# Some Properties of the Salt between Inosine and L-Lysine

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Among several basic amino acids, only the L-lysine free base was found to form a crystalline salt with inosine. A specific stereochemical arrangement of these two molecules, inosine and L-lysine, is considered to be involved in such a crystalline salt-formation on the basis of the fact that D-lysine did not form a crystalline salt with inosine. Part of the equilibria were determined at 10 °C for the inosine–L-lysine–water and inosine–L-lysine–60 w/w % aqueous ethanol systems. The salt was unstable against water, while it was stable against aqueous ethanol (containing more than about 40% ethanol). The solubility of the salt in water was summarized as follows: $\log S$ (w/w %) $= 0.0219\,t + 0.624$. The X-ray powder diffraction data of the salt are given.

In the course of a study of the interactions between nucleic acid components and amino acids, L-lysine and L-(or D-)tryptophan have both been found to form 1 : 1 crystalline compounds with inosine.[1,2] It has also been demonstrated that the optical resolutions of these two amino acids are performed through these crystalline compounds with inosine.[2,3] Such a crystalline compound of inosine with L-lysine is considered to be a salt, while with tryptophan it is considered to be a molecular complex, on the basis of the facts that lysine is basic and tryptophan is neutral. This paper will describe some properties of the crystalline salt of inosine with L-lysine.

## Results and Discussion

Inosine possesses a $pK_a$ value of 8.8, due to the hydroxyl group at the 6 position, and can form salts with various inorganic basic substances.[4,5] However, among several basic amino acids tested, only L-lysine formed a crystalline salt with inosine. An equimolecular solution of inosine and one of the basic amino acids (free bases) including L-arginine, L-histidine, L-ornithine, L-citrulline, and L-creatine was evaporated to dryness. The powder X-ray diffraction data revealed that the residue was an equimolecular mixture of crystals of inosine and individual amino acid. (The L-ornithine free base was dried in the amorphous solid.) The characteristic X-ray diffraction patterns, shown in Table 1, were obtained only when the L-lysine free base was employed.

Such a specific ability of the salt-formation of L-lysine with inosine cannot be interpreted merely in terms of the basicity of amino acid. L-Arginine shows no salt-formation despite the fact that basicity is higher than that of L-lysine.

The solubilities of amino acids (free bases) may explain to a certain extent such a salt-formation. The L-arginine free base or the L-histidine free base is not so soluble in water, and it is easily precipitated independently before the precipitation of the salt. On the other hand, L-lysine is very soluble and it is not easily

TABLE 1. THE X-RAY POWDER DIFFRACTION DATA OF THE SALT BETWEEN INOSINE AND L-LYSINE

| $d$ (Å) | $I/I_0$ [a] |
|---|---|
| 10.28 | 50 |
| 9.18 | 20 |
| 6.29 | 90 |
| 5.16 | 10 |
| 4.98 | 10 |
| 4.87 | 5 |
| 4.56 | 80 |
| 4.49 | 100 |
| 4.44 | 30 |
| 4.16 | 50 |
| 4.06 | 70 |
| 4.00 | 20 |
| 3.92 | 20 |
| 3.77 | 20 |
| 3.72 | 30 |
| 3.56 | 50 |
| 3.41 | 10 |
| 3.29 | 10 |
| 3.23 | 70 |

a) The scale of the intensity is so chosen as to make the most intense line have the value 100.

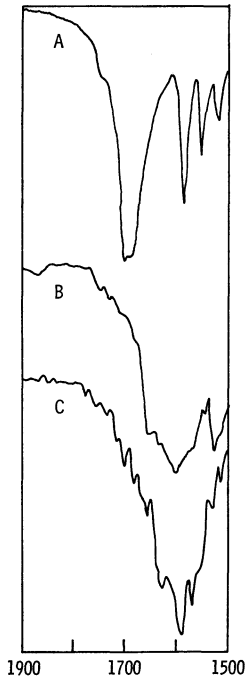


Fig. 1. Infrared spectra (KBr) of the crystals.
A: Inosine in the α-form.
B: The salt between inosine and L-lysine.
C: Sodium salt of inosine.

precipitated alone, and so there is a large possibility for the crystallization of the salt. However, the explanation in terms of the solubilities is not yet enough for the salt-formation, since L-ornithine was not associated with inosine in spite of its high solubility, like that of L-lysine.

It is also confusing that D-lysine cannot easily form a crystalline salt with inosine. This fact is advantageous for the resolution of racemic lysine.[3] When an equimolecular aqueous solution of inosine and D-lysine was evaporated to dryness, it gave an amorphous solid, or an almost amorphous solid plus a small quantity of inosine crystals in the α-form.[6] A specific stereochemical arrangement of these two molecules, inosine and L-lysine, may be involved in such a crystalline salt-formation, as is a molecular complex.

However, there is no doubt that an ionic bond is involved in this interaction. The infrared spectrum of the crystalline salt (Fig. 1) showed a strong absorbance at 1600 $cm^{-1}$, like that of sodium salt of inosine. This fact indicates an anionic dissociation of the hydroxyl group at the 6 position of the inosine molecule. The absence of the absorption band at 1680—1700 $cm^{-1}$ also indicates an absence of any carbonyl group in the inosine molecule.

A part of the equilibria for the inosine-L-lysine-water and inosine-L-lysine-60%(w/w) aqueous ethanol systems were determined at 10 °C. In order to attain to the equilibrium, the mixture of the crystals and the solution was shaken for 16 hr, 40 hr, or 48 hr; the different shaking times revealed no significant difference in the composition data of the solution phase with the shaking period.

Table 2 shows the composition data of a part of the equilibrium for the inosine-L-lysine-water system at 10 °C. Figure 2 illustrates the solution phase of this system. The solubility of inosine increases linearly with an increase in the amount of L-lysine until its L-lysine salt is precipitated (Point A in Fig. 2), and then it

Table 2. Composition data of the equilibrium for the inosine-L-lysine-water system at 10 °C

| Run No. | Solution (wt%) | | Wet residue[b] (wt %) | | Solid phase[a] |
|---|---|---|---|---|---|
| | Inosine | L-Lysine | Inosine | L-Lysine | |
| 1 | 0.95 | 0.00 | — | — | I2 |
| 2 | 4.55 | 2.48 | — | — | I2 |
| 3 | 6.65 | 4.78 | 90.3 | 2.59 | I2 |
| 4 | 9.34 | 6.55 | 87.2 | 1.57 | I2 |
| 5 | 9.37 | 6.25 | 75.8 | 3.24 | I2 |
| 6 | 15.1 | 10.6 | 84.8 | 2.91 | I2 |
| 7 | 19.9 | 15.7 | 84.0 | 3.30 | I2 |
| 8 | 20.8 | 15.5 | 88.7 | 4.60 | I2 |
| 9 | 25.1 | 19.4 | 69.2 | 29.0 | I2+I·L |
| 10 | 24.5 | 19.7 | 61.4 | 36.7 | I·L |
| 11 | 22.7 | 19.2 | 56.6 | 40.0 | I·L |
| 12 | 22.3 | 19.4 | 51.8 | 33.9 | I·L |
| 13 | 15.2 | 28.6 | 57.8 | 33.9 | I·L |

a) I2: Inosine dihydrate, $C_{10}H_{12}N_4O_5 \cdot 2H_2O$. I·L: L-Lysine salt of inosine, $C_{10}H_{12}N_4O_5 \cdot C_6H_{14}N_2O_2$. b) Inosine+L-Lysine+Water=100 (%).

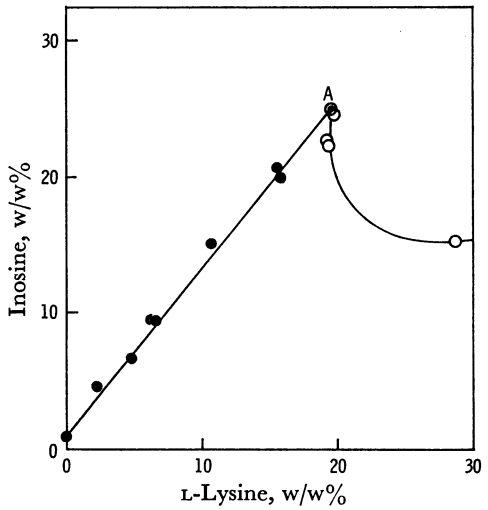


Fig. 2. An illustration of the solution phase of the equilibrium for the inosine-L-lysine-water system at 10 °C. (An extracted part from the phase diagram.) The symbolic marks of the residues are as follows:
●: Inosine dihydrate,
○: The salt between inosine and L-lysine.

decreases with a further increase in L-lysine. The slope of the initial linear increase is, however, not unity in relation to the molar ratio of inosine to L-lysine, but is, instead, about 0.7. The molar ratio at Point A is, consequently, also about 0.7. Accordingly, the L-lysine salt of inosine is unstable in the solution in which the molar ratio is unity; in other words, the salt is incongruent and unstable against water. When an excess amount of the salt is added to water at 10 °C, the suspended salt is changed into inosine dihydrate. For the stable crystallization of the salt, it is desirable that the solution possesses an excess amount of L-lysine; the molar ratio of L-lysine to inosine must be more than 1.4 (a reciprocal of 0.7).

On the other hand, the salt is stable against about 60%(w/w) aqueous ethanol, as is shown in Table 3 and Fig. 3. Table 3 shows the composition data of a part of the equilibrium for the inosine-L-lysine-60% aqueous ethanol system at 10 °C, while Fig. 3 illustrates

Table 3. Composition data of the equilibrium for the inosine-L-lysine-60% (w/w) ethanol system at 10 °C

| Run No. | Solution (wt %) | | Wet residue[b] (wt %) | | Solid phase[a] |
|---|---|---|---|---|---|
| | Inosine | L-Lysine | Inosine | L-Lysine | |
| 1 | 0.93 | 0.00 | — | — | I(α) |
| 2 | 1.47 | 0.48 | 99.3 | 0.7 | I(α) |
| 3 | 2.70 | 1.34 | 61.2 | 16.7 | I(α)+I·L |
| 4 | 2.39 | 2.12 | 64.8 | 34.4 | I·L |
| 5 | 2.55 | 4.00 | 62.7 | 36.2 | I·L |
| 6 | 2.58 | 5.92 | 66.9 | 33.2 | I·L |
| 7 | 2.52 | 8.05 | 62.0 | 32.1 | I·L |
| 8 | 2.47 | 13.8 | 64.1 | 35.7 | I·L |

a) I(α): Inosine in the α-form.[7] I·L: L-Lysine salt of inosine, $C_{10}H_{12}N_4O_5 \cdot C_6H_{14}N_2O_2$. b) Inosine+L-Lysine+Water=100 (%).

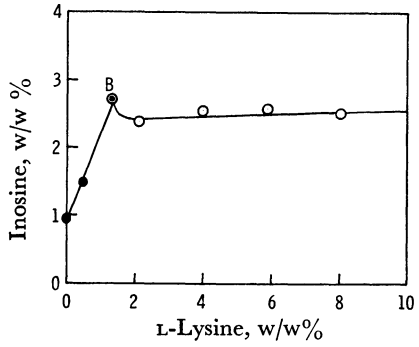


Fig. 3. An illustration of the solution phase of the equilibrium for the inosine–L-lysine–60 w/w% aqueous ethanol system at 10 °C. (An extracted part from the phase diagram.) The symbolic marks of the residues are as follows:
●: Inosine in the α-form,
○: The salt between inosine and L-lysine.

the solution phase of this system. The solubility of inosine in 60% ethanol increases linearly with an increase in the amount of L-lysine until its L-lysine salt is precipitated (Point B in Fig. 3). However, the molar ratio of inosine to L-lysine at Point B is about 1.1. Accordingly, the salt is stable against a 60% ethanol solution, whereas it is unstable against water.

A stabilizing effect in the presence of ethanol was observed when the concentration of ethanol was more than 40 w/w%. Therefore, the stable crystallization of the salt is performed from an equimolar solution of inosine and L-lysine by means of the addition of ethanol to make the concentration more than 40%.

Table 4. The solubility of inosine, and the L-lysine salt of inosine in water

| Temp. (°C) | Solubility (w/w %) | | |
|---|---|---|---|
| | Inosine in the α-form[7] | The salt, inosine·L-lysine | (Inosine-content in the solution of the salt) |
| 10 | 0.95 | 7.03 | ( 4.56) |
| 30 | 3.1 | 18.4 | (11.9 ) |
| 50 | 8.2 | 52.5 | (34.0 ) |

The precise determination of the solubility of the salt in water was difficult because of the instability of the salt against water. However, a brief determination was performed by the addition of the salt, a little at a time, to water at a constant temperature until no more salt was dissolved; we thus made clear solution. The results are summarized in Table 4, which also shows the solubility of inosine in the α-form for comparison of the inosine-content in the solution. The logarithm of the solubility is linear to the temperature; it is expressed as follows:

$$\log S\,(\%) = 0.0219\,t + 0.624$$

where $S$ represents the weight of the salt contained in 100 g of the solution and where $t$ is the temperature (°C). The inosine-content in the solution of the salt thus obtained is about four times as much as that in the saturated solution of inosine in the α-form.[7] An aqueous solution of a high concentration of inosine for medical use can be prepared by dissolving the salt of inosine with L-lysine into water.

## Experimental

*Materials.* The crystals of inosine, the L-arginine free base and the L-histidine free base used were of a commercial grade of the Ajinomoto Co., Inc. The aqueous concentrated solutions of the free bases of L-ornithine, L-lysine, and D-lysine were prepared from their respective hydrochlorides through the ion-exchange method. The free bases of L-citrulline and L-creatine were commercially obtained. The salt between inosine and L-lysine was prepared according to Suzuki *et al.*[1]

*Examination of the Salt-formation.* Inosine (0.005 M) and one of the basic amino acids (0.005 M) were dissolved in water, after which the mixture was evaporated to dryness *in vacuo*. The resulting solids were examined by the X-ray powder diffraction method. The IR spectra (KBr) were measured with a Nippon Bunkoh Model IR-S instrument.

*Analysis.* The inosine was determined with the absorbance at 250 nm in 0.1 M HCl, using the molar extinction coefficient of 11800. The lysine was determined according to the ninhydrin method by Yamagishi *et al.*[8]

*Determination of the Inosine–L-Lysine–Water System.* An excess amount of the crystals collected from inosine and the L-lysine salt of inosine were added to water or an aqueous solution of the L-lysine free base to make a slurry in tightly-capped glass bottles at 10±0.2 °C. The equilibria were achieved by tumbling the bottles for 16 hr (Runs No. 2, 3, 5, 7, 8, and 11) or 40 hr (Runs No. 4, 6, 9, 10, 12, and 13) at a constant temperature. A tumbling period of at least 16 hr was employed so as to avoid any more significant variation in the composition data of the solution phase. The prolonged period of 40 hr was employed for the convenience of the experimental schedule. Then, the mixtures were quickly filtered by means of a glass filter. The clear solutions and wet residues were submitted to analysis. The residues were examined by means of the X-ray diffraction method (Cu$K\alpha$) in order to identify the crystal form.

*Determination of the Inosine–L-Lysine–60% Ethanol System.* The same method was used except that the solvent was not water but 60 w/w% ethanol containing a definite amount of L-lysine. The tumbling period for the equilibrium was 48 hr.

*Stability of the Salt in Relation to Aqueous Ethanol.* An excess amount of the salt between inosine and L-lysine was added to an aqueous ethanol which had a definite concentration of ethanol. After the mixture had been shaken overnight at 10 °C, the suspended solid was examined by means of the X-ray method in order to confirm whether or not the form of the crystals was changed.

*The Solubility of the Salt in Water.* The salt between inosine and L-lysine was added to water, little by little, with vigorous shaking in order to dissolve it at a constant temperature (10, 30, or 50 °C). When a very small amount of a suspended solid remained undissolved in the solution, the mixture was filtered; the clear supernatant was then submitted to the analysis of inosine. The amount of the salt dissolved was calculated from the stoichiometry of 1 : 1.

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