EFFECT OF MYOSIN-ACTIN INTERACTION ON THE HEAT-INDUCED GELATION OF MYOSIN IN THE PRESENCE OF F-ACTIN

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INTRODUCTION

THE EXPERIMENTS of Fukazawa et al. (1961 a,b,c) showed myosin to be a key constituent with respect to the desirable binding quality in the experimental sausages. They also reported that the water-soluble proteins, actin and tropomyosin exerted almost no direct influence on the binding quality of meat. Subsequently, Samejima et al. (1969) found that the actomyosin seemed to play an important role in the binding properties of sausage emulsion. Actin itself did not exhibit any influence on the binding properties, but when F-actin and myosin were both present, the resulting binding properties were considerably improved (Samejima et al., 1969). Nakayama and Sato (1971), on the other hand, reported that the binding quality of the reconstituted actomyosin as well as myosin B superior to that of myosin alone, and on the basis of the results of their viscosity measurements, they concluded that native tropomyosin might relate to the binding quality of meat.

Lately, Cheng et al. (1979) also reported that effects of thermal processing on the gel textures appeared to be closely associated with degree of tropomyosin degradation from their results on fish tissue. However, Macfarlane et al. (1977) found the binding strength of myosin was higher than that of actomyosin under their experimental conditions. Siegel and Schmidt (1979) found that the mole ratio of myosin to actin in the extracted crude myosin fraction related to binding ability of adjacent pieces of meat.

Those findings implicate the importance of myosin-actin interaction to develop gelling properties of actomyosin systems and neccessity of a further study on whether native tropomyosin really play an important role in improving the heat-induced gel formability of myosin in the presence of F-actin. Thus, effect of native tropomyosin as well as those of blockage of myosin-actin interaction by chemical modifications of either myosin or actin on the heat-induced gelation of actomyosins has been explored in this report.

MATERIALS AND METHODS

MYOSIN was prepared by extracting fresh rabbit skeletal muscle according to the method described by Perry (1955). Natural actomyosin (NAM) of varied extraction time was prepared from fresh rabbit skeletal muscle by extracting with the Weber-Edsall solution. Desensitized actomyosin (DAM) was prepared from NAM according to the method described by Schaub et al. (1967). G-actin was prepared from acetone-treated rabbit muscle after applying to the method of Mommaerts (1952). Actin was modified by 2,4,6-trinitrobenzensulfonate (TNBS) by the method of Tonomura et al. (1962).

Measurements of rigidity of heat-induced gelation were carried out with a band type viscometer reported previously (Yasui et al., 1979). Scanning electron microscopic observations were made on the heat-induced gel of protein solutions using a Hitachi HHS-2R scanning electron microscope described previously (Yasui et al.,1979).

The procedure adopted for SDS-gel electrophoresis was essentially the same as that of Weber and Osborn (1969).

The binding of myosin to actin was measured using a preparative ultracentrifuge to separate bound myosin from free. Myosin was added to actin and samples were centrifuged at 100,000 x g for 120 min. About 1 ml of the supernatant was carefully removed and served for SDS-gel electrophoresis, and at the same time the protein concentration was measured. For conversion of the mixing ratio of myosin to actin from a weight basis to a molar basis, molecular weights of myosin and actin were taken as 480,000 (Tonomura, 1972) and 42,000 (Elizinga,1970), respectively.

RESULTS AND DISCUSSION

HEAT gelling properties of myosin were more improved in the presence of actin than in its absence (Samejima et al., 1969). This fact aroused our interest in the effect of actin on the heat-gelling abilities of myosin solutions. At the begining, therefore, we examined the gel-strength of NAM of various extraction times, because myosin A gradually turns into myosin B during prolonged extraction of muscle mince with a solution of neutral salt such as Weber-Edsall solution. Figure 1 depicts changes in the rigidity of the heat-induced gel and the ratio of myosin to actin of NAM and DAM isolated with Weber-Edsall solution at varying extraction times. Protein solutions (5 mg/ml) were heated at 65 °C in 0.6 M KCl and 20 mM phosphate buffer (pH 6.0). Rigidity of both NAM and DAM gradually increased in spite of the decreased in the ratio of myosin to actin with extraction period of time 0 to 24 hr.

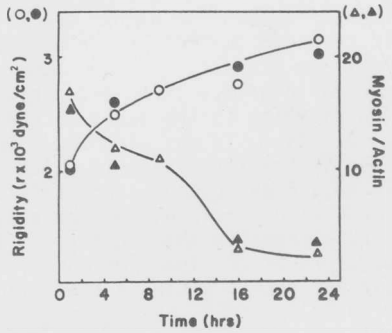

Figure 1. Changes in the rigidity of heat-induced gel and the ratios of myosin to actin of NAM and DAM extracted for varying times.
Rigidity: (○ :NAM, ● :DAM)
Myosin/Actin (△ :NAM, ▲ :DAM)

Figure 2 shows changes in the rigidity and the ratio of myosin to actin in NAM and DAM isolated with Weber-Edsall solution from aged muscle by 24 hr-extraction procedure. Rigidity of NAM extracted for 24 hr from fresh muscle (0 hr-aged) is 3,200 dyne/cm^2 and that of NAM obtained from 168 hr-aged muscle is approximately 2,600 dyne/cm^2. Correspondingly, the values of the ratio of myosin to actin are 3.0 for fresh NAM and 2.0 for 168 hr-NAM. The results suggest that myosin-actin interaction may be a key factor for signi-

ficant increase in the heat-induced gel formability. Cheng et al. (1979) recently reported that extent of myosin as well as tropomyosin degradation during thermal processing was closely related to texture of processed fish gels. It has been established in animal meat products that myofibrillar proteins are responsible for the desired textural properties in muscle-protein-based gel type comminuted products (Samejima et al., 1969; Nakayama and Sato, 1971; Siegel and Schmidt, 1979). Among the myofibrillar proteins, myosin is shown to be the primary agent for gel formation (Fukazawa et al.,1961 c; Samejima et al.,1969; Macfarlane et al.,1977) and, when combined with myosin, actin exhibits its complemental effect on the heat-induced gel formability of myosin (Samejima et al.,1969; Nakayama and Sato, 1971). In addition to roles of two major myofibrillar proteins, myosin and actin, the results of Cheng et al. (1979) seem to suggest the importance of tropomyosin or native tropomyosin in the heat-gelling properties of gel-type meat products. This view was already stressed by Nakayama and Sato (1971) who comparatively studied rheological properties of heat-set gels of isolated myosin B (NAM), DAM and reconstituted actomyosin.

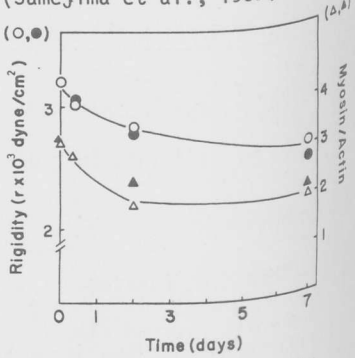

Figure 2. Changes in the rigidity of heat-induced gel and the ratios of myosin to actin of NAM and DAM isolated from aged muscle. Symbols are the same as Fig. 1

To confirm this close correlation between native tropomyosin and gel strength, we measured the rigidity of heat-induced gels of DAM prepared from NAM shown in Figs. 1 and 2, since it appears to be of particular interest if relatively small amount (7-8 % of myofibrillar proteins) of regulatory proteins which play significant roles in living muscle could participate in the gel formation of comminuted meat products as the second complemental agent other than actin. Actually, however, the results obtained (Fig. 1 and 2) were contradictory to the conclusion of Nakayama and Sato (1971) in the role of native tropomyosin for the gel textures.

Unlike actin, if the heat-induced gel was centrifuged after thermal treatment of the solution of actomyosin or myofibril suspension at high ionic strength (e.g. 3 % NaCl or KCl), a large part of tropomyosin was freed from the sediment (Samejima et al., unpublished data). Tropomyosin itself is a very heat stable protein (Woods, 1969) and did not show any sign of gelation upon heating (Samejima et al., unpublished data). Thus, it may be concluded that tropomyosin does not affect the gel texture induced by thermal treatment even though tropomyosin degradation looks coincident with myosin heavy chain degradation (Cheng et al., 1979). The apparent coincidence is merely a reflection of susceptibilities of those proteins to the proteolysis, and the finding that extent of myosin degradation is highly related to gel textural properties does not necessarily follow that tropomyosin acted as an enhancing agent for gel-formability of comminuted meat products.

Figure 3 shows changes in rigidity of reconstituted actomysoin solutions (5 mg/ml) equilibrated at various temperatures for 25 min at pH 6.0 in 0.6 M KCl. When the protein solutions were heated at temperatures ranging from 20° to 70 °C, the rigidity of the system increased as the temperature rises. The rigidity value which has reached maximum at 60 °C remains almost constant from 60 to 70 °C. These changes are similar to that of myosin solution described previously (Ishioroshi et al., 1979). Though the maximum value of the rigidity of myosin at pH 6.0 was 1,800 dyne/cm^2, those of actomyosins in which the corrected mole ratio of myosin to actin are 2.7 and 1.6 are formed to be 5,300 and 4,500 dyne/cm^2, respectively. These much higher values of the reconstituted actomyosin indicate the effectiveness of actin for myosin gel-formation by heating. As shown Fig. 3, while myosin alone revealed a typical temperature profile with two transition temperatures at 43 ° and 55 °C (Fig. 3 (a)), F-actin itself did not show any sign of gelation. Its rigidity showed higher values (500 dyne/cm^2) than those of any other reconstituted actomyosins at temperatures below 40 °C but showed relatively low rigidity at higher temperatures than 40 °C. This fact may be a refrection of the thixotropic nature of F-actin at or below body temperature of mammals (Tonomura,1972; Brotshi et al.,1978).

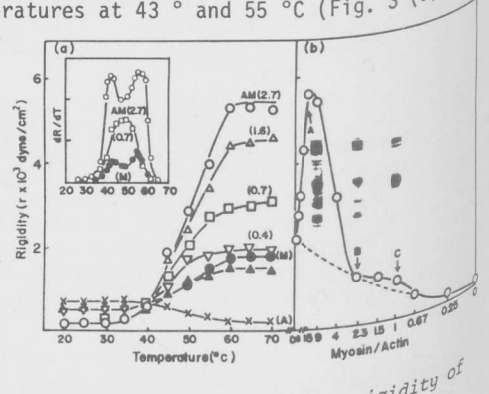

Figure 3. Changes in the rigidity of recostituted actomyosin. Changes in the rigidity of gel induced by stepwise heating are indicated in (a) and mole ratios of myosin to actin are shown in (b).
(a); (●):myosin alone, (×):actin alone Figures in parenthesis denote the corrected mole ratio. (Inset) Derivative plots as a function of temperature.
(b); Myosin alone (dotted line) and actomyosin (solid line). The gels are of supernatants at ratios indicated by arrows A,B and C after ultracentrifugation.

It would be expected than if there were no interaction between myosin and actin, the rigidity of the heat-induced gel from myosin-actin mixture at varying ratios would depend only on the myosin concentration in the system. When conbined with myosin, however, F-actin did exert a marked influence on the heat-induced gelation of myosin (Fig. 3 (a) and (b)). If the buffer alone was added in place of F-actin solution, the rigidity measurements were those expected for the dilution of the myosin (Fig. 3 (b) and Ishioroshi et al.,1979). Thus, under these experimental conditions, it was found that the greater the proportion of myosin the higher the gel formability as expressed by the rigidity up to the mole ratio of myosin to actin of about 2.7, where the maximum rigidity of 5,500 dyne/cm^2 was reached. Further increase in the ratio of myosin to actin brought about an almost linear decline of the rigidity to the level of control myosin which was 1,800 dyne/cm^2 (Fig. 3 (b)). It was also found that the rigidity developed upon heating by the actomyosin in which the mole ratio of myosin to actin was 1 or 1.6 exhibited essentially the same temperature profile as in the case with myosin alone, whereas, unlike the case with control myosin, changes in the rigidity of the actomyosin as a function of temperature at the lower mole ratio of 0.5 or 0.25 lacked the Tm_1 of myosin and showed a single transition curve whose Tm appeared at 50 °C (Fig. 3 (a)).

Investigations on the extent of myosin binding to F-actin were carried out by examining the protein concentration and qualitative protein distribution in the supernatant after removal of the actomyosin complex by ultracentrifugation at the three mole ratios of myosin to actin as specified in Fig. 3 (b). As shown by the electrophoretograms in Fig. 3 (b), the amount of free myosin in the supernatant decreased in the order of A,B and C and the converse relationship was seen with the amount of unpolymerized actin.

Figure 4 illustrates scanning electron microscopic structures of the heat-induced gels of myosin (a), reconstituted actomyosins (b-d) and F-actin (e) heated for 30 min at 65 °C in 0.6 M KCl and 20 mM phosphate (pH 6.0). The network structure with globular projections of myosin (Fig. 4 (a)) was transformed to smooth thread-like ones as the amount of actomyosin complex increases in the system (Fig. 4 (b)-(d)), and there was a progression in three dimensional ordering and a decrease in pore size. As mentioned earlier, F-actin alone did not gel upon heating under the present experimental conditions, but instead precipitated with liberation on water (Fig. 3 (a)). The scanning electron micrograph revealed that the protein formed beads-like aggregates and the spaces were too open to entrap water (Fig. 4 (e)). Thus, the presence of F-actin in excess made the morphology of the actomyosin gel similar to that of actin precipitates as shown in Fig. 4 (d). Those morphological changes from (a) to (d) in Fig. 4 are in accordance with the rigidity changes described above. The morphological aspects presented here suggest that the binding of myosin to F-actin, which aggregates and precipitates upon heating at temperature above 50 °C enables the complex to gel under the same conditions, forming thread-like three dimensional structure quite different from the original beads-like aggregates of F-actin.

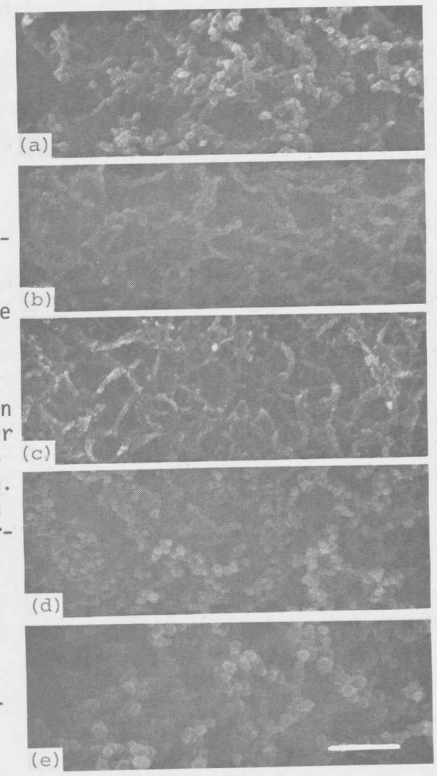

Figure 4. Scanning electron micrographs of myosin (a) and actomyosin (b-d) gels and actin precipitates (e).
Bar length is 2.0 μ.
The mole ratios of myosin to actin were 2.7 (b), 0.7 (c) and 0.18 (d), respectively.

The effect of pH on heat-induced gelation of reconstituted actomysoin is shown in Fig. 5 (a). At 65 °C, gel formed at pH 6.0 shows a highest value of shear modulus over the pH range 5.0-8.0. This trend is very similar to our previous report (Ishioroshi et al.,1979) about the heat-induced gelation of myosin. When the relative concentration of myosin in the system was much greater than that of F-actin, pH dependence of the developed rigidity of the system appeared to be substantially the same as that of myosin alone (Fig. 5 (a)). However, as the relative concentration of actin increased and that of myosin decreased, the peak shifted towards acidic side, i.e., from 6.0 to about 5.5. The maximum and minimum rigidities observed at any pH values tested were at the myosin/actin ratios of about 2.0 and 0.4, respectively, and the latter mole ratio corresponds to a stoichiometry for the myosin binding to F-actin; thus an improvement of the heat induced gel formability of myosin in the presence of F-actin was found to depend to a certain extent on the amount of actomyosin complex in the system whose gel formability is pH sensitive and fully exhibited at pH 5.5.

Changes in the rigidity under the standard assay conditions (pH 6.0 and 65 °C) were studied as a function of KCl concentrations (Fig. 5 (b)). As pointed out in our previous report (Ishioroshi et al.,1979), there is a dual dependency of the heat-induced gelation of myosin on KCl concentration, one is characterized by an extremely high rigidity at 0.1-0.2 M KCl and its dependency on storage time before heating and the other which has high rigidity at 0.4-1.0 M KCl and is independent of storage time. This trend was found to be the same with actomyosin reconstituted from varying mole ratios of myosin to actin as in the case of myosin alone (Fig. 5 (b)). Actomyosins in which fresh myosin (within 48 hr after preparation) had been used demonstrated the first peak with high rigidity values at 0.1-0.2 MKCl together with the second peak at 0.5-0.8 M concentration ranges, whereas the ones reconstituted with aged myosin (15 days after preparation) was devoid of the first peak, but revealed only the second one. It is worthwhile to note that the first peak specific for acto-fresh myosin appears to decrease its peak height with decrease of the myosin/actin mole ratio in the system and that the second peak which is common to both acto-fresh and acto-aged myosin demonstrates, with decrease in the myosin/actin ratio, the same changes as the rigidity changes shown in Fig. 3 (b). The origins of these decreasing and increasing effects of actin on the two peaks were explored by calculating % excess rigidity at KCl concentrations of 0.2 and 0.6 M, pH 6.0 and 65 °C.

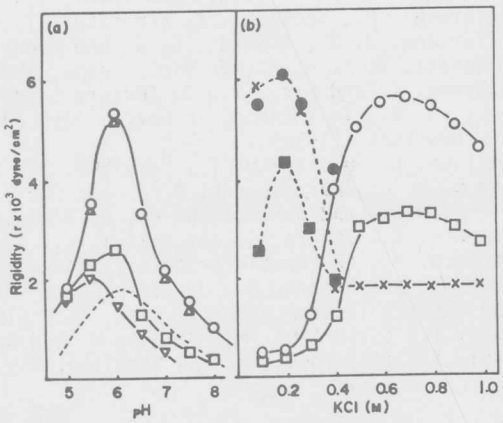

Figure 5. Effects of pH (a) and salt concentration (b) on changes in the rigidity of actomyosins and myosin treated for 20 min at 65 °C.
(a). In 0.6 M KCl. The mole ratios of (M) to accin (A) were; Myosin alone (dotted line), (○) M/A=2.7, (△) M/A=1.6, (□) M/A=0.7 and (▽) M/A=0.4.
(b). At pH 6.0. Dotted lines, fresh myosin and acto-fresh myosin. Solid lines, aged myosin and acto-aged myosin. (○, ●) M/A=2.7 and (□, ■) M/A=0.7 and (×) myosin alone.

It is well known that the SH groups of myosin is involved in binding with actin (Bailey,1954). Thus, myosin modified with PCMB lose binding ability with actin (Tonomura,1972). Actomyosin formation was also found to be inhibited, when actin was treated with TNBS (Tonomura,1972). Figure 6 shows the effect of chemical modification of myosin and actin on the rigidity of the gel formed upon heating. When myosin treated with a small quantity of PCMB (8 moles of PCMB to mole of myosin) was mixed with native actin, maximum value of rigidity was observed at 15 or 9 parts of myosin to 1 part of actin (weight ratio) as shown in Fig. 6 (a). This trend is similar to the results of the mixture of native myosin and native actin (Fig. 3 (a) and (b)), although its value is about a half of their values. However, if myosin treated with large quantity of PCMB (16 mole of PCMB to mole of myosin) was mixed with native actin, maximum value observed at optimal ratio of myosin and actin was depressed (Fig. 6 (a)). However, the enhancing effect of actin was no longer observed at any ratios of myosin to actin when native myosin and TNBS-treated actin were mixed (Fig. 6 (b)). On the other hand, when PCMB-myosin and TNBS-actin were mixed the rigidity of the resulting gels showed low values without any peak as shown in Fig. 6 (c). However, in experiments using TNBS treated actin, it must be noted that we can not exclude the possibility of modification of the added myosin with TNBS, because 30 fold molar excess TNBS was present in

the modified actin preparation and the reagent has been known to react easily and specifically with ε-amino groups of lysil residues on and in skeletal myosin (Tonomura,1972). The results obtained here, clearly indicates that there is an optimum myosin/actin ratio for actomyosin to develop the maximum heat-induced gel formability and that myosin must be bound with actin.

There appears to be no doubt about the fact that the enhancing effect of F-actin on the heat-induced gel formability of myosin is introduced by the binding of myosin to actin, because the addition of ATP or inorganic pyrophosphate which has been known to dissociate actomyosin to myosin and actin has abolished this effect (Yasui et al.,1980), though data are not shown here.

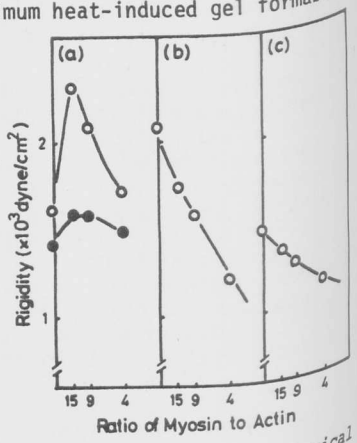

Figure 6. Effect of chemical modification of myosin and actin on the rigidity of reconstituted actomyosin gel induced by heating.
(a) PCMB-myosin and native actin.
○ : 8 M PCMB
● : 16 M PCMB
(b) Native myosin and TNBS-actin.
(c) PCMB (16 M)-myosin and TNBS-actin.

SUMMARY

NAM, DAM, myosin and actin were prepared from rabbit skeletal muscle and the rigidity of their heat-induced gels as measured by the band type viscometer. to study the gelation of myosin in the presence of F-actin, reconstituted actomyosin which prepared by mixing in varying ratio of myosin to actin was tested for rigidity and scanning electron microscopy of the gel.

We could not find essential difference between NAM and DAM in the extent of their heat-induced gel strength. Thus, it may be considered that native tropomyosin does not exert any influence on the gel formability. The highest value of rigidity of the reconstituted actomyosin was obtained when the large excess of myosin compared to actin concentration was present, i.e., mole ratio of myosin to actin being about 2.0. Myosin must be bound with actin in the optimum ratio of myosin to actin to develop desirable gel strength, since gel strength becames weaker in the systems of PCMB-myosin with native actin and TNBS-actin with native myosin than in that of native myosin and actin.

The SEM studies revealed progressive changes in three demensional ordering as actin concentration in the actomyosin varied. These were in concordance with the results of gel strength.

REFERENCES

Bailey, K., in "The Proteins" ed. by H. Neurath and K. Bailey, Academic Press, New York, Vol 2, (1954).
Brotshi, E. A., Hartwig, J. H. and Stossel, T. P., J. Biol. Chem., 253:8988 (1978)
Cheng, C. S., Hamann, D. D. and Webb, N. b., J. Food Sci., 44:1080 (1979)
Elizinga, M., Biochemistry 9:1365 (1970)
Fukazawa, T., Hashimoto, Y. and Yasui, T., J. Food Sci., 26:331 (1961 a).
Fukazawa, T., Hashimoto, Y. and Yasui, T., J. Food Sci., 26:541 (1961 b).
Fukazawa, T., Hashimoto, Y. and Yasui, T., J. Food Sci., 26:550 (1961 c).
Ishioroshi, M., Samejima, K. and Yasui, T., J. Food Sci., 44:1280 (1979).
Macfarlane, J. J., Schmidt, G. R. and Tunner, R. H., J. Food Sci.,42:1603 (1977).
Mommaerts, W. F. H. M., J. Biol. Chem., 198:445 (1952).
Nakayama, T. and Sato, Y., J. Texture Studies 2:475 (1971).
Perry, S. V., in "Methods in Enzymology" ed. by S. P. Colowick and N. O. Kaplan. vol 11, 582, Academic Press, New York. (1955).
Samejima, K., Hashimoto, Y., Yasui, T. and Fukazawa, T., J. Food Sci., 34:242 (1969).
Schaub, M. C., Hartoshorne, D. J. and Perry, S. V., Biochem. J., 104:263 (1967).
Siegel, D. G. and Schmidt, G. R., J. Food Sci., 44:1129 (1979).
Tonomura, Y., Tokura, S. and Sekiya, K., J. Biol. Chem., 237:1074 (1962).
Tonomura, Y., in "Muscle proteins, muscle contraction and cation transport". Univ. of Tokyo Press, Tokyo. (1972).
Weber, K. and Osborn, M., J. Biol. Chem., 244:4406 (1969).
Woods, E. F. Intern. J. Prot. Res., 1:29 (1969).
Yasui, T., Ishioroshi, M., Nakano, H. and Samejima, K., J. Food Sci., 44:1201 (1979).
Yasui, T., Ishioroshi, M. and Samejima, K., (in print, 1980).