, Toshima-ku, Tokyo.*
3) Y. Mizushima and M. Kobayashi, *J. Pharm. Pharmacol.*, **20**, 169 (1968); B. Silvestrini and B. Catanese, *Arzneimittel Forsch.*, **18**, 425 (1968); K. Tanaka and Y. Iizuka, *Biochem. Pharmacol.*, **17**, 2023 (1968); A.G. Radwan and G.B. West, *Brit. J. Pharmacol.*, **33**, 193 (1968); A.D. Inglot and E. Wolna, *Biochem. Pharmacol.*, **17**, 269 (1968); M.W. Whitehouse, *Biochem. Pharmacol. Suppl.*, 293 (1968).
4) T. Förster, "Fluoreszenz Organisscher Verbindungen", p. 83, Vandenhoeck & Ruprecht, Gottingen, 1951.
5) G. Karreman, R.H. Steele, and A. Szent-Györgyi, *Biochim. Biophys. Acta*, **25**, 280 (1957).

where $\varepsilon_A(\tilde{\nu})$ represents the molar extinction coefficient of the acceptor for the wave number $\tilde{\nu}$ and $\varepsilon_F(2\tilde{\nu}_0-\tilde{\nu})$ is the emission intensity of the donor, for the wave number $2\tilde{\nu}_0-\tilde{\nu}$, in the same units as the extinction coefficient. Fluorescence spectra of aromatic amino acids were obtained from the literature,[6] and the values of $\tilde{\nu}_0$ and τ used for the calculation are listed in Table I.[5]

Table I. τ and $\tilde{\nu}_0$ of Aromatic Amino Acids in Water[5]

	$\tau\times10^8$ (sec)	$\tilde{\nu}_0\times10^{-3}$ (cm^{-1})
Tryptophan	0.20	32.6
Tyrosine	0.91	34.4
Phenylalanine	1.1	37.1

Experimental

Measurements of Spectra

The absorption spectra of non-steroidal anti-inflammatory drugs were determined with a Hitachi Model EPU-2A photoelectric spectrophotometer. The solvents used for the measurements of absorption spectra were a G.R. grade of ethanol purchased from the Junsei Pure Chemicals Co., Ltd., and M/15 phosphate-buffered solution of pH 7.0. The absorption spectra are shown in Fig. 1.

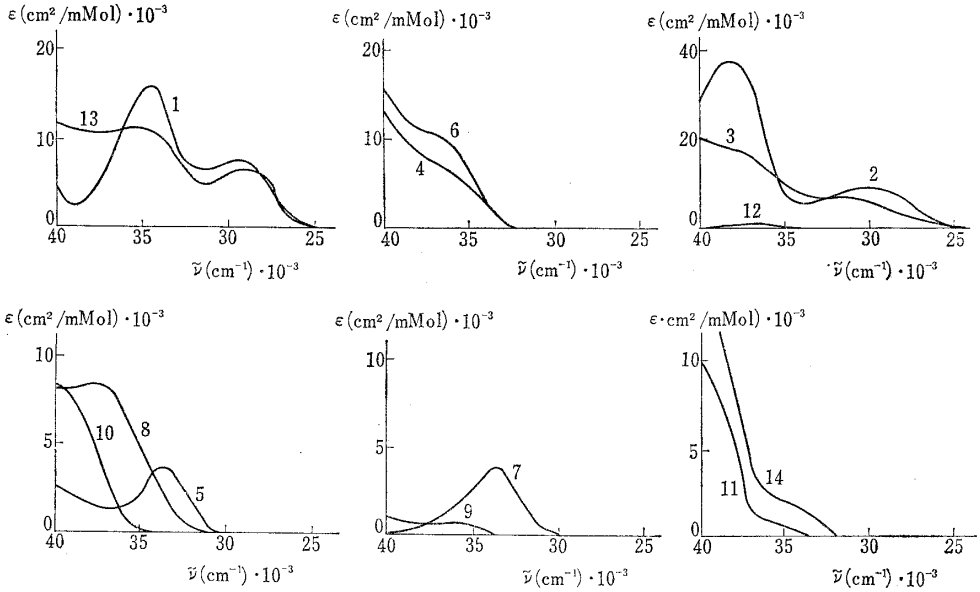

Fig. 1. Absorption Spectra of Anti-Inflammatory Drugs

1: flufenamic acid in ethanol
2: cinchophene in ethanol
3: indomethacin in ethanol
4: phenylbutazone in ethanol
5: benzydamine in ethanol
6: oxyphenbutazone in ethanol
7: salicylic acid in ethanol
8: aminopyrine in pH 7 phosphate buffer
9: acetylsalicylic acid in water
10: antipyrine in pH 7 phosphate buffer
11: phenacetin in pH 7 phosphate buffer
12: ibufenac in ethanol
13: mefenamic acid in ethanol
14: acetaminophen in ethanol

Results and Discussion

The values of R_0 for non-steroidal anti-inflammatory drugs are shown in Table II. In this connection, the aromatic amino acids should be considered not as simple monomers but as residues of some proteins when *in vivo*, and their fluorescence spectra and the lifetime of

6) F.W.J. Teale and G. Weber, *Biochem. J.*, **65**, 476 (1957).

Table II. R_0 and $J_{\tilde{\nu}}$ between Aromatic Amino Acids and Non-Steroidal Anti-Inflammatory Drugs

Substance	Tryptophan R_0 (Å)	Tryptophan $J_{\tilde{\nu}}\times10^{-8}$ (cm³)	Tyrosine R_0 (Å)	Tyrosine $J_{\tilde{\nu}}\times10^{-8}$ (cm³)	Phenylalanine R_0 (Å)	Phenylalanine $J_{\tilde{\nu}}\times10^{-8}$ (cm³)
Flufenamic acid[a]	26	1525	27	551	20	84
Cinchophene[c]	26	1526	26	410	21	113
Indomethacin[a]	24	1041	26	399	20	85
Phenylbutazone[a]	11	9	20	79	18	50
Benzydamine[a]	18	184	23	200	16	19
Salicylic acid[b]	14	44	21	125	15	18
Aminopyrine[b]	11	12	20	78	17	32
Acetylsalicylic acid[b]	8	1	11	3	11	3
Antipyrine[b]	0	0	13	7	15	17
Phenacetin[b]	0	0	9	1	13	8
Ibufenac[a]	0	0	0	0	9	1
Mefenamic acid[a]	25	1419	26	453	20	79
Acetaminophen[b]	9	3	18	43	16	19

a) Supplied from Dr. Y. Mizushima of Tokyo Univeristy.
b) Iwaki Pharmaceutical Co., Ltd., Tokyo
c) Tokyo Kasei Co., Ltd., Tokyo
d) T. Hoffman la Roche Co., Ltd., New Jersey

excited states may be a little different. It should also be assumed that the absorption spectra of acceptors may change when they are bound to biological constituents *in vivo*. In addition, the solvents used for the measurements of their spectra are not standardized to the water. However, all of these effects were disregarded in the present work.

Then, relationship between the R_0 values of non-steroidal anti-inflammatory drugs and their anti-inflammatory activity was examined, as shown in Table III. Relative anti-inflammatory activity[7] and ED_{50} in carrageenin-induced oedema[8] were used as an indicator for anti-inflammatory activity. Judging from Spearman's coefficient of rank correlation,[9] γ_s,

Table III. Spearman's Coefficient (γ_S)[a] of Rank Correlation between Values of R_0 (x_i) and Anit-Inflamamatory Activity (y_j)

	Values of R_0 (Å)	Number of samples (n)	$\sum(x_i-y_j)^2+T+U$	γ_S
(A)[b]	R_0 (Phe)	14	210.97	0.5363[d]
	R_0 (Tyr)	14	228	0.4989
	R_0 (Try)	14	252	0.4462
	R_0 (Phe)+R_0 (Tyr)[e]	14	200	0.5556[d]
	R_0 (Phe)+R_0 (Try)	14	240	0.4725
	R_0 (Try)+R_0 (Tyr)	14	246	0.4593
	R_0 (Phe)+R_0 (Try)+R_0 (Try)	14	228.1	0.4987
(B)[c]	R_0 (Phe)	11	86.05	0.6089[d]
	R_0 (Tyr)	11	89.05	0.5952[d]
	R_0 (Try)	11	113.15	0.4857
	R_0 (Phe)+R_0 (Tyr)[e]	11	79.1	0.6405
	R_0 (Phe)+R_0 (Try)	11	113.05	0.4861[d]
	R_0 (Try)+R_0 (Tyr)	11	105.05	0.5225
	R_0 (Phe)+R_0 (Tyr)+R_0 (Try)	11	109.05	0.5043

a) $\gamma_S=1-\frac{6[\sum(x_i-y_j)^2+T+U]}{n^3-n}$, $T=\frac{1}{12}\sum_t(t^3-t)$, $U=\frac{1}{12}\sum_u(u^3-u)$, where "t" and "u" are the number of samples in same rank in x_i and in y_j, respectively.
b) In the case of relative anti-inflammatory activity
c) In the case of ED_{50} in carrageenin-induced oedema
d) significant at 5% level
e) As shown in Fig. 2.

the correlation are significant at 5% level in some cases and γ_s tend to increase in the case of summation of tyrosine and phenylalanine. Therefore some complementary relationship may exist between each amino acid. These relationships are shown in Fig. 2. However, the

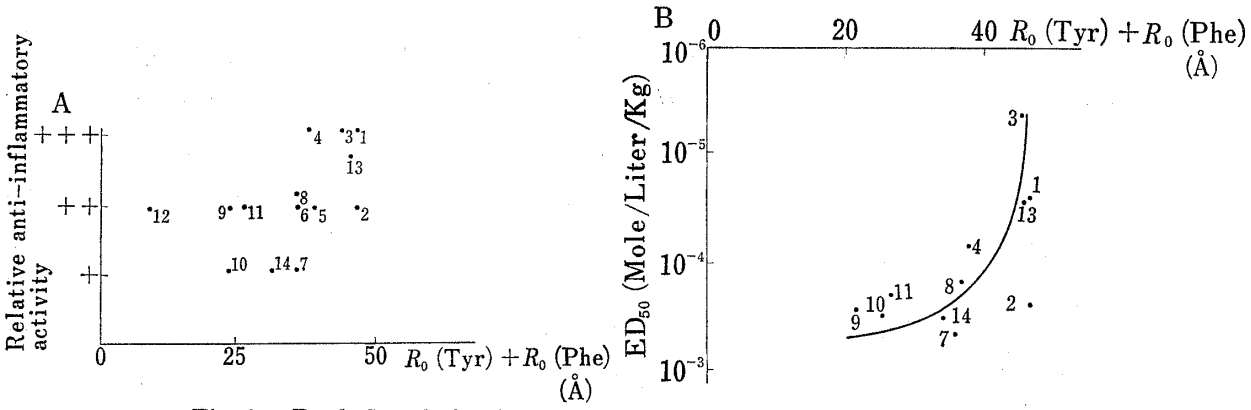

Fig. 2. Rank Correlation between Anti-Inflammatory Activity and Values of R_0 in the case of Summation of Tyrosine and Phenylanine

A: relative anti-inflammatory activity B: ED_{50} in carrageenin-induced oedema

1: flufenamic acid	6: oxyphenebutazone	11: phenacetin
2: cinchophene	7: salicylic acid	12: ibufenac
3: indomethacin	8: aminopyrine	13: mefenamic acid
4: phenylbutazone	9: acetylsalicylic acid	14: acetaminophen
5: benzydamine	10: antipyrine	

correlation is not significant in the case of summation of tryptophan and tyrosine or phenylalanine, and there seems to be some kind of a relationship here but details are not yet clear.

Changes in R_0 values by the metabolism of some non-steroidal anti-inflammatory drugs are listed in Table IV. These results show that the R_0 values of both phenylbutazone and

Table IV. R_0 and $J_{\tilde{\nu}}$ of Some Metabolites

Substance	Tryptohan		Tyrosine		Phenylalanine	
	R_0 (Å)	$J_{\tilde{\nu}} \times 10^{-8}$ (cm³)	R_0 (Å)	$J_{\tilde{\nu}} \times 10^{-8}$ (cm³)	R_0 (Å)	$J_{\tilde{\nu}} \times 10^{-8}$ (cm³)
Oxyphenbutazone	12	13	20	91	17	37
Gentisic acid	20	402	21	146	14	9

salicylic acid have a little tendency to decrease corresponding to their metabolites, oxyphenbutazone and gentisic acid, respectively, except in the case of tryptophan. Therefore, it is expected that the R_0 values of the metabolites of non-steroidal anti-inflammatory drugs would be about the same as the original values in the case of tyrosine and phenylalanine. Hence, it becomes clear from these facts that some relationships may be present between the R_0 values of non-steroidal anti-inflammatory drugs and their activity. However, some of the aromatic compounds, which have chemical structure similar to that of non-steroidal anti-inflammatory drugs, or some other drugs which have no anit-inflammatory activity, also have higher R_0 values.

Therefore, for aromatic compounds to have anti-inflammatory activity it is necessary that they have higher values of R_0 in the case of tyrosine and phenylalanine, and also some factors which keep the concentration of the compound sufficiently in action point, such as penetration of the membrane, velosity of metabolism, affinity to protein, *etc.*, must be furnished.

7) Y. Mizushima, "Inflammation and anti-inflammatory drugs," Nanzan-Do, Tokyo, 1967, p. 90, 120.
8) C.J.E. Niemegeers, *J. Pharm. Pharmacol.*, **16**, 810 (1964).
9) G.U. Yule and M.G. Kendall, "An introduction to the theory of statistics," Ch. Griffin, London 1965, p. 266.

From these results, it is suggested that the resonance transfer of excitation energy between some proteins, which will contain tyrosine and phenylalanine residues, and non-steroidal anti-inflammatory drugs would occur at the action point of these drugs, and that such a mechanism may play an important role in their anti-inflammatory effect. However, perhaps the correlation between the value of R_0(tyrosine+phenylalanine) and the anti-inflammatory activity is not only a reflection of the resonance transfer of energy but also the aromaticity of the drug. This would suggest perhaps that Van der Waals interactions are also important for the formation of a tight complex at the site of action of these drugs. However, the present results show that the value of R_0 between tyrosine or phenylalanine and various aromatic compounds will in future become an indicator to find out non-steroidal anti-inflammatory drugs. On the other hand, purine and pyrimidine bases can act as energy donor but they do not because they do not emit fluorescence in the range of neutral pH at room temperature[10] and, thereore, nucleic acid has been omitted from the present work.

Finally, how is the electronic excited states of molecules such as aromatic amino acid residues produced in a dark cell without light? As a suggestion for this question, the spontaneous extra-weak ultraviolet bioluminescence from the living organisms may considered. Konev[11] and Tarusov[12] observed a very weak spontaneous luminescence in the ultraviolet region from the living organisms such as frog heart, frog mucsle tissues, and the liver of mice, and the authors also succeeded in determining the spontaneous extra-weak bioluminescence from the living organisms recently.[13] Therefore, it appears reasonable to postulate that various normally occurring and foreign aromatic compounds can be excited electronically with these spontaneous ultraviolet ray in living cells. Hence, this work also presents an interesting problem in connection with the possible existence of electronic excited state in some biochemical processes *in vivo*.

Acknowledgement We thank Dr. Y. Mizushima, Faculty of Medicine, University of Tokyo, for the supply of non-steroidal anti-inflammatory drugs.

10) S. Udenfriend and P. Zaltzman, *Anal. Biochem.*, 3, 49 (1962).
11) S.V. Konev, "Fluorescence and phosphorescence of proteins and nucleic acids," Plenum press, New York 1967, p. 177.
12) B.N. Tarusov, *Radiobiologiya*, 1, 150 (1961).
13) K. Kumaki, S. Hata, K. Mizuno, and S. Tomioka, to be published elsewhere.