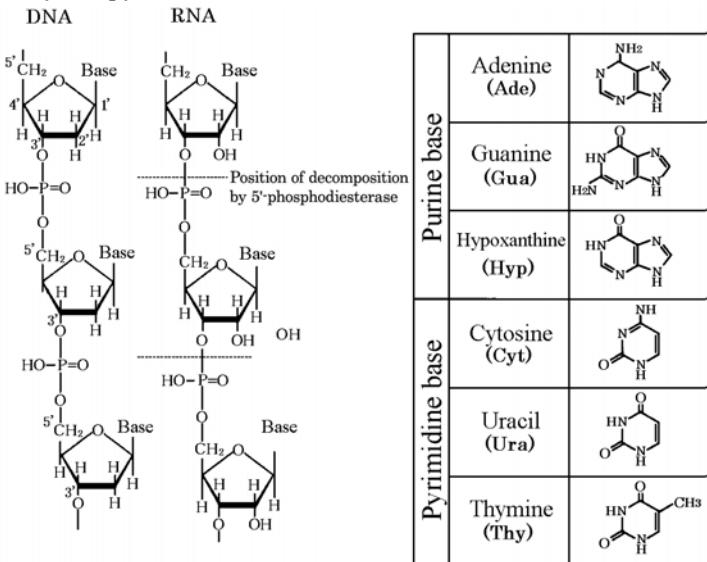


Separation of Nucleic acid constituents

Nucleic acids do exist in nuclei and protoplasm of biological cells and control the growth and heredity. There are two kinds of nucleic acids; DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). There are six kinds of nucleotides formed of sugar, phosphoric acid and purine base or pyrimidine base, and four kinds of such nucleotides bond each other in various orders and constitute large molecules: poly-nucleotides or nucleic acids. Fig.VIII-7-1 illustrates such bond-structures of DNA and RNA and six kinds of bases that constitute them.

Whereas DNA consists of four kinds of deoxy nucleotides the sugar component of that is 2-deoxy-D-ribose, deoxyadenylic acid (d-AMP), deoxyguanylic acid (d-GMP), deoxycytidylic acid (d-CMP) and deoxythymidylc acid (d-TMP), RNA consists of four kinds of ribo-nucleotides the sugar component of that is D-ribose, adenylic acid (AMP), guanylic acid (GMP), cytidylic acid (CMP) and uridylic acid (UMP). Another difference between DNA and RNA is that RNA holds uracil (Ura) instead of thymine (Thy) as pyrimidine base.



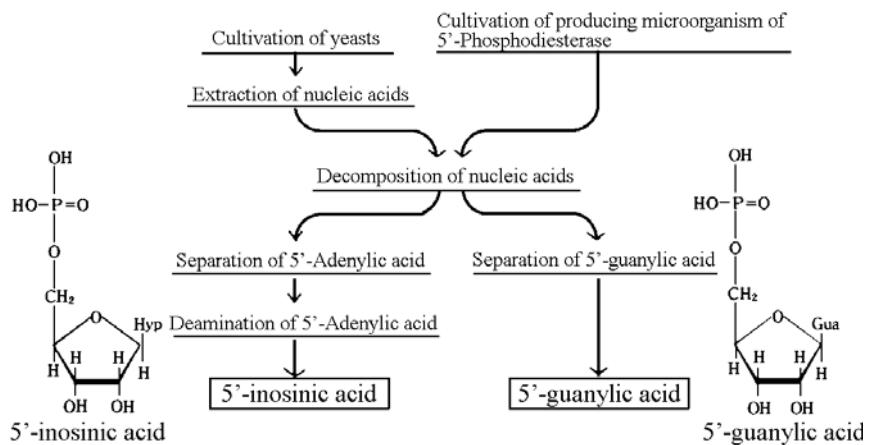
DNA: Most of the bases are Ade, Gua, Cyt, Thy

RNA: Bases are Ade, Gua, Cyt, Ura

[Fig.VIII-7-1] Structures of nucleic acids

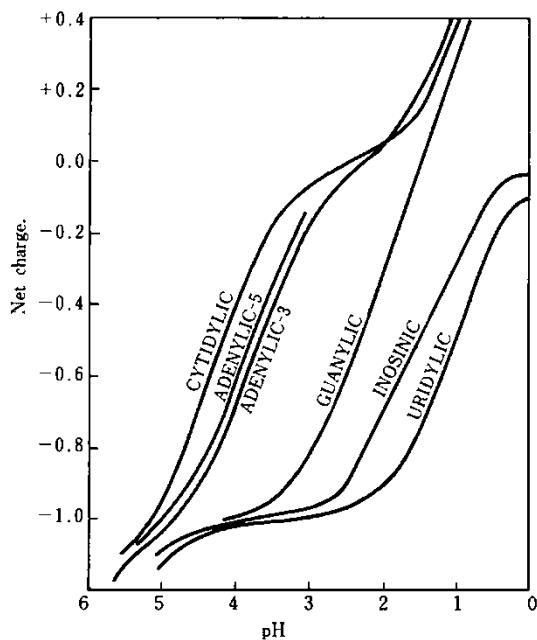
Nucleic acids are used as written below:

Firstly, taste nucleotides that are produced by enzymatic hydrolysis of RNA extracted from yeasts with NaCl or NaOH solutions are used as nucleic seasonings. Secondly, nucleotides are used as pharmaceuticals, e.g. anticancer agents, antiviral agents, blood circulation insufficiency and cerebrovascular disease therapeutic agents. Yeast RNA produces, with yeast 5'-phosphodiesterase produced by *Penicillium citrinum* or *Streptomyces aures*, 5'-adenylic acid (5'-AMP), 5'-guanylic acid (5'-GMP), 5'-cytidylic acid (5'-CMP) and 5'-uridylic acid (5'-UMP), and 5'-AMP is then converted into 5'-inosinic acid (5'-IMP). (Please see Fig.VIII-7-2.) 5'-GMP and 5'-IMP have not only the taste of shiitake mushroom and the one of dried bonito respectively but also synergistic taste effect with sodium glutamate, and thus are used as compound seasonings.



[Fig.VIII-7-2] Manufacturing of 5'-inosinic acid and 5'-guanylic acid

Sodium salts of 5'-inosinic acid, 5'-uridylic acid, 5'-guanylic acid, 5'-cytidylic acid and 5'-ribonucleotide and calcium 5'-ribonucleotide are defined in Food Sanitation Law. They are used not only as mixtures of ribonucleotides obtained by decomposition of RNA but utilized as single materials separated with IERs.

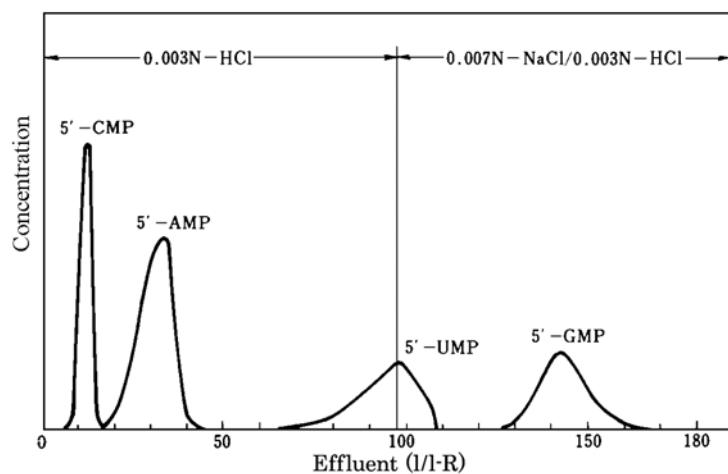


[Fig.VIII-7-3] Net charge per molecule of ribonucleotide as a function of pH
(calculated from data of Levene).⁽¹⁰⁰⁾

The separation with IERs is carried out based on the differences among the electric charges of nucleotides. The dissociations of amino groups in the base parts are different depending on bases, although the dissociation of phosphoric acid group is the same in all nucleotides. The correlation curves of the electric chargea of various nucleotides with pH are demonstrated in Fig.VIII-7-3. Electric charge differences are the largest in the pH range of 2 ~4, and thus separations are usually done in this pH range. However, the actual elution order is sometimes different from this order of electric charges, e.g. purine bases have stronger nonpolar affinity than pyrimidine bases, or thymine has stronger affinity than uracil since it has more methyl groups. Fig.VIII-7-4 illustrates the separation with SBAERs, e.g. DIAION™ MA01SS. The elution order is different from the one in Fig.VIII-7-3 partly because of nonpolar affinity.

As regards to SBAERs, anions or ion strengths in the eluents also affect the elution order. The elution positions can be estimated on the elution positions of 2'-AMP in formic acid, hydrochloric acid and acetic acid buffer solutions as shown in Fig.VIII-7-1 and on the relative elution positions of 2'-AMP against other nucleotides at various pHs as shown in Fig.VIII-7-5.

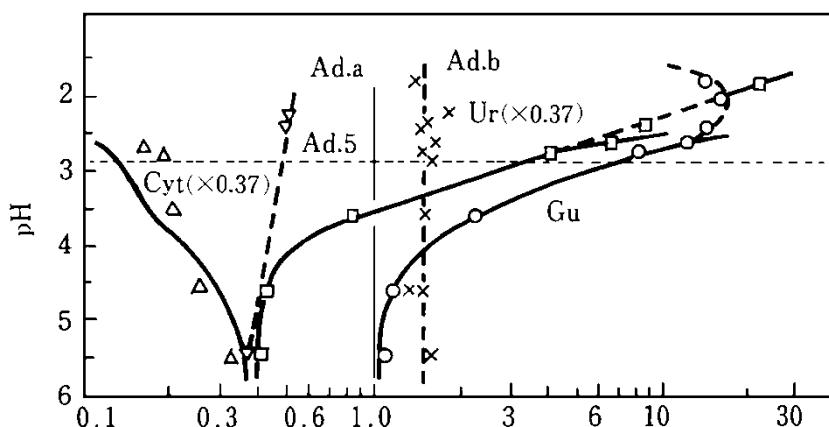
Regarding SACERs on the other side, all nucleotides but 5'-UMP that has no positive charge can be separated by elution with water after adsorption with SACERs in acidic conditions, pH ≤1.



[Fig.VIII-7-4] Separation of 5'-Mononucleotide with DIAION™ MA01SS⁽¹⁰¹⁾

[Table VIII-7-1] ML. TO PEAK OF ADENOSINS; PHOSPHATE (ADENYLIC ACID a) FOR DOWEX-1 COLUMN, APPROXIMATELY 0.74 SQ. CM. × 13.5CM. (10ML)⁽¹⁰⁰⁾

Solution	Concentration (M)	pH	ML. to peak
Formic acid	1	1.8	25
	0.25	2.2	100
	.1	2.4	340
HCl	.005	2.3	70
	.004	2.4	110
	.002	2.7	600
	.0016	2.8	900
0.01 M acetate buffer plus NaCl	.01	3.6	800
	.01	4.6	1800
	.02	4.6	800
	.02	5.6	2100
	.10	5.6	150



[Fig.VIII-7-5] Observed relative distribution coefficients (point, broken lines) and calculated relative negative charges (solid lines) of nucleotides as functions of pH